MOLECULAR CHARACTERIZATION AND TISSUE DISTRIBUTION OF FATTY ACID BINDING PROTEIN (FABP) ENCODING GENE IN *SCHISTOSOMA MEKONGI*

By
Mrs. Hong Thi Cam DO

A Thesis Submitted in Partial Fulfillment of the Requirements
for Master of Science (BIOSCIENCE FOR SUSTAINABLE AGRICULTURE)
Graduate School, Silpakorn University
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การศึกษาคุณลักษณะทางโมเลกุลและการกระจายตัวของยีน Fatty Acid Binding Protein (FABP) ในพยาธิใบไม้เลือด *Schistosoma mekongi*

โดย
Mrs.Hong Thi Cam Do

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรวิทยาศาสตรมหาบัณฑิต
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By	Hong Thi Cam DO
Field of Study	(BIOSCIENCE FOR SUSTAINABLE AGRICULTURE)
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MRS. HONG THI CAM DO : MOLECULAR CHARACTERIZATION AND TISSUE DISTRIBUTION OF FATTY ACID BINDING PROTEIN (FABP) ENCODING GENE IN *SCHISTOSOMA MEKONGI* THESIS ADVISOR : ASSISTANT PROFESSOR NARIN PREYAVICHYAPUGDEE, Ph.D.

The cDNA encoding SmekFABP of adult *Schistosoma mekongi* was cloned and sequenced. The nucleotide sequence of SmekFABP was 582 bp in length. The nucleotide sequence of SmekFABP showed an open reading frame encoding FABP containing 132 amino acids. The SmekFABP amino acid sequences showed the highest degree of identity with the *S. japonicum* (GenBank: AAA64426) at 95.4%. The identity of SmekFABP amino acid sequences with other schistosome species (*S. mansoni*, and *S. haematobium* showed at 91.7 and 90.2% respectively. The expected molecular weight of rSmekFABP determined from its constituent amino acids is 14.82 kDa and the predicted molecular weight of rSmekFABP protein with histidine tag is 15.5 kDa. In this study we got one major band at 26 kDa, that could be rSmekFABP that form a complex protein, and the faint band that was 15.5 kDa, which is possible to be the rSmekFABP. A high degree of similarity and identity of *S. mekongi* with *S. japonicum* FABP in both nucleic and amino acid, and similarity in localization of worm tissue, indicates that they share a common ancestor. The low degree of conservation observed from amino acid sequences of mammalian hosts could reveal its applicable for using as the vaccine candidate against the schistosome infection which may not interfere with the hosts' FABP molecule during the vaccination. One immunogenic epitope was predicted by five programs (Hopp & Woods; Welling, Parker, B-EpiPred and ABCpred), it was 87-DSESKITQTQKDAKN-101. Base on bioinformatics approach, this region of amino acid sequence could possible to be a vaccine candidate that have dual protection against infection of both *S. mekongi* and *Fasciola* spp., and it could have a potential to be CTL and Major Histocompatibility Complex-I (MHC-I) epitope.

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Hong Thi Cam DO

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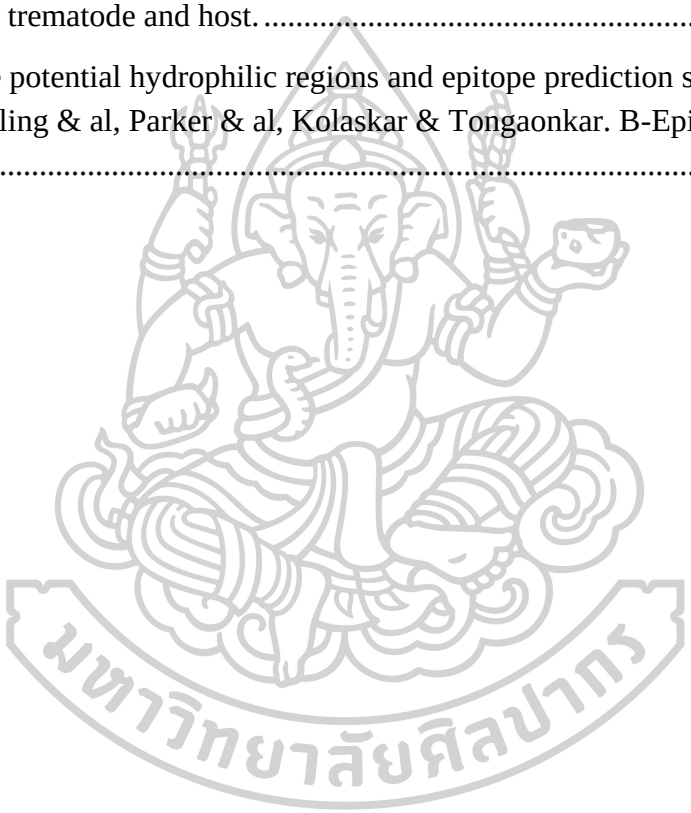

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CHAPTER I

INTRODUCTION

1.1 Introduction to the research problem and its significance

Schistosomiasis is a chronic debilitating disease caused by trematode flatworm of genus *Schistosoma* which are causative agents in human and animal in tropical region. According to the World Health Organization (WHO), annual death due to schistosomiasis is could be high as 200,000, making schistosomiasis second only to malaria among tropical disease as a cause of morbidity (Chitsulo et al., 2000; Wei et al., 2009). At least six species cause human schistosomiasis: *Schistosoma haematobium*, *Schistosoma intercalatum*, *Schistosoma guineesis*, *Schistosoma japonicum*, *Schistosoma mansoni*, and *Schistosoma mekongi* (Gordon et al., 2019). In Asia, there are three schistosome are endemic including *S. japonicum* (occurring in China, the Philippines and some area in Indonesia) is the most prevalent, *S. malayensis* (Malaysia) and *S. mekongi* (Mekong river basin such as Lao People's Democratic Republic (Lao PDR) and Cambodia, along Mekong river near the border between Southern Laos and Northern of Cambodia, there are approximately 1.5 million people are at risk of infection by *S. mekongi* (Attwood et al., 2008).

In 1978, *S. mekongi* was first identified as a new species (Voge et al., 1978). Even though the morphology is very similar to *S. japonicum*, but the differences between two species are geography distribution and morphology. Schistosomiasis mekongi is a board endemic in Mekong river basin, and a small focus was found in Thailand (Harinasuta & Kruatrachue, 1962; Sornmani et al., 1971). Humans are the definitive host for the parasite; dogs and domestic pigs serve as a reservoir. (Lorette et al., 1983; Strandgaard et al., 2001). The intermediate host of *S. mekongi* is *Neotricula aperta* (previously *Lithoglyphopsis aperta*). The lifecycle of *S. mekongi* is complex involving sexual reproductive phases in vertebrate hosts (often a mammal) and asexual reproductive phases in invertebrate hosts (freshwater snails).

Transmission occurs most frequently during the dry season, and human interaction with water is more frequent (Kitikoon et al., 1973). Poor knowledge about the disease, poor sanitation condition, and a lack of effective health systems promote

the transmission of schistosomiasis in endemic area. So successful strategy to control this schistosomiasis are required, including management of the human definitive host, animal reservoir hosts and environment.

Schistosomiasis is caused by immunologic reactions to *Schistosoma* eggs trapped in tissues. The eggs are release the antigens that result in stimulate a granulomatous reaction involving T cells, macrophages, and eosinophils that lead to clinical signs of disease. The number and location of eggs trapped in the tissues is caused symptoms and signs. Clinical feature of schistosomiasis expresses as two distinct stages. Frist, the prepatent stage begins as a skin rash caused by an immune response to the penetrating cercaria. Second the patent stage relates with schistosome egg production and can be further divided into acute and chronic schistosomiasis (Da Silva, 1991). Acute disease is commonly seen in naïve persons, whereas chronic disease, is more likely seen in resident in schistosome-endemic areas. Chronic disease occurs due to retention of eggs in the liver, spleen, and intestinal walls. Entrapment of eggs in the tissues is the beginning of inflammation and causes of granuloma in Schistosomiasis. Eggs in the tissues secret product that elicit immune reaction that end with granuloma formation and severe fibrosis in various tissue. This can result in hepatosplenomegaly, portal hypertension, abdominal pain, and bloody diarrhea (Da Silva et al., 2005).

The current drug of choice for treatment of schistosomiasis is Praziquantel (PZQ). The drug has been used in mass treatment and remains a primary tool in the war against the disease (Knopp et al., 2013). Praziquantel, a pyrazinoisoquinoline derivative, it is a broad spectrum, safe and highly efficacious anti-schistosomal which is principally active against the adult stage of all the schistosome species (Gray et al., 2011; Utzinger & Keiser, 2004; Utzinger et al., 2003; Vale et al., 2017) although its high efficacy, schistosomiasis is spreading into a new area and repeated use of the same chemotherapeutic agent in the mass treatment campaigns may concern about the development of parasite resistance to PZQ (Geerts et al., 1997). Consequently, vaccine strategies represent an essential component for the future control of schistosomiasis as an adjunct to chemotherapy and sustainable strategy for long term controlling the transmission of the disease (Botros et al., 2005; Doenhoff & Pica-Mattoccia, 2006; Fonseca et al., 2012).

Currently, Fatty acid binding proteins (FABPs) is one of six candidate vaccine antigens that was selected by the WHO to study against schistosomiasis (Bergquist N, 1995), which display various degrees of therapeutic effects (Bergquist N, 1995). FABP is a protein belongs to large family of intracellular lipid-binding proteins; having the low molecular weight (14 – 15 kDa) and the functional role for the synthesis of the unique outer membrane that is continually shed and renewed. Although their requirement for fatty acids is particularly high; however, they cannot synthesize these de-novo condition. In addition, lipids taken from the host play an immune-protective role (Bennett & Caulfield, 1991). In 2003, Valiar et al., selecting epitope from *Fasciola hepatica* and *S. mansoni*; the percentage of protection 42-50% (Vilar et al., 2003). Furthermore, the latest researching from Rahmani et al., FABP is one of immunogenic epitope that have been chosen to be construct a multi-epitope vaccine against *S. mansoni*. And based on immune-informatics analysis, this multi-epitope molecule could be stimulated T and B cell medicated immune responses (Rahmani et al., 2019).

This study focuses on gene cloning, characterization, tissue distribution and prediction immunogenic B-cell epitope of SmekFABP which could provide a promising vaccine against *S. mekongi* for future solution to eliminate schistosomiasis in endemic area.

1.2 Goal objective of the study

1. To clone and characterize FABP gene from *S. mekongi*.
2. To predict the potential immunogenic epitope of FABP from *S. mekongi*.
3. To study the distribution of SmekFABP encoding gene in parasite tissues by *In situ* Hybridization techniques.

1.3 Hypothesis of the study

Cloning, characterization and identification of Fatty acid binding protein gene from *Schistosoma mekongi* and predicted the potential immunogenic epitope of this molecule.

CHAPTER II

LITERATURE REVIEW

2.1 Schistosomiasis

Neglected Tropical Disease (NTDs) are diseases that primarily affect the world's poorest and most vulnerable populations. These diseases are caused by infectious and parasitic agents that affect billions of people per year. According to the World Health Organization, Schistosomiasis, a major of the Neglected Tropical Disease (NTDs) disease, is considered the most important helminthic disease of humanity in terms of morbidity and mortality rates for developing countries in Africa, South America, the Caribbean, the Middle East, and Asia (Greer et al., 2018; Hotez et al., 2014; WHO, 2002).

Schistosomiasis (known as Bilharziasis) is caused by the *Schistosoma* genus that live in the bloodstream of humans and animals. According to WHO, in tropical and subtropical areas schistosomiasis is only second to malaria in terms of the number of cases, socio-economic importance, and public health (Santos, 2011).

Schistosomiasis is considered to be a "man-made disease" since human are both the victims and the source of infections (El-Garem, 1998). Insanitary habits among humans such as defecation in canal water and at the same time exposing themselves to this polluted water by bathing, swimming for recreation, washing utensils and clothes, and other forms of contact characterize endemic areas (El-Garem, 1998). In addition, the parasitosis is characterized as being asymptomatic, but may evolve into more severe clinical forms, and may even cause death (Sah et al., 2015; Steinmann et al., 2006; Weerakoon et al., 2015). Schistosomiasis is highly prevalent in sub-tropical regions of Africa, the Americas, and Asia (Cavalcanti et al., 2013).

Globally, an estimated 4,400 to 200.000 people die annually caused by schistosomiasis (Thétiot-Laurent et al., 2013). WHO reports that by 2014, at least 258 million people worldwide required regular and frequent preventive treatment for schistosomiasis. Transmission is interrupted in some countries (WHO, 2010). However, environmental changes that result from the development of water resources

and population growth and migration can facilitate the spread of schistosomiasis. Recently, the disease has appeared in Europe in the French island of Corsica (Berry et al., 2016). Additionally, the WHO International Agency for Research on Cancer has confirmed that infection with *S. haematobium*, it causes a specific type of bladder cancer, with the incidence of schistosomiasis-linked bladder cancer being 32 times higher in some areas of Africa compared to the of simple bladder cancer in the USA. (WHO, 1996).

Schistosomiasis is caused by immunologic reactions to *Schistosoma* eggs trapped in tissues. The eggs are release the antigens that result in stimulate a granulomatous reaction involving T cells, macrophages, and eosinophils that lead to clinical signs of disease. The number and location of eggs trapped in the tissues is caused symptoms and signs. Initially, the inflammatory reaction is readily reversible. In the latter stages of the disease, the pathology is related to collagen deposition and fibrosis, resulting in organ damage that may be only partially reversible.

Eggs can end up in the skin, brain, muscle, adrenal glands, and eyes. As the eggs penetrate the urinary system, they can find their way to the female genital region and form granulomas in the uterus, fallopian tube, and ovaries. Central nervous system (CNS) involvement occurs because of embolization of eggs from the portal mesenteric system to the brain and spinal cord via the paravertebral venous. The groups most vulnerable to this infection are children and pregnant women, due to the forms of contagion and also because these are risk groups more prone to reinfection (Othman & Soliman, 2015; Webster et al., 2013).

2.2 Epidemiology of schistosome species

Schistosomes exists in many developing countries in Africa, Asia, South America (Sundaraneedi et al., 2017), and several Caribbean islands (Alemu et al., 2018), where it is in poor communities without access to safe drinking water and adequate sanitation. This disease ranks second beneath malaria on the list of parasitic diseases (Alemu et al., 2018; Mohamed et al., 2018) and the transmission has been existed in 75 to 76 countries (Mohamed et al., 2018; Sundaraneedi et al., 2017).

Schistosomiasis remains of significant public health important world, with an estimated 779 people are at risk of infection (Chala & Torben, 2018) and infects more

than 230 to 250 million people annually (Alemu et al., 2018; Wei et al., 2018). This disease causes 280,000 deaths annually (Sundaraneedi et al., 2017) and a worldwide burden of 3.3 million disability-adjusted life years (Alemu et al., 2018; Braun et al., 2018).

At least, 6 trematode species are known infect humans. There are *S. mansoni* (occurring in Africa, South America, the Caribbean, and the Middle East), *S. haematobium* (mainly occurring in Africa and the Middle East, with recent autochthonous transmission observed in Corsica, France), *S. intercalatum* (the species confined to a few countries in Central Africa), *S. japonicum* (Asia), *S. mekongi* (Mekong Delta including Cambodia, lao People's Democratic Republic (Lao PDR), and previously Thailand whose current stratus in transmission interruption (Boissier et al., 2016; Tchuenté et al., 2003). A sixth species, *S. malayensis*, which is thought to be closely related to *S. mekongi*, is endemic in Malaysia. These species differ not only biological from one another, but also in their geographical distribution and in the types of symptoms they produce (Table 1). All of species are primary responsible for the two major forms of schistosomiasis including intestinal and urogenital (Gryseels et al., 2006).

Table 1. Summarizes the *Schistosoma* species, regions of prevalence, and disease.

Species	Regions of prevalence	Disease
S. mansoni	Africa, Middle East, Caribbean, South America	Intestinal and hepatic
S. japonicum	China, Southeast Asia (Philippines, Indonesia)	Intestinal and hepatic
S. mekongi	Cambodia, Lao People's Democratic Republic	Intestinal and hepatic
S. heamatobium	Africa, The Middle East, Southern Europe (Corsica, France)	Urogenital
S. intercalatum	Central and West Africa	Urogenital
S. malayyensis	Malaysia	Intestinal and hepatic

S. mansoni (causing intestinal schistosomiasis) is the major species of *Schistosoma*. It is endemic in 55 countries and the most prevalent in is widely distributed in Africa, South America, the Caribbean, and Brazil, Venezuela and Suriname (Chitsulo et al., 2000; Schistosomiasis, 2010). More than 88 million people were given medical treatment. Approximately 393 million Africans are at risk of infection from *S. mansoni*, of which about 55 million are infected at any moment. Annual death due to *S. mansoni* is about 130,000 (M. J. Van der Werf et al., 2003). Zoonotic transmission is possible with these species, because the parasite infects not only human but also wild rodents.

S. haematobium is the species responsible for urogenital schistosomiasis. It is a recognized carcinogen and the 2nd leading cause of bladder cancer worldwide. It is also an underdiagnosed cause of infertility and predisposes chronically infected individuals to HIV (Antoni et al., 2017; Van Tong et al., 2017). *S. haematobium* is prevalent in many countries in sub - Saharan Africa, as well as some parts of the Middle East (Nicolls et al., 2008). This parasitosis inhabits the vesical plexus of veins around the human bladder and urinary tract and only occasionally the veins of the rectum and portal systems. In a survey in 2000, it was estimated that 70 million individuals out of 682 million had experienced haematuria and 32 million had dysuria associated with *S. haematobium* infection. Renal failure accounts for a large percentage of the estimated 150,000 deaths from urinary tract schistosomiasis. Significant association was observed between major bladder wall pathology and squamous cell carcinoma (van der Werf et al., 2003).

S. intercalatum is found in parts of Western and central African countries (King et al., 2005). There are two strains including the Lower Guinea strain and the Zaire strain. (Tchuenté et al., 2003). In 1999, the noted number of *S. intercalatum* infections was 1.73 million (Crompton, 1999). Environment factors and possible mating with the more predominant species *S. intercalatum* contribute to the limited species distribution and number.

S. japonicum is an important parasite and one of the major infectious agents of schistosomiasis. It is occurred in China, the Philippines, Indonesia and Southeast Asia. It is the cause of schistosomiasis japonica, a disease that still remains a significant health problem especially in lake and marshland regions. The severity of

S. japonicum arises in 60% of all neurological diseases in schistosomes due to the migration of schistosome eggs to the brain. Approximately 60 million individuals are risk of infection and close two million are currently infected. The disease has been eradicated in Japan through integrated multi-disciplinary. Symptoms an infected person might experience include fever, cough, abdominal pain, diarrhea, hepatosplenomegaly and eosinophilia.

S. mekongi was first identified as a new species in 1857 (Attwood et al., 2008). Humans are the definitive host for the parasite, which causes damage to the liver, spleen, gastrointestinal, and esophagus. Other than humans, other animals which serve as a reservoir for *S. mekongi* are dogs and domestic pigs (Lorette et al., 1983; Strandgaard et al., 2001). Additionally, the rate of disease due to *S. mekongi* infection is higher among children than in adults (Gryseels et al., 2006). As of 2002, these numbers estimate that 60,000 people are at risk in the river delta of Laos, with a case prevalence of 11,000. Meanwhile the 80,000 people are at risk in Cambodia (Urbani et al., 2002). While infection in animal reservoir hosts to the transmission of *S. mekongi* has not been established (Jacobson & Abel, 2007). Integrated control measures have greatly the incidence of human infection.

S. malayensis is an emerging species of *Schistosoma*, which is endemic in Malaysia (Greer et al., 1988) is more closely related to *S. mekongi* (Webster et al., 2006). It is primarily a parasite of the rodent *Rattus muelleri* and from the early studies identified the intermediate host of *S. malayensis* as *Robertsiella karporensis* (Blair et al., 1997; Greer et al., 1988). Serologic surveys showed at 3.9% prevalence in rural populations for schistosomiasis due to *S. malayensis* (Latif et al., 2013).

S. japonicum, *S. mekongi* and *S. malayensis* is three of *Schistosoma* species currently occurring in Asia. To date, Japan is the only country in Asia to have eliminated schistosomiasis, while Thailand is waiting verification of transmission interruption by the World health Organization (Rollinson et al., 2013). Currently, there are six Asian countries schistosomiasis is endemic including in China, the Philippines, Indonesia, Lao PDR, Cambodia, and Malaysia, and is merging in a seventh – Myanmar (Soe et al., 2017).

2.3 Life cycle of Schistosome species

Unlike other trematodes, schistosomes are dioecious (i.e. they have separate hosts); an adult female worm laying up 3.000 eggs per day depending on the species. The life cycle of schistosome is complex that involves in two hosts: fresh-water snails that act as intermediate host and mammals as definitive host (Ross et al., 2002). Schistosomes have co-evolved with their molluscan and mammalian host leading to a well-balanced and highly efficient means of transmission (Waine & McManus, 1997).

All six species of schistosome are contracted in the same way; that is, by direct contact with infested surface water containing free-living forms of the parasite known as cercariae, which can penetrate the skin (Figure 1.) Schistosome cercariae consist of a tail, used for motility in the water, and a head region, which contains suckers for attachment to host skin and glands containing proteolytic enzymes to facilitate penetration of the skin.

2.3.1 Cycle of Schistosome in the Molluscan Intermediate host

Eggs that are present in the human excreta hatch on contact with water and release microscopic larvae called miracidia. In other to survive, the tiny larvae must find and penetrate a specific water snail. In fresh water, these eggs form miracidia, which hatch and infect snails of the genus *Bulinus* (*S. haematobium* and *S. intercalatum*), *Biomphalaria* (*S. mansoni*), *Oncomelania* (*S. japonicum*) and *Neotricula* (*S. mekongi*). Once inside the host snail, miracidia directly penetrate the soft tissue of snail. Inside the snail, they lost their cilia and develop into mother sporocysts. The sporocysts rapidly multiply by asexual reproduction, each forming numerous daughter sporocysts. The daughter sporocysts move to the liver and gonads of the snail, where they undergo further growth (Inobaya et al., 2014). Within 2 -4 weeks, they undergo metamorphosis and give rise to fork-tailed cercariae. Stimulated by light, hundreds of cercariae penetrate out of the snail into water (Gryseels et al., 2006). Snails can shed hundreds of cercaria daily; about 200 for S. haematobium, 15 to 160 for S. japonicum, and 250 to 600 for *S. mansoni* (Braun et al., 2018). This asexual multiplication allows the parasite to increase dramatically in numbers, enhancing considerably the chances of re-infecting humans (Cheng, 1987).

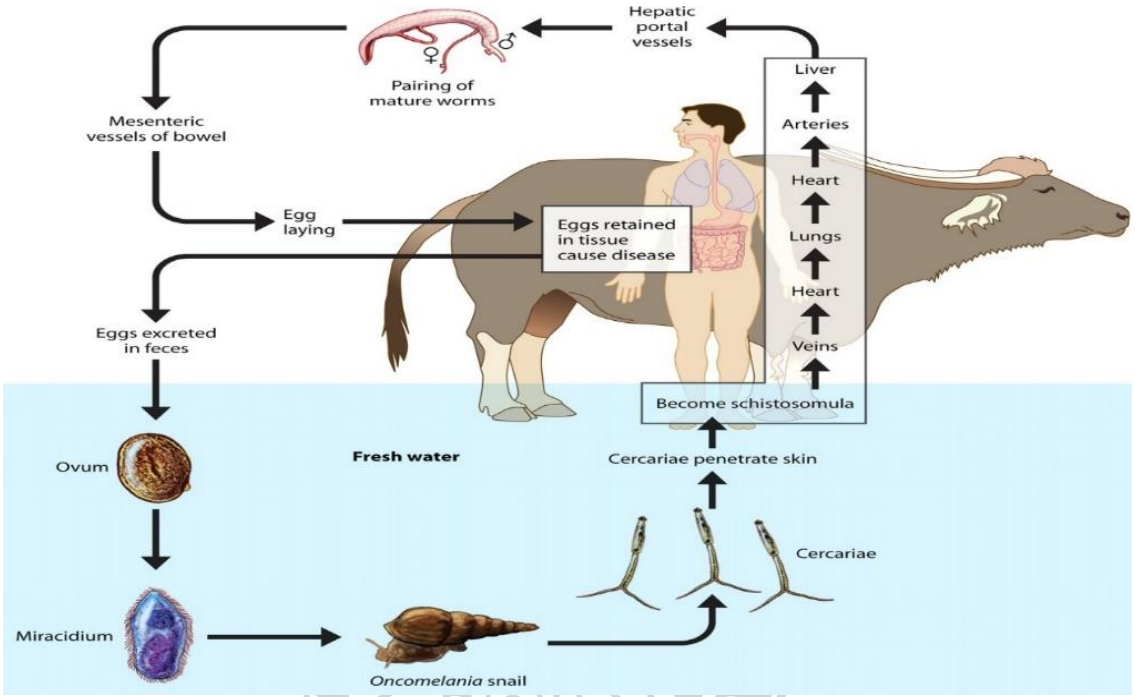

Figure 1. The life cycle of schistosomes
From: McManus et al. (2010)

2.3.2 Cycle of schistosome in the Mammalian Host

People are infected with cercariae by contact with infested water during their normal daily activities, such as hygiene, recreation, fishing, or farming by irrigation. A minute or less of contact is sufficient for the larvae to pierce the horny layer of the host and penetrate the human skin (Stirewalt & Hackey, 1956; Stirewalt & Kruidenier, 1961). During penetration, the cercariae shed their bifurcated tails, and the resulting schistosomula enter capillaries and lymphatic vessels. Within 12 hours of infection, people may complain about a tingling sensation or light rash, commonly referred to as “swimmer’s itch”, because of the irritation at the point of entrance. The schistosomula migrate throughout the body’s tissue through blood circulation. Schistosomula grow into schistosomes and adult worms (Chala & Torben, 2018; Gurarie et al., 2018).

Through the heart, schistosomula migrate to the lungs by the second or third after penetration (Gobert et al., 2007). By the 15th day, they can be found accumulated in the liver, where they feed on portal blood and undergo rapid growth. These young larval and then schistosomes digest red cells. Their development takes

three to four weeks after penetration. Then, mature worm pair off, with the male worm wrapped around the female. The mature male and female worm pair, and then, depending on species, migrate to the vessels of the bowel or bladder where egg production occurs. For example, the male transports his mate to the mesenteric venous plexus for *S. mansoni*, *S. japonicum* and *S. intercalatum* or to the vesical plexus for *S. haematobium*. The female is located under the digestive or vesical mucous membrane and lays 200 to 2000 eggs each day, starting from the 40th day after penetration. Eggs pass from the lumen of blood vessels into adjacent tissues and many are shed in the feces (*S. mansoni*, *S. japonicum*, and *S. intercalatum*) or urine (*S. haematobium*) (Cheng, 1987). Many eggs pass through the intestinal or bladder wall and are excreted in the faeces or urine. The schistosome life cycle is completed when the eggs hatch, releasing free-swimming miracidia, which, in turn, re-infect freshwater snail. The life span of the worm is five to ten years, and it is capable of evading the immune response of the host by a variety of different mechanisms (Boros, 1989). Water containing cercariae can cause human schistosomiasis (Braun et al., 2018).

2.4 Pathology of schistosome species.

After penetration in human skin, cercariae become schistosomules, which are susceptible to the attack of the cellular effector response mediated by antibodies that recognize antigens expressed on their surface, as they alter their biochemistry allowing them to escape from the immune system (Santos, 2011).

The larvae that are not destroyed can enter the vessel and are taken to the heart and lungs. In nine days, they migrate to the liver, where they feed and develop, reach adulthood, and finally reach the mesenteric veins, where they copulate and begin the production of eggs. These can be retained in the organs or can be eliminated by the feces, being the eggs of *S. haematobium* eliminated by the urine. Adult worms can render their integument resistant to immunoregulatory effector mechanisms that cleave immunoglobulins and inhibit the action of lymphocytes and mast cells (Lee et al., 2014; Santos, 2011).

Schistosomiasis is unusual amongst helminth diseases for two reasons: much of the pathogenesis is due to the eggs (rather than larvae or adults); and most of the

pathology is caused by host immune responses (delayed-type hypersensitivity and granulomatous reactions). Schistosomiasis is caused by immunologic reactions to *Schistosoma* eggs trapped in tissues. The eggs are release the antigens that result in stimulate a granulomatous reaction involving T cells, macrophages, and eosinophils that lead to clinical signs of disease. The number and location of eggs trapped in the tissues is caused symptoms and signs. Initially, the inflammatory reaction is readily reversible. In the latter stages of the disease, the pathology is related to collagen deposition and fibrosis, resulting in organ damage that may be only partially reversible. The course of infection is often divided into three phases: migratory, acute and chronic. The acute phase is usually asymptomatic, and in the chronic phase the most common manifestations of this disease are fever, chills, weakness, weight loss, headaches, nausea, vomiting, diarrhea, hepatomegaly, splenomegaly, and eosinophilia. Intestinal neoplasia and granulomatous disease of the intestine are considered to be ulcerative colitis (Santos, 2011).

The migratory phase occurs when cercariae penetrate and migrate through the skin. This is often asymptomatic, but in sensitized patients, it may cause transient dermatitis (‘swimmers itch’), and occasionally pulmonary lesions and pneumonitis.

The acute phase (sometimes called Katayama fever) is coincident with first egg release and is characterized by allergic responses (serum sickness due to overwhelming immune complex formation), resulting in pyrexia, fatigue, aches, lymphadenopathy, gastrointestinal discomfort and eosinophilia.

The chronic phase occurs in response to the cumulative deposition of fluke eggs in tissues and the host reactions that develop against them. Not all the eggs laid by female worms successfully penetrate the gut or bladder walls, many are swept away in the circulation and become trapped in organs where they elicit strong granulomatous responses. Eggs become surrounded by inflammatory cells forming characteristic pseudotubercles, which may coalesce to form larger granulomatous reactions (polyps). The encapsulated eggs die and eventually calcify. The resultant effects on host organs and tissues are manifold, and include intestinal polyposis, abdominal pain, diarrhea, glomerulonephritis, pulmonary arteritis, cardiovascular problems including heart failure, and periportal (Symmer’s clay pipe-stem) fibrosis. Portal hypertension often leads to hepatomegaly, splenomegaly, ascites, and

sometimes gross enlargement of esophageal and gastric veins (varices) which may burst. Cerebral granulomas have been associated with focal epileptic convulsions, while spinal cord granulomas may cause transverse myelitis. Infections by *S. haematobium* often cause haematuria (blood in urine) and progressive disruption of the bladder wall may lead to carcinoma.

The groups most vulnerable to this infection are children and pregnant women, due to the forms of contagion and also because these are risk groups more prone to reinfection (Othman & Soliman, 2015; Webster et al., 2013). Chronic schistosomiasis, one of the major health problems in tropical and sub-tropical countries, affects more than 200 million people worldwide, and the majority of cases occur in Sub-Sahara Africa (Chala & Torben; Steinmann et al., 2006).

2.5 Treatment for schistosomiasis – drugs

Although, the eradication and control of schistosomiasis has been successful in a number of countries including Japan, Tunisia, Puerto Rico, controlled to an acceptable level Egypt and reduced to only small foci in the Caribbean Island and most of the Middle East (Chitsulo et al., 2000). However, it remains a major public health challenge in many other countries, where the severe lack of clean water and sanitation with a simultaneous increase in socio-economic status; reduction in serious morbidity and intensity of infection has relied on treatment alone.

Several drugs have been used in the treatment of *Schistosoma* infection, and notable among them are praziquantel, metrifonate, oxamniquine and artemisinin derivatives (Conder & Campbell, 1995; Ross et al., 2002). All of these drugs are useful for treatment for both individual patient and for mass drug administration for communities in endemic area.

Oxamniquine (OXA) is activated in Schistosoma via the sulfotransferase mechanism, after which oxamniquine binds to the DNA (Albonico et al., 2015). This drug has been commercially referred to as Mansil®, where the first clinical trials with this drug were performed (Katz & Coelho, 2008). It is only effective in the case of *S. mansoni* infection, which prevents the occurrence of chronic *S. mansoni* infection and posture (Jauréguiberry et al., 2010). However, the main limitation of OXA is that it is not active against *S. haematobium* or *S. japonicum*, a fact that discouraged its use

outside South America, the only place where *S. mansoni* is prevalent and some cases of resistance to OXA have been reported (Cioli et al., 2014). Oxamniquine is used in large scale only in Brazil (Ridi El & Tallima, 2013).

Artemisinin is an antimalarial, which has been shown to have anti-schistosomal properties, with the best outcomes artesunate and artemether. In general, these types of compounds are more active against the immature form of the Schistosome than against adults. Results of clinical trials show that artesunate alone presents lower cure rates when compared to PZQ, whereas the combination of a praziquantel-artemisinin derivative is more effective than PZQ itself (Cioli et al., 2014; Jauréguiberry et al., 2010; Ridi El & Tallima, 2013). A limitation for the use of artemisinin against schistosomiasis is the risk that it may favor the development of drug-resistant plasmodia in co-endemic areas (Cioli et al., 2014).

Metrifonate can be used to treat against urinary schistosomiasis for the treatment of urinary schistosomiasis (WHO, 1995). Its use is hindered by a complex administration schedule with multiple doses required over a 2 week. However, at present there is no longer commercially available (Ross et al., 2002)

Praziquantel**,** a pyrazinoisoquinoline derivatives**,** is currently the drug of choice for the treatment of schistosomiasis and is the only commercially available anti-schistososomal medicine. Laboratory studies and clinical have shown that is highly efficacious against all six schistosome species that parasitize humans (Geary et al., 2010), although it is less active against juvenile schistosomes compared with adult worms and eggs (Gray et al., 2011; Sabah et al., 1986; Utzinger & Keiser, 2004; Utzinger et al., 2003; Vale et al., 2017).

Praziquantel is also the mainstay for preventive chemotherapy for morbidity control of schistosomiasis. Praziquantel is cost-effective for treating schistosomiasis. The World Health Organization (WHO) recommends a single dose of 40 mg/kg for all species and ages (Zwang & Olliaro, 2014). However, this recommendation has a limitation: treatment with praziquantel does not prevent reinfection (Wu et al., 1994), and is therefore relatively ineffective at interrupting the transmission cycle. Praziquantel is principally aimed at reducing the prevalence and intensity of infection and to control morbidity over the longer term. Some concern has been expressed that praziquantel- resistant schistosomes may develop, most likely in Africa (Cupit &

Cunningham, 2015; Wang et al., 2012), and there is thus a pressing need to develop new anti-schistosomal drugs (Bergquist et al., 2017) and other non-pharmaceutical interventions.

2.6 Prevention of schistosomiasis – vaccines

In spite of mass chemotherapy with Praziquantel (PZQ) has been the strategy of choice for the control schistosomiasis because of its efficacy, its ease of administration, its tolerable side-effects, and its cost (Cioli, 1998). They are limited, in sufficient and present a series of problems such as low efficacy, high toxicity, and the emergence of resistant strains (Dias et al., 2013). Furthermore, the construction of dams and the development of important irrigation schemes are often followed by impressive epidemic outbreaks.

Therefore, a more suitable option would be development of schistosomiasis vaccine, is one of strategies to prevent and control schistosomiasis. The combination strategy between vaccine and chemotherapy would decrease both morbidity and reduce the case of re-infection. Such novel control program for schistosomiasis would improve significantly on the current strategy, which is based on chemotherapy alone.

A schistosomiasis vaccine could create a long-term decrease in illness spectrum and transmission (Ricciardi et al., 2018). Currently, there are no vaccine available for schistosomiasis disease (Tebeje et al., 2016). According to WHO, there are six vaccine candidates for schistosomiasis; and vaccine candidates for *Schistosoma* infections are in different phase of development. Some vaccines have reached in pre-clinical trial phase while other are in phase one and two clinical trials. Examples include *S. haematobium* 28 kDa antigen glutathione S-transferase (ShGST) and *S. mansoni* 14 kDa of Fatty acid binding protein of (Sm14-FABP) (Santini-Oliveira et al., 2016). However, there are studies involving vaccine formulations with the purified antigen, attenuated cercaria and excretory-secretory antigens (Othman & Soliman, 2015).

Nevertheless, strong immunologic evidence in both humans and animal models exist to support the feasibility of developing of an effective vaccine for schistosomiasis control and/or elimination (Colley & Secor, 2014; Fonseca et al., 2015).

2.7 Lipid Binding protein in Plathyhelminth

Long chain fatty acids (LCFA) are related to the different cellular processes such as membrane synthesis, protein modification, and control of energy supply. LCFA also have functions as signaling and regulatory molecules; they facilitate the interplay of extracellular medium, cell membrane, cytoplasmic and nucleus to control many biological activities. LCFA get poorly soluble in aqueous solution due to the hydrophobic feature; therefore, their intracellular transport to metabolism sites and action is believed to be mediated by lipid binding proteins.

Parasitic helminths express high levels of lipid-binding proteins but they are incapable of synthesizing fatty acids and cholesterol de novo (Smyth & McManus, 2007). They survive dependently on the sequestration and utilization of host lipids during infection. So, these parasites must have an efficient binding system for the uptake and transport of key hydrophobic molecules. In metabolic pathway, lipid-binding proteins play an important role in the exchange of lipids between parasite and host. These proteins might also be involved in the uptake, transfer, and storage of hydrophobic ligands.

Two groups of lipid binding proteins have been studied extensively in platyhelminth parasites including hydrophobic ligand binding proteins (HLBPs) and fatty acid binding proteins (FABPs). Although, these members share the ability to bind lipids, they differ in their ligand binding specificity, sequence, structure, and putative function. The results of phylogenetic studies show that HLBPs and FABPs evolved via different pathways and had individually evolutionary origins. Members of both groups are putative targets for chemotherapy, immunodiagnosis and vaccine development.

2.7.1 Hydrophobic ligand binding proteins (HLBPs)

HLBPs form a family of lipoproteins specific to the cestode. Two main classes of HLBP have been described: one consists of molecules confined to the cytoplasm, while the other consists of secreted and/or excreted molecules. HLBPs are defined as abundant, immunogenic, and high molecular mass oligomers, whose monomers are helix-rich subunits of about 7–11 kDa.

HLBPs play an important role in the biological function of cestodes by controlling the sequestration of lipids from the host organism and also by regulating drug sequestration. Moreover, HLBPs might act as messenger molecules. Particularly, HLBPs could bind to signaling lipids and participate subsequently in cell activation and/or differentiation processes that are required for parasite adaptation to host immune responses.

Members of the HLBP family have been identified in *Echinococcus granulosus* (EgAgB) (Oriol et al., 1971); in *Taenia solium* (TsHLBPs) (Kim et al., 2011; Lee et al., 2007; Sako et al., 2000), in *Moniezia expansa* (MeHLBP) (Barrett et al., 1997; Janssen & Barrett, 1995), in *Himenolepys diminuta* (H-HLBP) (Saghir et al., 2000), and in *Taenia crassiceps* (Tc-HLBP) (Zarlenga & Rhoads, 1994). Genes that share a high sequence identity with other members of the HLBP family have been identified in *Taenia hidatigena* (ThLBPs) and *Taenia multiceps* (TmHLBPs); their sequences were submitted to GenBank (Jia et al., 2011). HLBPs are capable of binding to fatty acids and their CoA-esters, triacylglycerols, sterols, lysophospholipids, phospholipids, non-polar organic ions, and anthelmintic drugs (Alvite & Esteves, 2012).

2.7.2 Fatty acid binding proteins (FABPs)

FABPs are a family of the intracellular lipid binding proteins that have been to study. These proteins have a highly immunogenic character serving to confer significant levels of protection against challenge infections, and has been supposed as vaccine candidates. These proteins are widely distributed across animal species, and the results of experimental work conducted in vertebrates have contributed to our understanding of their role in parasites.

The first platyhelminth FABP that be described was isolated from the parasite *S. mansoni* and was designated Sm14 based on its apparent molecular mass (Moser et al., 1991). Homologous proteins were subsequently isolated and characterized from *S. japonicum* (Sj-FABPc) (Becker et al., 1994), *S. bovis* (SbFABP) (GenBank Accession Number: AY615730), *Fasciola hepatica* (Fh15) (Rodríguez-Pérez et al., 1992), *Fasciola gigantica* (FgFABP) (Estunningsih et al., 1997), *E. granulosus* (EgFABP1 and EgFABP2) (Esteves et al., 1993; Esteves et al., 2003), *Mesocestoides vogae* (MvFABPa and MvFABPb) (Alvite et al., 2008), and *Taenia*

solium (TsFABP) (GenBank Accession Number ABB76135). Platyhelminth FABP showed low amino acid sequence identity compared to vertebrate FABP. Furthermore, they do not contain common protein sequence motifs. However, the 3D structures that have been resolved or predicted for platyhelminth FABP are similar to the typical-barrel structures previously resolved for their FABP counterparts (Angelucci et al., 2004; Jakobsson et al., 2003).

The Sm14 protein from *S. mansoni*, which is derived from a cloned gene exhibits an affinity for fatty acids and can protect exotic mice and rabbits from challenges with *S. mansoni* cercariae.Further research leading to the application of expression of *Pichia pastoris* and use of the synthetic GLA-SE excipient, was used in phase I clinical trials (Tendler et al., 2015). In addition, Sm14 showed 44% identity with rFh15 from *Fasciola hepatica* (Hillyer, 1995). Essentially identical three-dimensional structures and shared discontinuous epitopes were observed. Furthermore, Sm14 induces elimination of liver damage in mice, sheep and goats against *F. hepatica* infection in the experiment (Hillyer, 2005; Mossallam et al., 2015). Native NFh12 and recombinant FABP rFh15 from *F. hepatica* were shown to be protective in terms of reducing worm burden and liver damage when using Freund adjuvant in C57/BL6 mice against *S. bovis* infection (Aban et al., 1999; Abáné et al., 2000). Moreover, a reduction in the large parasitic burden, improved liver damage, and anti-fertility effects were observed in BALB/c mice and golden hamsters vaccinated with rFh15 using the adjuvant adaptation vaccination system against *S. bovis* (Vicente et al., 2015; Vicente et al., 2014). Furthermore, FABP 14.6 kDa purified from *Fasciola gigantica* was shown to reduce parasitic count and liver damage against *S. mansoni* infection in CD1 mice (Aly et al., 2012).

CHAPTER III

MATERIALS AND METHODS

3.1. Collection of adult parasites

Schistosoma mekongi (Laotian strain) is maintained in *Neotricula aperta* snails and mice (ICR strain) at the Applied Malacology Unit, Department of Social Medicine and Environment, Faculty of Tropical Medicine, Mahidol University, Bangkok. Fifty of the ICR mice are individually infected with thirty of *S. mekongi* cercariae derived from experimentally infected *N. aperta* by the looping methods. At fifty-six days post-infection, mice are sacrificed by over inhalation of CO_2 and adult flukes are collected by perfusion using 0.85% normal saline solution. After washing the flukes several times with 0.85% normal saline solution, as judged by the normal macroscopic appearance and active motility, are selected and stored at −80 ◦C until use. The protocol involves in using laboratory animals was approved by the Animal Care and Use Committee, Faculty of Tropical Medicine, Mahidol University, (FTM-ACUC 006/2005).

3.2. Cloning, identification and characterization of *S. mekongi* Fatty Acid Binding Protein encoding gene

3.2.1 Cloning and sequence of *S. mekongi* FABP (SmekFABP)

cDNA of the adult *S. mekongi* from project “Development of Serodiagnosis for *S. mekongi* in Mice by Sandwich ELISA and Localization of Cathepsin B on the Worm”. which was approved by Animal Care and Use Committee of Faculty of Tropical Medicine, Mahidol University (FTM-ACUC 023/2017). Briefly, total RNA was isolated from the whole adult *S. mekongi* worms by TRIzol reagent (Molecular Research Center, Inc.) using the protocol provided by the manufacturer, and stored at −80 ◦C. The partial cDNA sequence of the SmekFABP was amplified by PCR using the following set of primers: forward primer (5’-ACT TTA GGC GTT CAG TCA ATC GGA A-3’) and reverse primer (5’-GCA ATG TTT ATT GAA CAA AAG TGA AGC TG-3’). These Primers were designed by based on GenBank: FN315763.1 of *S. japonicum*. The PCR was performed in 35 cycles at 94

◦C for 30 s, 50 ◦C for 30 s, and 72 ◦C for 90 s. The PCR product are ligated into the pGEM-T easy vector (Promega, USA) and used for sequencing.

3.2.2 Bioinformatics identification and characterization of SmekFABP

SmekFABP DNA sequences generated from cDNA clones and the deduced protein sequences were subjected to search against the nucleic acid and protein databases using the basic local alignment search tool (BLAST) (http://ww.ncbi.nih.gov/BLAST) and swiss-Prot database (https://swissmodel.expasy. org/). Multiple alignment of homologous sequences from closely related *Schistosoma* species were carried out by Clustal Omega program (https://www.ebi.ac.uk/Tools/msa /clustalo/). Using this alignment, neighbor-joining (Saitou & Nei, 1987) analysis was performed using the Mega X (Kumar et al., 2018) with bootstrap resampling using 1000 repetitions (Felsenstein, 1985). The prediction of 3-D model was visualized the I-TASSER program (Roy et al., 2010; Yang & Zhang, 2015; Zhang, 2008) and use SignalP program for predict signal peptide (Petersen et al., 2011).

3.3 Expression of recombinant SmekFABP in *Escherichia coli*

Protocol was performed according to method described by (Sripa et al., 2017). Briefly, a full DNA sequence of FABP with 6-hisditine tag at C-terminus is amplified from cDNA of *S. mekongi* adult worm using the forward primer of 5'FABPNdeIF: 5'-CAT ATG GCG ACT TTG GGT ACT GGG ATG A-3' and reverse primer: 3'FABPXhoIR: 5'-CTC GAG TTA ATG ATG ATG ATG ATG ATG AAC ATT CTC ATA TTC TTT TAT TTG TCG-3', with amplified product of 420 bp. The PCR product is ligated into PET-17b vector (Figure 2) with NdeI and XhoI digestion prior to transforming into *E. coli* (BL21). The correct FABP DNA sequencing is verified by DNA sequencing. To produce the recombinant *S. mekongi* fatty acid binding protein (rSmekFABP), the bacteria is inoculated into LB-broth containing 1 μg/ml ampicillin and grown at 37 ◦C until the OD_{600} reading of 0.6–0.8, then 1 mM of IPTG is added to the culture medium and incubated at 30 ◦C for 6 h. rSmekFABP is obtained by lysing bacteria cells with the lysis buffer (50 mM NaH2PO4, 300 mM NaCl, 8 M urea, 6 M guanidine hydrochloride and 20 mM Immidazole pH 8.0), and the protein is purified by binding to 1 ml of Ni-NTA Agarose resin (Qiagen, Germany) in a 15 ml column, washed with washing buffer

containing 20 mM Imidazole and eluted from the resin with elution buffer containing 250 mM Imidazole. The purified rSmekFABP is dialyzed using dialysis tubing which excludes MW 15.4 kDa in cold-PBS for 3 hours before use.

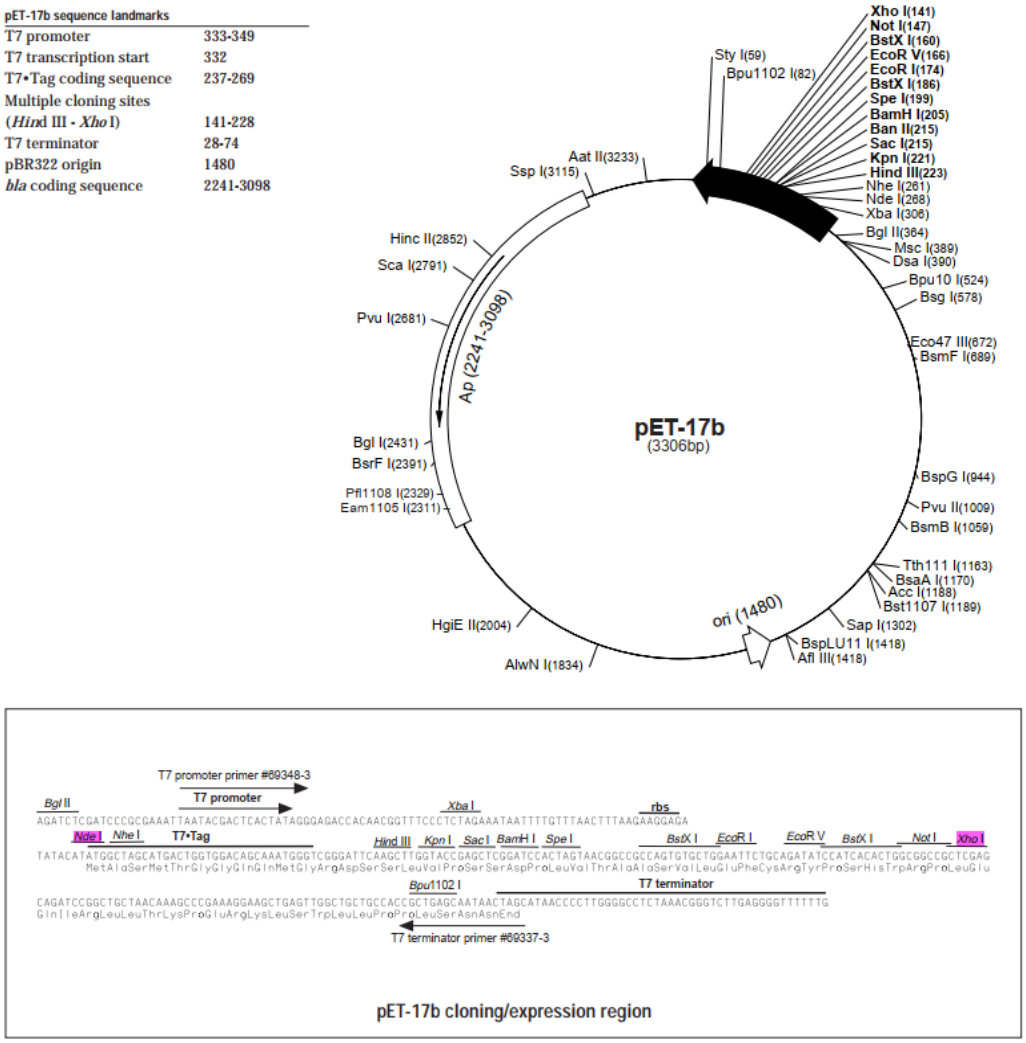

Figure 2 Diagram of pET17b cloning/expression region

From: Novagen

3.4 Prediction of antigenic sites

In this study work, the potential hydrophilic antigenic epitopes of SmekFABP were found out in order to identify the antigenic determinants. Antigenic epitopes are determined using several prediction methods, for example, Hopp and Woods (Hopp & Woods, 1981); Welling & al (Welling et al., 1985); HPLC/Parker & al (Parker et al., 1986); Kolaskar & Tongaonkar antigenicity (Kolaskar & Tongaonkar, 1990); B-

EpiPred Server (Larsen et al., 2006) and ABCpred Prediction Server (Saha & Raghava, 2006).

3.5 The distribution of SmekFABP in parasitic tissue by in situ hybridization techniques

The specific primers (SmekFABP_Forward and SmekFABP_Reverse in section 3.2.1) for FABP gene are used for localization of expression of SmekFABP mRNA by performing hybridization. The adult worms were collected and fixed in 2% paraformaldehyde at 4^0C for 18h. The tissue is then processed through routine tissue processing before being embedded in paraffin blocks. Tissue section (5 μm–thickness) is cut and mounted onto silane-coated slides. The specific primers are used to produce DIG-labeled probes by DIG-oligonucleotide labeling kit (Roche, Germany) from cDNA of adult *Schistosoma mekongi*. DIG-labeled sense and antisense single strand DNA probes are added to the hybridization, the *S. mekongi* infected tissues are sectioned, and then incubated at 42^0C for overnight. After hybridization, these sections are incubated with alkaline phosphatase conjugated anti-digoxigenin antibody for 1 h, the signal was developed by addition of NBT-BCIP substrate. Counterstain sections for 1-2 min in 0.5% Bismarck brown and photographs are taken with Leica compound microscope equipped with a digital camera (Leica, Germany).

CHAPTER IV

RESULT

4.1 Molecular cloning and analysis of *S. mekongi* FABP (SmekFABP) encoding gene

The sequence encoding SmekFABP was amplified by PCR from an adult stage *S. mekongi* cDNA library with degenerated primers. These forward and reverse primers were designed from the conserved amino acid sequences of *S. japonicum* (GenBank accession no. FN315763.1). The SmekFABP RT-PCR product was 582 bp in length (Figure 3). The nucleotide sequence of SmekFABP showed an open reading frame encoding fatty acid binding protein containing 132 amino acid residues with the predicted molecular weight 14.82 kDa (Figure 4).

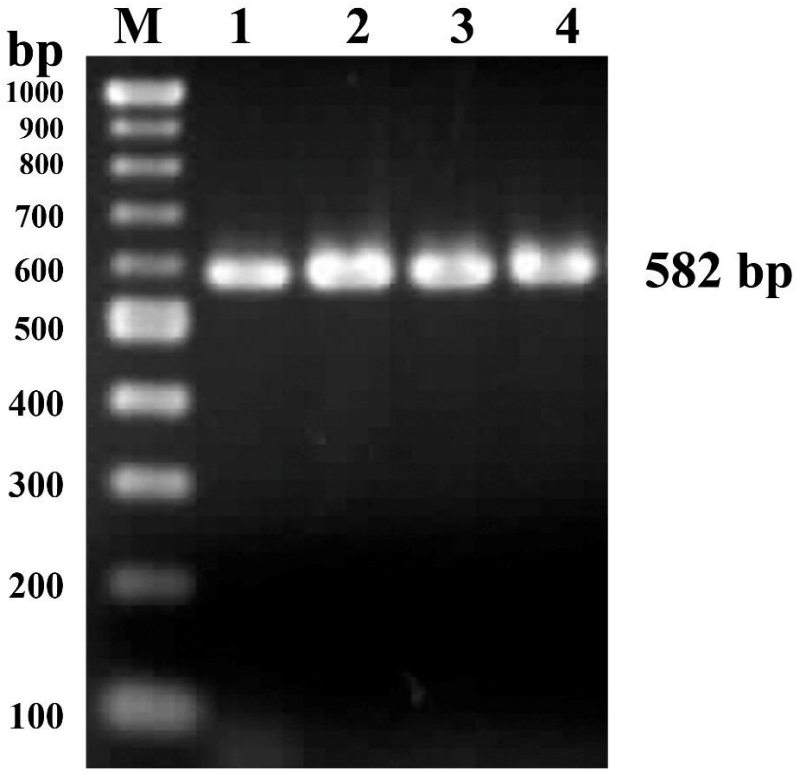

Figure 3. The PCR product specific band of SmekFABP cDNA fragment.
Lane M: 100 bp molecular marker and Line 1-4: SmekFABP cDNA fragment.

```
55  ACTTTAGGCGTTCAGTCAATCGGAAATAATGTCTTCTTTTTTGGGAAAGTGGAAA
                                  M  S  S  F  L  G  K  W  K    9
109 CTGAACGAATCACACAACTTCGATGCTGTTATGTCAAAGCTCGGTGTTTCGTGG
    L  N  E  S  H  N  F  D  A  V  M  S  K  L  G  V  S  W   27
163 GCGACCCGACAGATTGGGAACACAGTGACGCCAACTGTCACTTTTACAATGGAT
    A  T  R  Q  I  G  N  T  V  T  P  T  V  T  F  T  M  D   45
217 GGGGATACGATGACCATGCTGACAGAGTCAACTTTCAAGAACCTCTCAGTCACG
    G  D  T  M  T  M  L  T  E  S  T  F  K  N  L  S  V  T   63
271 TTCAAATTCGGTGAGGAATTCGATGAGAAAACCAGTGATGGTAGGAACGTTAAG
    F  K  F  G  E  E  F  D  E  K  T  S  D  G  R  N  V  K   81
325 TCAGTCGTTATCAAAGATTCAGAGTCAAAGATAACTCAAACTCAGAAGGATGCT
    S  V  V  I  K  D  S  E  S  K  I  T  Q  T  Q  K  D  A   99
379 AAGAACACAACTGTAATCGTTCGTGAAATAATAGGTGATACTATGAAAACTACT
    K  N  T  T  V  I  V  R  E  I  I  G  D  T  M  K  T  T  117
433 GTAACTGTTGATGATGTTACGGCTATTCGGAATTACAAACGATTATAACCCCTG
    V  T  V  D  D  V  T  A  I  R  N  Y  K  R  L  *        132
487 CAAGCTGACAGACTGGTCAAATGTTTCTTGAAAGCGCTTGTATTTCAACTGACA
541 TTATCACAAAAATGACCACCGGTGGACGCATTTTGTCACGCATGTGTCATTCCA
582 TATTTAATTAATCAGCTTCACTTTTGTTCAATAAACATTGC
```

Figure 4. The DNA sequence and the deduced amino acid sequence of *S. mekongi* FABP.

The start (ATG) and stop (TAA) codon underlined. The 2D-structure was predicted by I-TESSER program, and it contained two α-helix (highlight in gray) and ten β-sheet chain (underlined).

The multiple alignment of nucleotide sequences and deduced amino acid sequences of SmekFABP with FABPs from other related trematode and host FABPs showed in Figure 5 and 6 respectively. The percentage of identity between nucleotide and amino acid sequences of SmekFABP with FABPs from related trematode and host FABPs were analyzed by Sequence Identity Matrix as showed in Table 2 and 3 respectively. The identity between nucleotide sequences of SmekFABP and FABPs from *Schistosoma* spp. (SjFABP_L23322.1 from *S. japonicum*; SmFABP_M60895.1 from *S. mansoni*; ShFABP_AB114679.1 from *S. haematobium* and SbFABP_AY615730.1 from *S. bovis*) were 88.7, 63.0, 62.6 and 57.2% respectively (Table 2) while and identity at amino acid level were 89.4-95.4% (SjFABP_AAA64426 from *S. japonicum*; Sm14FABP_2POA_A from *S. mansoni*; ShFABP_BAF62288 from *S. haematobium* and SbFABP_AAT39384 from *S. bovis*) (Table 3). The highest degree of identity was found with *S. japonicum*

(SjFABP_AAA64426). The percentages of identical residues shared between SmekFABP with FABPs from *Fasciola* species (Fh_AJ250098.1 from *F. hepatica* and Fg_U52908.1 from *F. gigantica*) were 36.2-47.2% identity at nucleic acid level and (Fh_CAB65015 from *F. hepatica* and Fg_AAB06722 from *F. gigantica*) 39.3.-48.4% identity at amino acid level (Table 2 and 3). When compared SmekFABP with FABPs from host (RatFABP_BC086947.1 from Rat, HumanFABP_BC032801.1 from human), showed 26.6-27.1% identity at nucleic acid level and (RatFABP_2JU3_A from Rat, HumanFABP_3STN_A from human) 25.7-27.2% at amino acid level (Table 2 and 3).

Phylogenetic analysis through the Bio Edit program (NEIGHBOR-Joining with 1,000 replicates) showed that SmekFABP was in one cluster with FABPs from *S. japonicum* (SjFABP_AAA64426 and SjFABP_AAG50052) (Figure 5). The next neighbors of SmekFABP in the phylogenetic tree were the *S. mansoni*, *S. haematobium and S. bovis* (Sm14FABP_2POA_A from *S. mansoni*; ShFABP_BAF62288 from *S. haematobium* and SbFABP_AAT39384 from *S. bovis*). Whereas SmekFABP was distant relative to group of animal and human FABP (Figure 7).

The secondary structure of *S. mekongi* FABP was predicted by I-TESSER program (Zhang, 2008). Ten β-strands were shown in yellow while two α-helices were shown in pink. The blue represents turns in the peptide backbone (TM-score = 0.91±0.06, RMSD = 1.9±1.6Å) (Figure 8). The protein shared a common tertiary structure as present in other FABPs, consisting of 10 anti-parallel β-strands forming a clam shell-like β-barrel structure, which together with two α-helices, enclose an internal cavity which form the ligand binding site for hydrophobic molecule (Zimmerman A, et al., 2002). Analysis of SmekFABP nucleic acid sequence by the Signal P program showed that it had no signal peptide which would be non-secretory protein. (Figure 9).

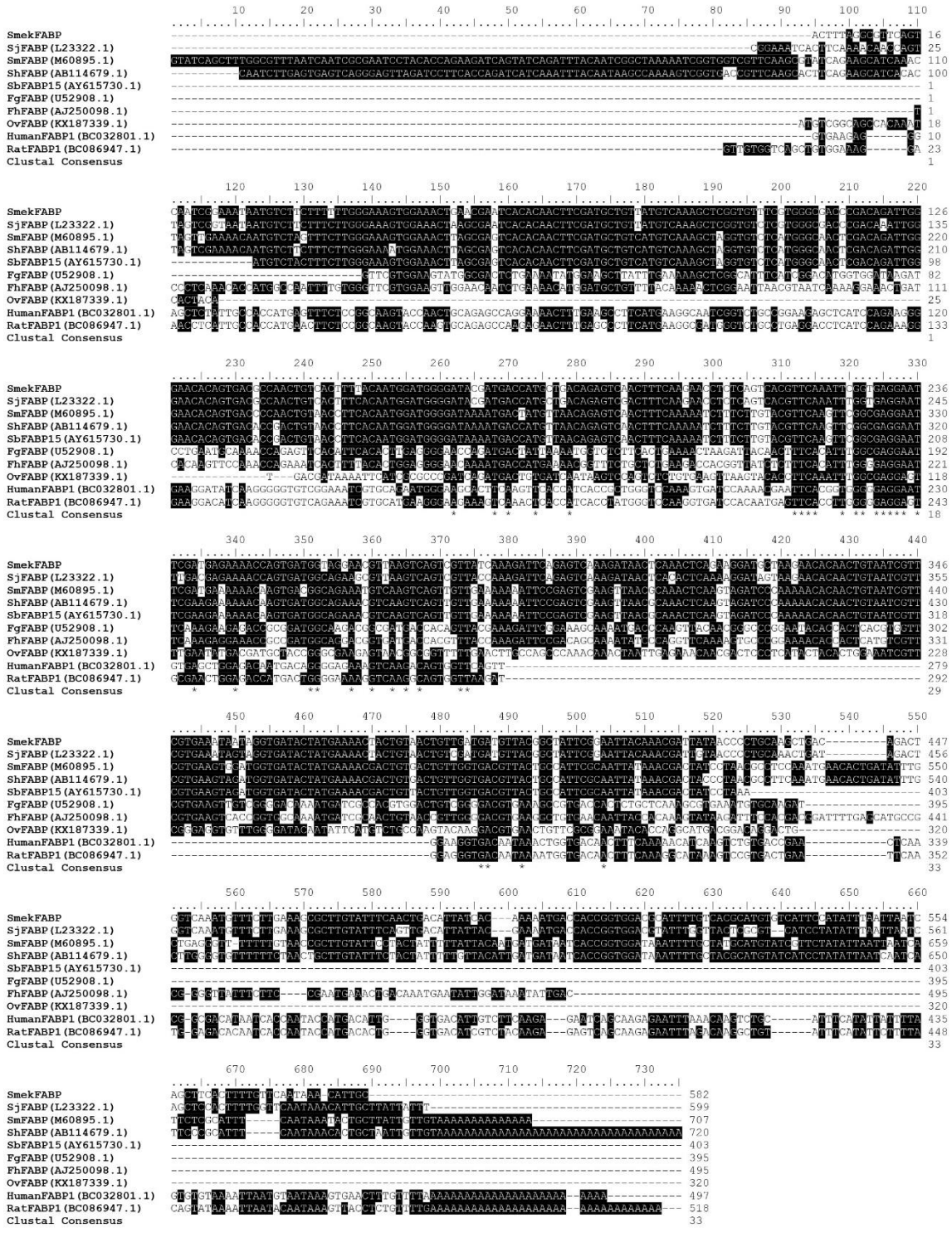

Figure 5 Multiple sequences alignment of the nucleotide sequences of SmekFABP with FABPs from related schistosome species and host.

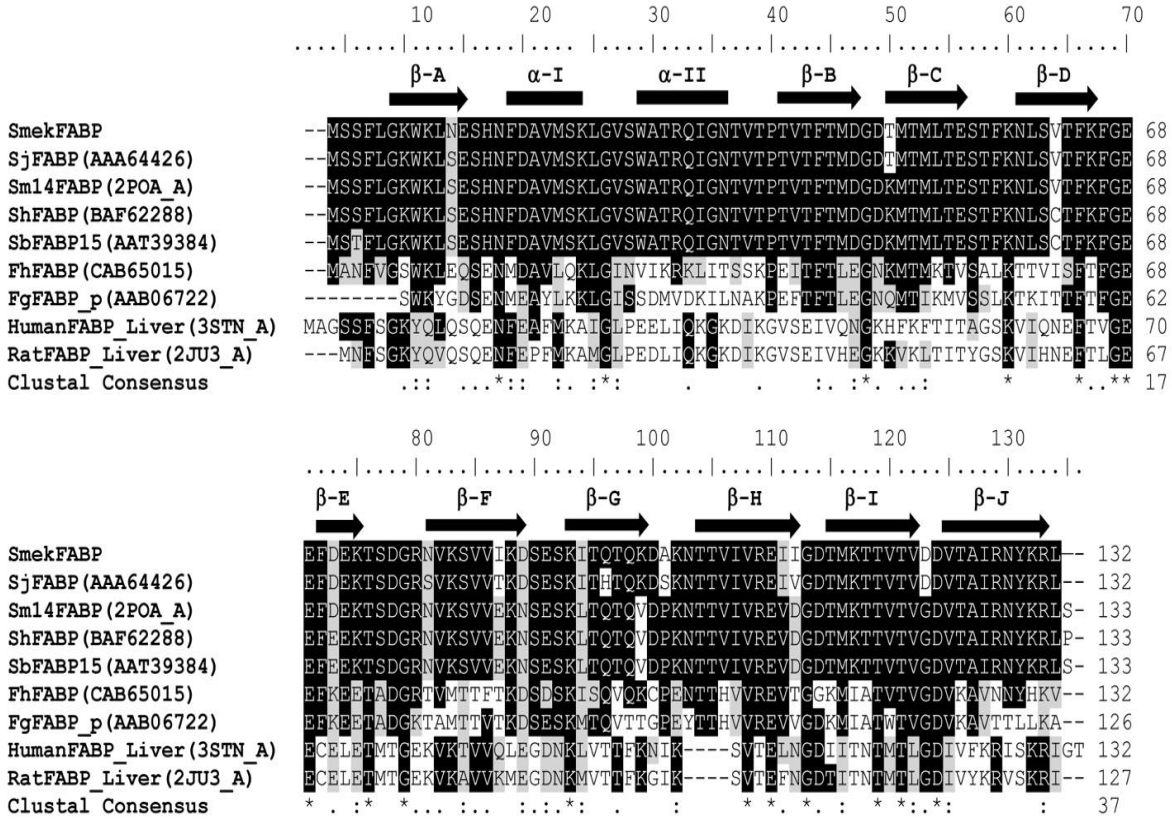

Figure 6. Multiple sequences alignment of the deduced amino acid sequences of SmekFABP with FABPs from related schistosome species and host.

The amino acid sequences of SmekFABP with other FABPs showing highly conserved amino acids by grey and black outlines. The asterisk (*) indicates identical amino acids, two dots (:) indicates conserved amino acid substitutions and dot (.) indicate semi-conserved amino acid substitutions. Arrows and rectangle indicate β-strands and α-helices in SmekFABP. Database accession numbers for the proteins aligned here: SjFAPB: AAA64426; Sm14FABP: 2POA_A; ShFABP: BAF62288; SbFABP15: AAT39384; FhFABP: CAB65015; FgFABP_p: AAB06722; Human FABP_Liver: 3STN_A; RatFABP_liver: 2JU3_A.

Table 2. Sequence identity matrix of SmekFABP nucleotide sequence with FABP protein from trematode and host.

Sequence		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Smek FABP	ID															
2	Sj FABP (L23322.1)	0.887	ID														
3	Sm FABP (M60895.1)	0.630	0.639	ID													
4	Sh FABP (AB114679.1)	0.626	0.640	0.865	ID												
5	Sb FABP15 (AY615730.1)	0.572	0.554	0.555	0.552	ID											
6	Fg FABP (U52908.1)	0.362	0.357	0.303	0.297	0.497	ID										
7	Fh FABP (AJ250098.1)	0.475	0.448	0.368	0.367	0.442	0.585	ID									
8	Ts FABP (HQ259679.1)	0.382	0.384	0.334	0.355	0.285	0.224	0.307	ID								
9	Ov FABP (KX187339.1)	0.258	0.256	0.215	0.207	0.343	0.335	0.286	0.179	ID							
10	Cs FABP (AF527454.1)	0.468	0.488	0.421	0.432	0.411	0.340	0.402	0.384	0.255	ID						
11	Human FABP1 (BC032801.1)	0.271	0.267	0.245	0.245	0.300	0.273	0.222	0.234	0.210	0.301	ID					
12	Pig FABP1 (NM_001004046.2)	0.285	0.280	0.235	0.233	0.227	0.324	0.293	0.225	0.248	0.286	0.750	ID				
13	Rat FABP1 (BC086947.1)	0.266	0.263	0.245	0.272	0.294	0.263	0.253	0.228	0.202	0.292	0.812	0.691	ID			
14	Cattle FABP (X86904.1)	0.263	0.254	0.215	0.211	0.357	0.331	0.284	0.190	0.246	0.251	0.663	0.804	0.627	ID		
15	Dog FABP1 (NM_001287051.1)	0.240	0.245	0.265	0.271	0.257	0.227	0.222	0.199	0.181	0.267	0.715	0.644	0.686	0.579	ID	
16	Mouse FABP2 (NM_007980.3)	0.187	0.187	0.228	0.242	0.155	0.133	0.161	0.204	0.108	0.190	0.196	0.159	0.201	0.151	0.228	ID

Smek: *S. mekongi*; Sj: *S. japonicum*; Sm: *S. mansoni*; Sh: *S. haematobium*; Sb: *S. bovis*; Fh: *F. hepatica*; Fg: *F.gigantica*; TS: *Taenia solium*; Ov: *Opisthorchis viverrine*; Cs: *Clonorchis sinensis*.

Table 3. Sequence identity matrix of SmekFABP amino acid sequence with FABP protein from trematode and host.

Sequence		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Smek FABP	ID															
2	Sj FABP (AAA64426)	0.954	ID														
3	Sm14 FABP (2POA_A)	0.917	0.909	ID													
4	Sh FABP (BAF62288)	0.902	0.894	0.977	ID												
5	Sb FABP15 (AAT39384)	0.894	0.887	0.977	0.984	ID											
6	Fg FABP (AAB06722)	0.393	0.401	0.406	0.406	0.406	ID										
7	Fh FABP (CAB65015)	0.484	0.484	0.488	0.488	0.488	0.628	ID									
8	Ts FABP (ADZ72848)	0.373	0.373	0.373	0.373	0.373	0.253	0.328	ID								
9	Ov FABP (AND62924)	0.240	0.240	0.270	0.277	0.277	0.213	0.211	0.145	ID							
10	Cs FABP (RJW 73596)	0.481	0.481	0.462	0.455	0.455	0.345	0.473	0.365	0.189	ID						
11	Human FABP (3STN_A)	0.272	0.272	0.286	0.294	0.286	0.213	0.213	0.153	0.158	0.240	ID					
12	Pig FABP (P49924.1)	0.265	0.265	0.278	0.285	0.285	0.221	0.219	0.164	0.169	0.240	0.856	ID				
13	Rat FABP (2JU3_A)	0.257	0.257	0.270	0.278	0.278	0.229	0.219	0.179	0.169	0.218	0.787	0.818	ID			
14	Cattle FABP (CAA60506)	0.250	0.250	0.263	0.270	0.270	0.221	0.204	0.141	0.169	0.203	0.772	0.834	0.795	ID		
15	Dog FABP (NP001273980)	0.265	0.265	0.278	0.285	0.285	0.213	0.219	0.164	0.169	0.240	0.863	0.913	0.850	0.842	ID	
16	Mouse FABP (NP032006.1)	0.270	0.278	0.283	0.276	0.276	0.250	0.315	0.274	0.167	0.201	0.227	0.227	0.234	0.250	0.227	ID

Smek: *S. mekongi*; Sj: *S. japonicum*; Sm: *S. mansoni*; Sh: *S. haematobium*; Sb: *S. bovis*; Fh: *F. hepatica*; Fg: *F.gigantica*; TS: *Taenia solium*; Ov: *Opisthorchis viverrine*; Cs: *Clonorchis sinensis*.

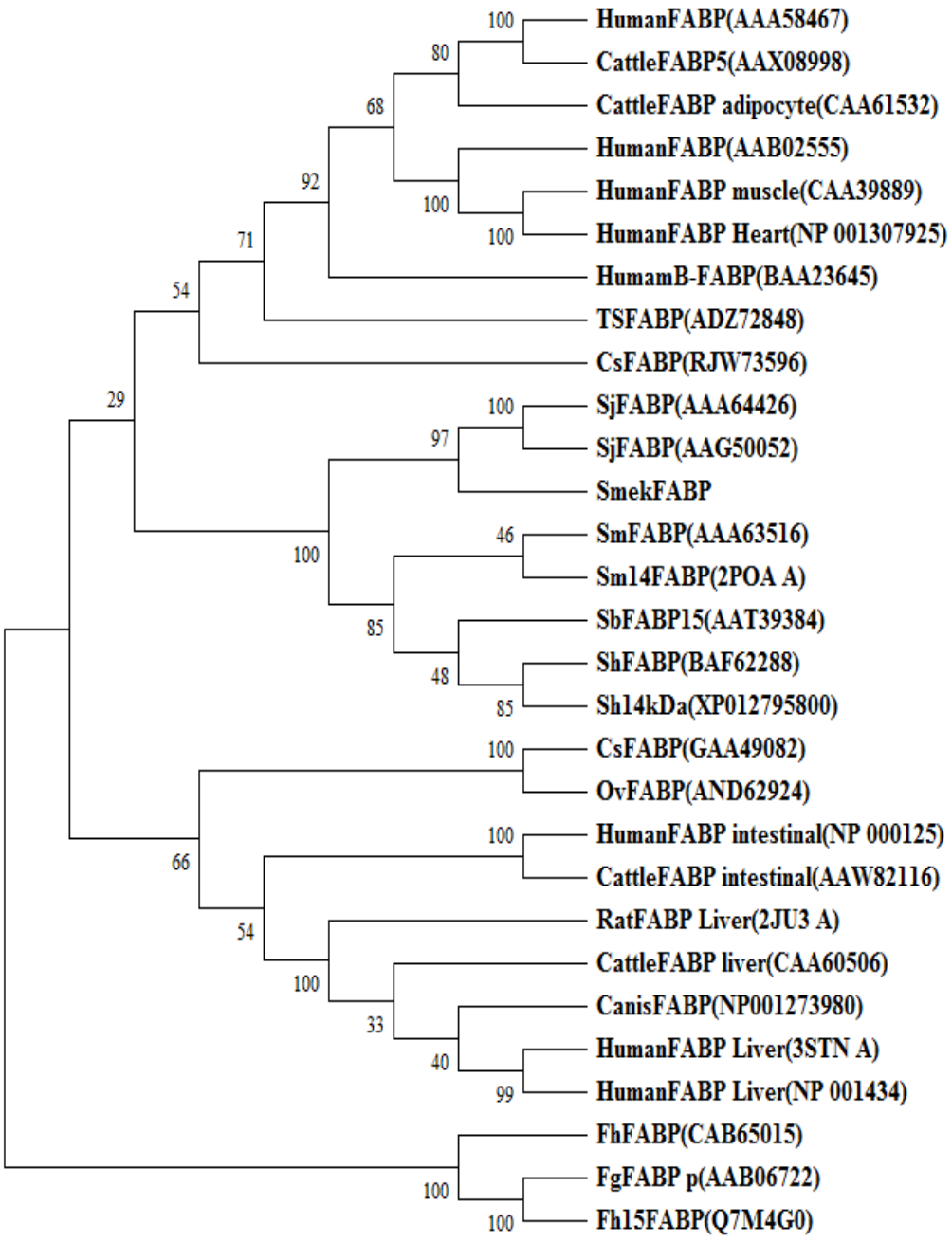

Figure 7. The phylogenetic analysis of SmekFABP with other schistosome, trematode and host. Numbers at the branching point indicate percent bootstrap value.

Figure 8. Three-dimensional structural modeling of SmekFABP by I-TESSER program.

Ten β-strands were shown in yellow while two α-helices were shown in pink. The blue represents turns in the peptide backbone (TM-score = 0.91±0.06, RMSD = 1.9±1.6Å).

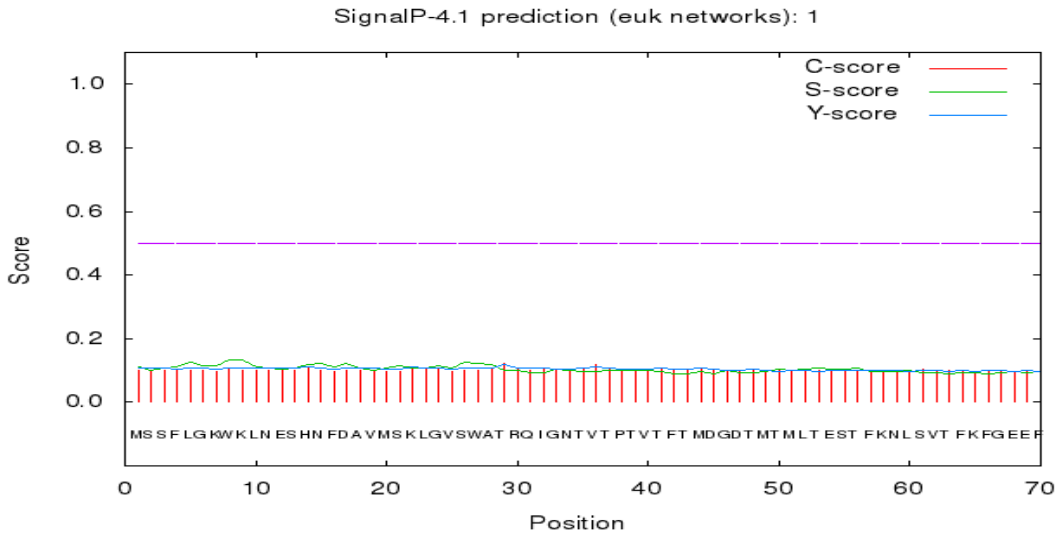

Figure 9. SignalP result for the signal peptide of SmekFABP protein

Analysis of FABP nucleic acid sequence by the Signal P program (http://www.cbs.dtu.dk/services/SignalP-4.1/) showed that it had no signal peptide which would be non-secretory protein. Score C, S and Y represent cleavage site score, signal peptide score and combination score (derived from C and S scores), respectively.

4.2. Production of recombinant SmekFABP protein (rSmekFABP)

The recombinant SmekFABP was expressed in the bacterial expression system. The recombinant protein (rFABP) was then purified by using Ni^{+2}-charged resin under denaturing condition. SDS-PAGE profiles of rSmekFABP showed a single band of rFABP at molecular weight (MW) of 26 kDa and minor band of 23 kDa (Figure 10). And after dialysis, the purified recombinant protein that showed the minor band at 15.5 kDa (Figure 11).

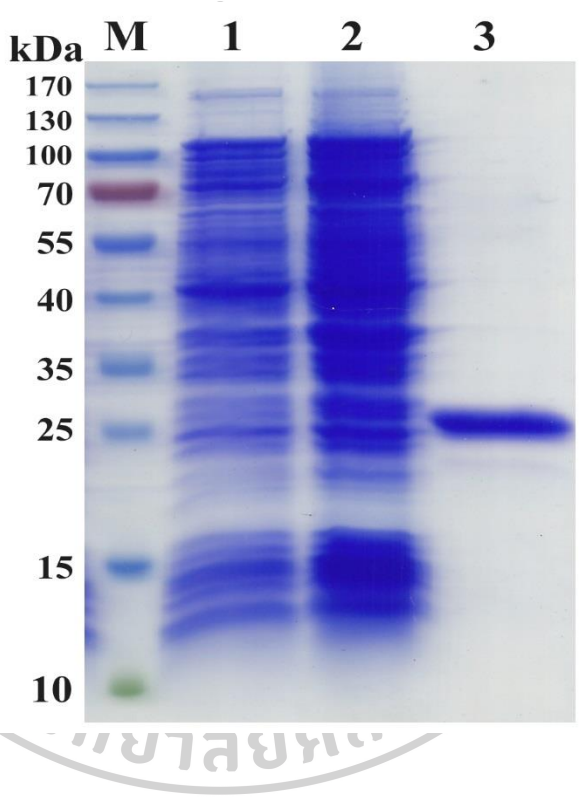

Figure 10. Electrophoretic pattern of rSmekFABP expressed from the bacterial system.

Each fraction was analyses by 12.5% SDS-PAGE stanned with Coomassie Blue. Lane M: PageRuler Prestained Ladder; Lane 1: Total protein from *E. Coli* BL21/pET17b-SmekFABP before induce; Lane 2: Total protein from *E. Coli* BL21/pPET17b-SmekFABP after induce; Lane 3: rSmekFABP purified with Ni-NTA Column.

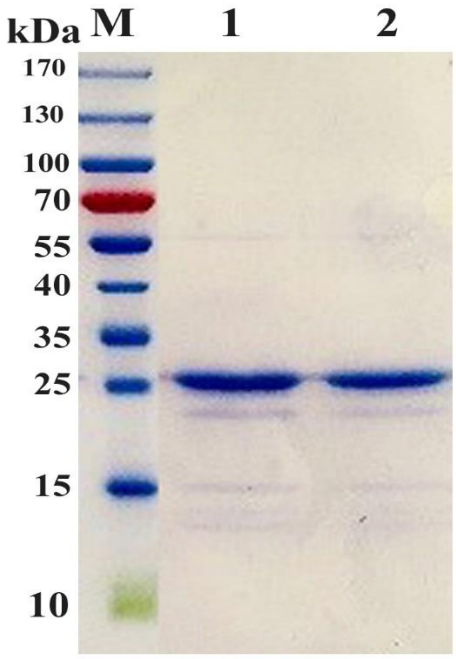

Figure 11. Electrophoretic pattern of rSmekFABP after dialysis.

Each fraction was analyses by 12.5% SDS-PAGE stanned with Coomassie Blue. Lane M: PageRuler Prestained Ladder; Lane 1 and 2: rSmekFABP purified after dialysis.

4.4 Determination of antigenic epitope on SmekFABP

Advances in bioinformatic analysis and database access to the DNA and protein sequences of *Schistosoma* species, are becoming an important tool in prediction of candidate immunogenic epitope for diagnosis and vaccine development. In the present study, Hopp and Woods, Welling & al, Parker & al, Kolaskar and Tongaonkar, B-EpiPred Server and ABCpred Server, antigenicity scales were designed to predict antigenic epitope region in SmekFABP protein of the human blood fluke *S. mekongi*.

By analyzing graphical and numerical data, it was found that according to Hopp and Woods scale the regions 9-11, 13-16, 49-51, 57-58, 67-104, 108-112, 114-119, 123-128 contained the potential hydrophilic regions (Hydrophilicity: score > 0). The analysis found high in position between 67-104 (Maximum Score 1.711) in a protein (Figure 12).

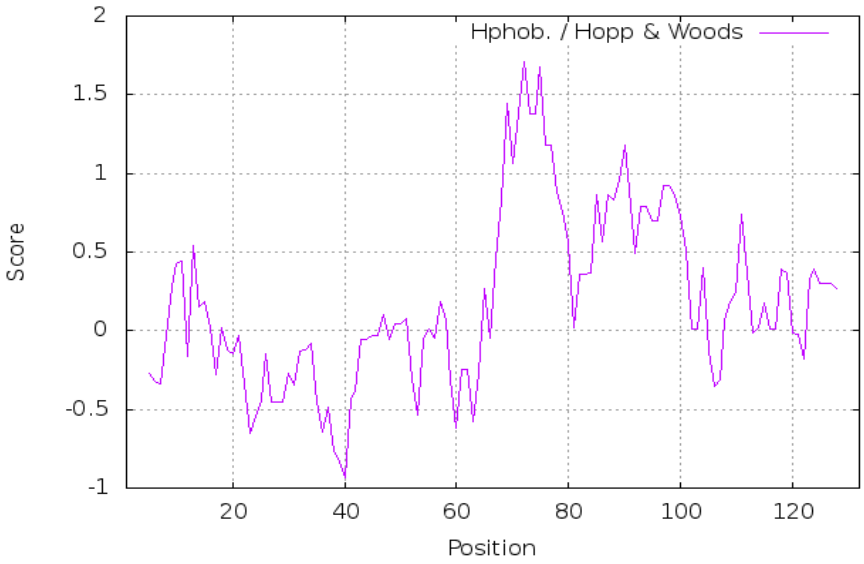

Figure 12. Graphical representation of antigenic peptide evaluation by Hopp and Woods

In the Welling et al., 1985 antigenicity plot gives value as the log of the quotient between percentage in average proteins and percentage in a sample of known antigenic regions, the predicted hydrophilic regions were 6-7, 9-15, 61-62, 82-85, 94-101, 126-128. The high score shows in position 9-15 (Maximum Score 0.482) (Figure 13).

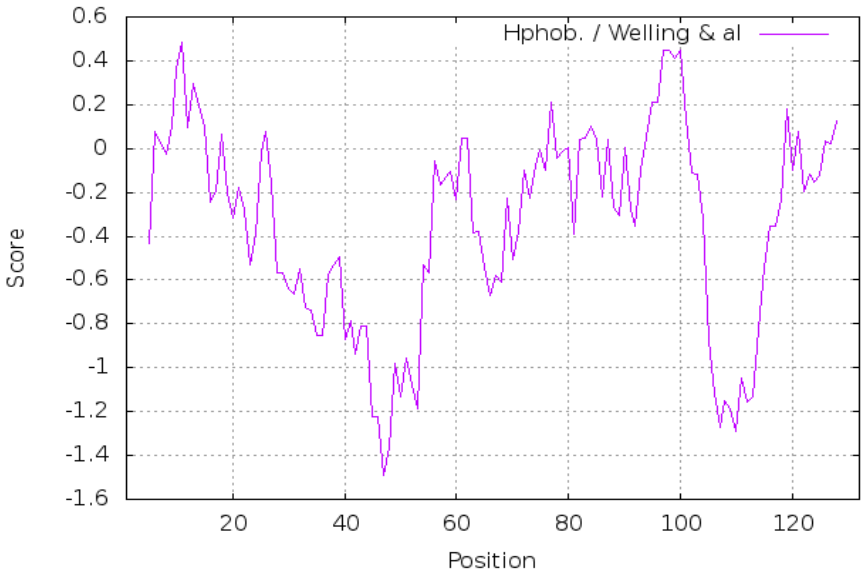

Figure 13. Graphical representation of antigenic peptide evaluation by Welling & al

We also study the hydrophobicity plot of HPLC/Parker and the predicted hydrophilic regions were 9-22, 25-59, 65-105, 109-128. The highest peak is found in position between 65-105 (Maximum Score 6.900) (Figure 14).

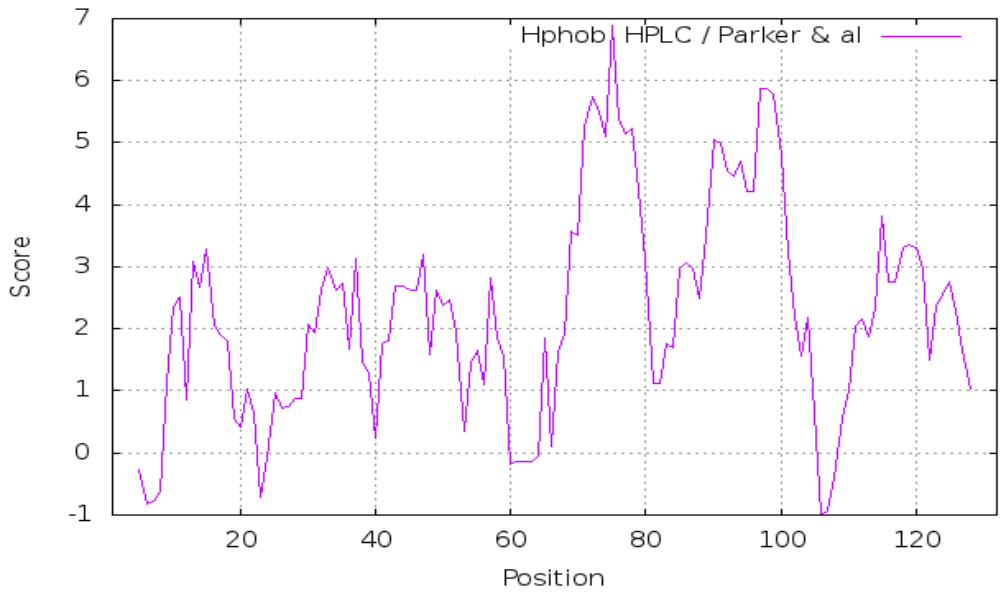

Figure 14. Graphical representation of antigenic peptide evaluation by HPLC/Paker & al

According to Kolaskar and Tangaonkar scale, at 0.995 as the threshold level, the most likely antigenic determinants were at 16-FDAVMSKLGVSWA-28, 58-KNLSVTFK-65, 80-VKSVVIKD-87, 102-TTVIVREI-109, and 117-VTVDDVTAI-126 (Figure 15). B-BepiPred predicts the location of linear B-cell epitopes result found that between 30-RQIGNTVTPT-39, 41-TFTMD-45, 69-EFDEKTSDGRN-79, 87-DSESKITQTQKDAKN-101 and 115- KTTVTV-120. The maximum score (1.754) is found at the position 75 (Figure 16). The ABCpred server was used to predict the B-cell epitopes (16 amino acid) and a total of 10 epitopes for the three proteins (23-38, 61-76 and 88-103) that showed the highest score was selected for designing the subunit vaccine design along with suitable linkers and an adjuvant.

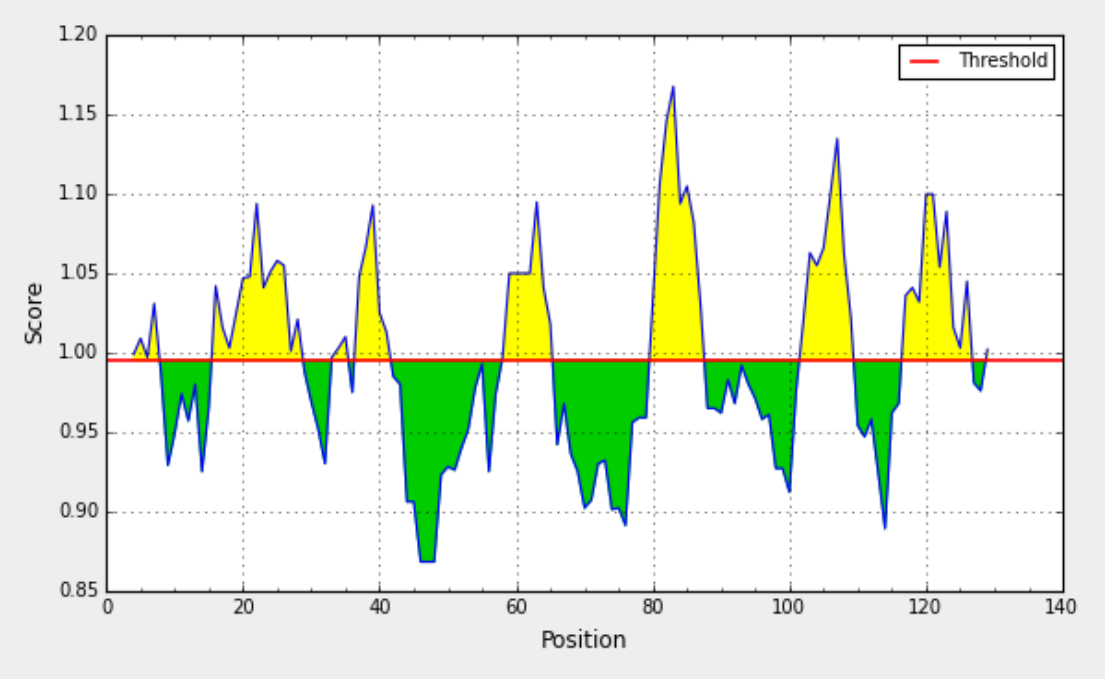

Figure 15. Graphical representation of antigenic peptide evaluation by Kolaskar and Tongaonkar Antigenicity for SmekFABP.

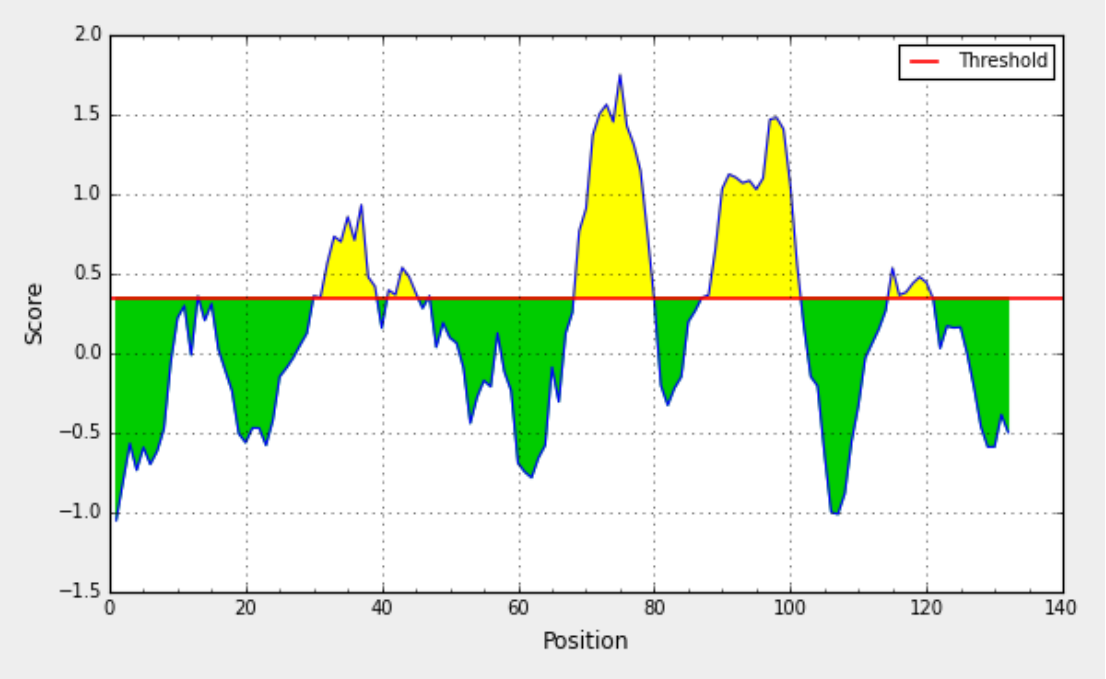

Figure 16. Graphical representation of antigenic peptide evaluation by Bepipred Linear Epitope Prediction for SmekFABP.

Prediction of immunogenic region which exposed on the surface of the protein is a necessary step for epitope-based vaccine design. In this study, the hydrophilic regions of SmekFABP proteins which are supposed to be antigenic and exposed to the surface of the protein were identified for antigenic determinants. The sequences of predict epitopes have been summarized in Table 4. One major overlapping region which were hit by 5 programs from all 6 programs (Hopp & Woods; Welling, Parker, B-EpiPred and ABCpred), is region 87-DSESKITQTQKDAKN-101. The region is located on β-strands (Figure2: βF and βG) which forming a clam shell-like β-barrel structure of the ligand binding site for hydrophobic molecule. This epitope is present on β-sheets regions, which have high antigenic response than helical region as previously reported by (Zimmerman & Veerkamp, 2002). Besides, (Vilar et al., 2003) have demonstrated that the immunogenic epitope conferred protection against at least 2 species of trematode*; S. mansoni* and *F. hepatica*. The protection is between 42-50%.

Table 4. The potential hydrophilic regions and epitope prediction sites from Hopp & Woods, Welling & al, Parker & al, Kolaskar & Tongaonkar. B-EpiPred and ABCpred program.

<table>
<tr><th>Hopp & Woods</th><th>Welling & al</th><th>Parker & al</th><th>Kolaskar & Tangaonkar</th><th>B-Epipred</th><th>ABCpred</th></tr>
<tr><td>9-11,13-16, 18</td><td>6-7, 9-15, 18</td><td>9-22</td><td>13</td><td></td><td></td></tr>
<tr><td></td><td>26</td><td rowspan="2">25-59</td><td>16-28</td><td>30-39, 41-45</td><td>23-38</td></tr>
<tr><td>47, 49-51, 55, 57-58</td><td></td><td rowspan="2">58-65</td><td>47</td><td></td></tr>
<tr><td rowspan="2">65, 67-104</td><td>61-62, 77</td><td rowspan="2">65-105</td><td>69-79</td><td>61-76</td></tr>
<tr><td>80, 82-85, 87, 90, 94-101</td><td>80-87</td><td>87-101</td><td>88-103</td></tr>
<tr><td>108-112, 114-119</td><td>119</td><td rowspan="2">109-128</td><td rowspan="2">102-109, 117-126</td><td>115-120</td><td></td></tr>
<tr><td></td><td>121, 126-128</td><td></td><td></td></tr>
</table>

4.5 The distribution of FABP in parasitic tissues by in situ hybridization techniques

Paraffin longitudinal sections of adult *S. mekongi* were hybridized with an antisense SmekFABP RNA probe. Positive hybridization signals were detected in parenchyma cells (Figure 17). Hybridization signals were not observed in control section.

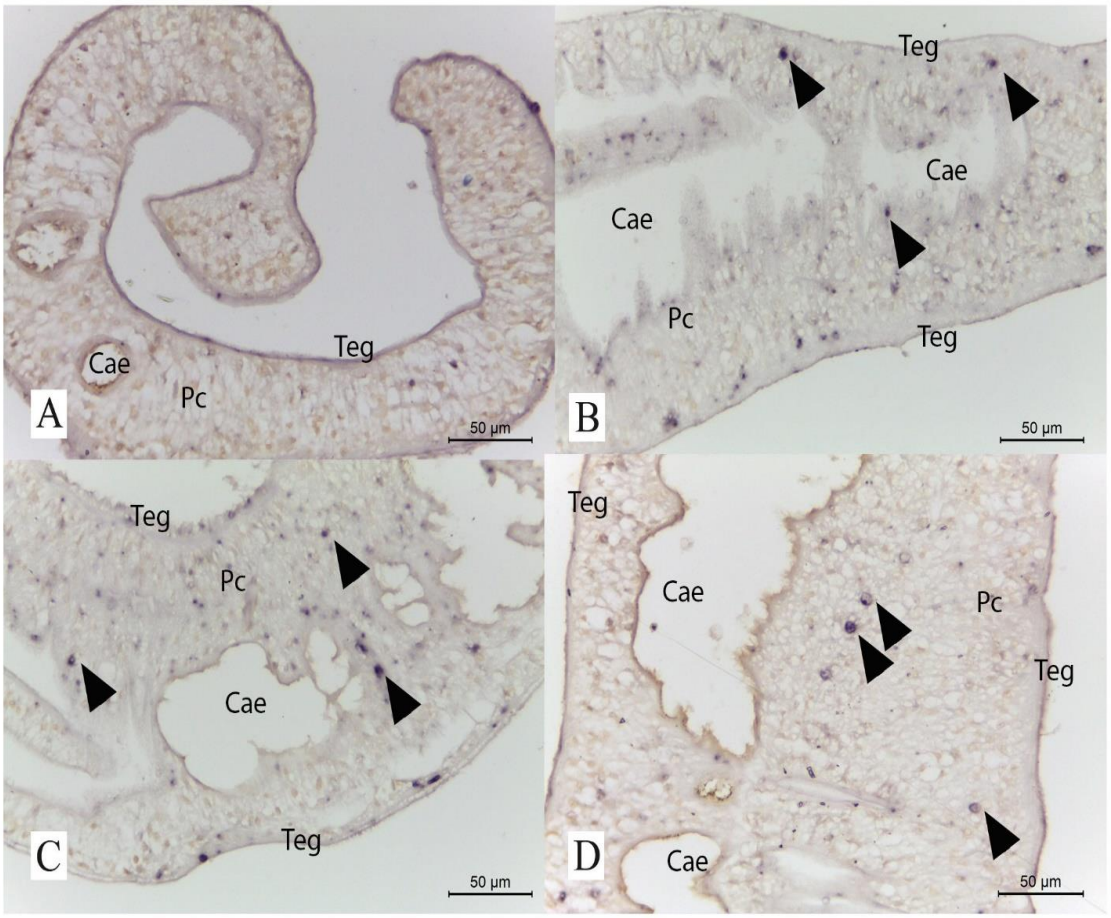

Figure 17. *In situ* hybridization of *S. mekongi* adult stage longitudinal-sections with SmekFABP RNA probes.

A. Control section probed with Bismarck brown Y for counter stain. No labelling in any part of the worm is seen. (Scale bar = 50 µm)

B. Medium magnification micrograph showing staining in the parenchyma cell (Pc), No labelling in the tegument (Teg) and caecum (Cae) (Scale bar = 50 µm)

C. Medium magnification micrograph showing staining in the parenchyma cell (Pc), No labelling in the tegument (Teg) and caecum (Cae) (Scale bar = 50 µm)

D. Medium magnification micrograph showing staining in the parenchyma cell (Pc), No labelling in the tegument (Teg) and caecum (Cae) (Scale bar = 50 µm)

CHAPTER V

DISCUSSION

5.1 Cloning, identification and expression of *S. mekongi* FABP

The results of comparing the identity of nucleotide and amino acid sequences through Clastal Omega sequence analysis and Sequence Identity Matrix Alignment (BioEdit), showed that the amino acid sequences of *S. mekongi* FABP had high level identities with those Trematode parasite species indicated that they had close relationship between them. The SmekFABP amino acid sequences showed the highest degree of identity with the *S. japonicum* (GenBank: AAA64426) at 95.4%. The identity of SmekFABP amino acid sequences with other schistosome species (*S. mansoni*, and *S. haematobium* showed at 91.7 and 90.2% % respectively). A high degree of similarity and identity of *S. mekongi* and *S. japonicum* FABP indicates that they share a common ancestor. The immunogenic property of FABP molecule from *S. japonicum* FABP have been previously reported, and conferred the protection against challenge infection (Tu et al., 2014; Wei et al., 2009). So, it is possible that the molecule of *S. mekongi* FABP could be one of candidate vaccine molecules against the *S. mekongi* infection. By contrast, the amino acid sequences for the homologous genes of mammalian hosts, showed low identity with *S. mekongi* FABP which ranged from 25-27.2%. According to the phylogenetic analysis in this study, it revealed that *S. mekongi* FABP exhibited distant evolutionary relationship from the mammalian host species. The low degree of conservation observed from amino acid sequences of mammalian hosts could reveal its applicable for using as the vaccine candidate against the schistosome infection which may not interfere with the hosts' FABP molecule during the vaccination.

The Signal p program (Nielsen et al., 1997) rained on eukaryotic sequences found no evidence of a cleavable signal peptide in the amino acid sequence of any of the encoded proteins. This is also true for other members of the cytosolic Lipid Binding Protein family (Kennedy et al., 2000), with the exception of an unusual subfamily from nematodes (Mei et al., 1997; Plenefisch et al., 2000).

The recombinant SmekFABP (rSmekFABP) protein fusion with 6X-histidine tag was produced in bacterial system following the induction with 1 mM IPTG. The

recombinant protein was purified, analyzed on SDS-PAGE and stained with Coomassie blue. After purification, the protein represented one major band at 26 kDa and 2 faint bands at 23 kDa and 15.5 kDa on stained SDS-PAGE gels, while the predicted molecular weight of rSmekFABP protein with histidine tag was 15.64 kDa. Previously report demonstrated that cytosolic FABPS usually have molecular weight between 13-15 kDa (Sripa et al., 2017). The major band in this result at 26 kDa could be rSmekFABP which form complex protein may be dimer or binding with some protein in bacterial cell lysate. And the faint band at the 15.5 kDa could be possible to be the rSmekFABP. We have a plan to confirm this protein. First, we will run this protein under more reducing condition than previous, with more than 10% β-Mercaptoethanol (previously we used 1 and %10% β-Mercaptoethanol ethanol, the major band at 26 kDa was still present (data not shown). The second, we would confirm the band of expression protein by using anti-histidine tag antibody to recognize the protein. Third, we will produce polyclonal antibody against rSmekFABP, and then using the polyclonal antibody to probe on crude extract of *S. mekongi* which could detect the native band of FABP at 13-15 kDa.

5.2 Prediction of antigenic epitope on SmekFABP

Vaccination against schistosomiasis is one of strategies to eliminate and eradicate schistosomiasis. It could be used alone or in combination with anthelminthic drug to reduce the reinfection in an endemic area. FABP is one of six immunogenic candidate vaccine antigens that have been selected from WHO (Bergquist, 1995) to apply against schistosomiasis. In this study, we predicted the immunogenic epitope on molecule of SmekFABP by using bioinformatic tool. The result showed that the region 87-DSESKITQTQKDAKN-101, is an overlapping epitope which was hit by 5 programs (Hopp & Woods; Welling, Parker, B-EpiPred and ABCpred) from all 6 programs, which one region of this sequence “SESKITQ” (in an underline: 87-D<u>SESKITQ</u>TQKDAKN-101) was the same sequence that present in the peptide of *S. mansoni* FABP (EKN<u>SESKLTQ</u>) which (Vilar et al., 2003) demonstrated that the sequence has a dual protection against infection of both *S. mansoni* and *F. hepatica*. They found that the sequence “EKNSESKLTQ” which conserved in FABP from both *S. mansoni* (sm14) and *F. hepatica* (Fh15), were capable of inducing levels of

protection equivalent to the recombinant form of Sm14 whole molecule. The peptides sequences induced the 42-50% protection in Swiss mice challenged infection with *S. mansoni* cercaria or *F. hepatica* metacercaria (Vilar et al., 2003). Rahmani et al. (2019) developed chimeric vaccine based on helper T-cell and Cytotoxic T lymphocyte (CTL) epitopes for induction of strong immune response against *S. mansoni* using immune-informatics approaches. One of the immunogenic epitopes that was constructed in the chimeric vaccine molecule was chosen from *S. mansoni* FABP, the epitope was "TQTQVDPKNI", which have a potential to be CTL and Major Histocompatibility Complex-I (MHC-I) epitope. The epitope was similar with our predicted SmekFABP immunogenic epitope, as showed in underline "87-DSESKITQTQKDAKN-101". Waghmare and Chavan (2012) the of Major Histocompatibility Complex Binding Peptides and Epitopes from Fatty-Acid-Binding Protein of *S. japonicum*. One of predicted epitope was 84-VTKDSESKITHTQKDSKNT-102, which also has the similar amino acid sequence as our predicted epitope in SmekFABP "87-DSESKITQTQKDAKN-101". So, SmekFABP predicted epitope "87-DSESKITQTQKDAKN-101" could be one of candidate immunogenic peptide from *S. mekongi* FABP that could be used as vaccine against *S. mekongi* or *Fasciola* spp. infection that need further study the vaccine potential in animal model in the future.

5.3 Localization of SmekFABP by *In situ* hybridization technique

This preliminary study demonstrated that the SmekFABP was present in the parenchyma cell of male adult worm. This finding was similar to localization of FABP in *S. japonicum* by (Gobert et al., 1997). They studied the localization *S. japonicum* Fatty acid-binding protein (SjFABP) by using polyclonal antibody labelled with colloidal gold particles raised against recombinant Sj-FABP, under transmission electron microscopy. They found that Sj-FABP was localized within parenchyma cell and lipid droplets below the subtegumental region of the male parasite. Additionally, Sj-FABPc was present in the vitelline droplets of the vitelline glands of female parasites. There were no detectable levels of Sj-FABP on the surface or within the tegument of male or female parasites. Brito et al. (2002) demonstrated that 14 kDa-Fatty acid binding protein of *S. mansoni* (Sm14) is localized in tissues

near the interfaces of parasite/host contact. Such as the basal lamella of the tegument and the epithelium of the gut. This suggests a possible role for FABP as a second element in the transport of fatty acids, but it is not clear whether the uptake occurs through the tegument or the gut because both tissues are associated with the flow and demand for fatty acids (Brito et al., 2002). Hockley and McLaren (1973) demonstrated that the captured fatty acids in *S. mansoni* was presented in the basal lamella of the tegument and are stored in the gut or in the esophageal gland. In the study by Sirisriro et al. (2002) localization of FABP from *F. gigantica* by immunoperoxidase technique showed the highest concentration of FABP in the parenchymal tissue, and parenchymal cells appear to have different quantities of FABP. Viriya et al. (2006), demonstrated that parenchymal cells in adult *F. gigantica* can be classified into three types based on their ultrastructural features and different quantities of FABP being stored.

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CHAPTER VI

CONCLUSIONS

6.1 Cloning, identification and expression of SmekFABP

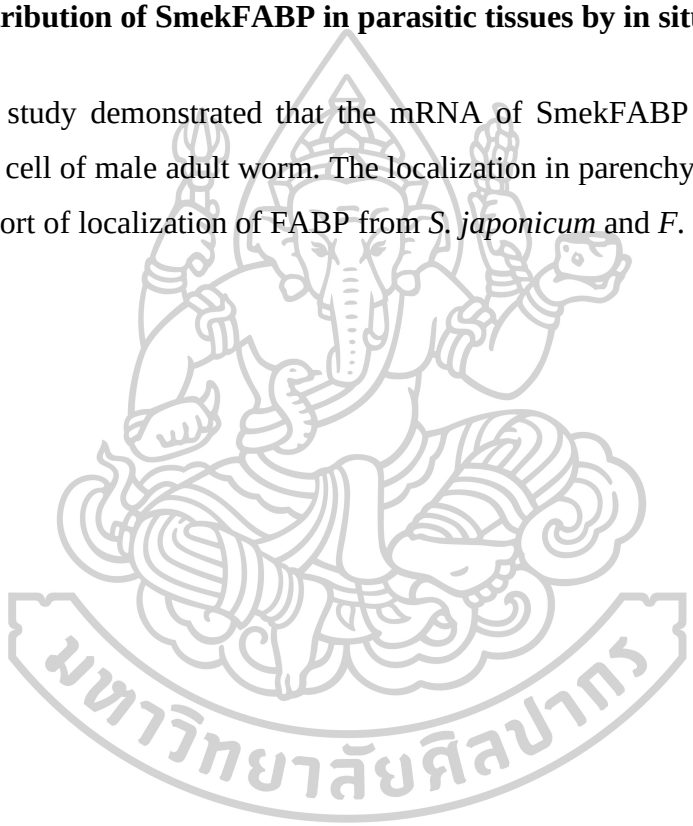
1. The cDNA encoding SmekFABP of adult *S. mekongi* was cloned and sequenced. The nucleotide sequence of SmekFABP was 582 bp in length. The nucleotide sequence of SmekFABP showed an open reading frame encoding FABP containing 132 amino acids.
2. SmekFABP sequences showed the high identity values with FABPs from Schistosome spp. (57.2% - 88.7% for nucleic acid and 89.4% -95.4% for amino acid sequences) and Fasciola spp. (36.2% - 47.5% for nucleic acid and 39.3% 48.4% for amino acid sequences).
3. SmekFABP sequences showed lower level of identity with the FABP from the rodent and mammalian hosts including mouse, rat, Pig, Cattle, Dog, and human (18.7% -28.5% for nucleic acid sequences and 25% - 27.2% for amino acid sequences).
4. Phylogenetic analysis showed that SmekFABP exhibited distant evolutionary relationship from the mammalian host's FABPs. The low degree of conservation observed from amino acid sequences of mammalian hosts could reveal its applicable for using as the vaccine candidate against the schistosome infection which may not interfere with the hosts' FABP molecule during the vaccination.
5. The expected molecular weight of rSmekFABP determined from its constituent amino acids is 14.82 kDa and the predicted molecular weight of rSmekFABP protein with histidine tag is 15.5 kDa. It is possible that we have got the monomer of rSmekFABP (with histidine tag) at 15.5kDa. However, we need to confirm this recombinant protein by runing this protein under reducing condition (more than 10% β-Mercaptoethanol) or immunoblotting by using anti-histidine antibody to probe with the recombinant protein or producing polyclonal antibody against rSmekFABP to probe with native FABP in immunoblot.

6.2 Prediction of antigenic sites Prediction of B-cell antigenic epitope

One immunogenic epitope was predicted by five programs (Hopp & Woods; Welling, Parker, B-EpiPred and ABCpred), it was 87-DSESKITQTQKDAKN-101. Base on bioinformatic, this region could possible to be a vaccine candidate that could have dual protection against infection of both *S. mekongi* and *Fasciola* spp., and it could has a potential to be CTL and Major Histocompatibility Complex-I (MHC-I) epitope.

6.3 The distribution of SmekFABP in parasitic tissues by in situ hybridization techniques

This study demonstrated that the mRNA of SmekFABP was present in the parenchyma cell of male adult worm. The localization in parenchyma cell is similar to previous report of localization of FABP from *S. japonicum* and *F. gigantica*.



APPENDIX: Research Proceeding

Attended and presented (oral presentation) my work at the 46th Congress on Science, Technology and Technology-based Innovation. The 46th Congress on Science, Technology and Technology-based Innovation (STT46) is jointly organized by the Science Society of Thailand under the Patronage of His Majesty the King, under the theme "The Power of Science to Achieve the SDGs" at Ramkhamhaeng University, Bangkok, during 5-7 October, 2020

MOLECULAR CLONING AND B-CELL EPITOPES OF THE GENE ENCODING FATTY ACID BINDING PROTEIN FROM BLOOD FLUKE (*SCHISTOSOMA MEKONGI*)

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Abstract

Schistosomiasis is one of zoonotic disease that affected people in Lao PDR and Cambodia in Mekong river basin. World Health Organization (WHO) has recognized the Fatty acid binding protein is one of vaccine candidates against human schistosomiasis. In the present study, we cloned, characterized and predicted immunogenic epitope of Fatty acid binding protein from *Schistosoma mekongi*, a novel gene namely SmekFABP from *S. mekongi*. Our results showed that the partial of SmekFABP contains 582 bp nucleotides and, and its open reading frame encoded for 132 amino acid with the predicted molecular weight 14.82 kDa. The protein alignment was performed by using Clustal Omega and BioEdit indicated that the FABP sequence of *S. mekongi* showed a highest degree of identify with *S. japonicum* at 95.4% and its resembled epitope regions 87-DSESKITQTQKDAKN-101 of SmekFABP from *S. mekongi* based on hydrophilicity scale and recognized by B-cell. This immunogenic epitope could be target for vaccine development against *S. mekongi* in the future.

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