Cloning and Characterization of *Schistosoma mansoni* Glucose Phosphate Isomerase gene

Thesis

SUBMITTED FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN BIOCHEMISTRY

By

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ABSTRACT

Name : Samah Mamdouh Abd El – Hafiz

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This work has been carried out to investigate that: Schistosomes are blood–dwelling flukes that infect 200 million people worldwide and are responsible for hundreds of thousands of deaths annually. There is no vaccine available for this disease. This parasite is heavily dependent on energy generated from glucose metabolism. In this study, we report the isolation and characterization of the full-length coding sequence of *Schistosoma mansoni* glucose phosphate isomerase (SMGPI). The complete DNA sequencing of SMGPI cDNA consists of 2204 nucleotides encoded a 554-amino-acid protein. The deduced amino acids of SMGPI and GPI of different organisms showed a high degree of similarity (55-80%). Recombinant SMGPI protein (62 KDa) was produced in *E. coli* BL21 cells. The SMGPI could be a candidate for schistosomiasis vaccine

Key words : Schistosoma mansoni, Glucose phosphate isomerase, Complete DNA sequence, Recombinant SMGPI protein.

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Samah Mamdouh Mohamed

LIST OF ABBREVIATIONS

Ab	Antibody
Ag	Antigen
AGPC	Acid guanidinium phenol chloroform
Amp	Ampicillin
AMV	Avian myeloblastosis virus reverse transcriptase
AP	Alkaline phosphatase
BCIP	5-Bromo-4-chloro-3-indolyl-1-phosphatase
BLAST	Basic local alignment search tool
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
CIAP	Calf intestinal alkaline phosphatase
CMV	Cytomegalovirus
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
dNTP	2'-Deoxyribonucleoside 5'-tri-phosphate
E. coli	*Escherichia coli*
EDTA	Ethylene diamine tetraacetic acid
ESTs	Expressed sequence tags
F6P	Fructose-6-phosphate
G3PDH	Glyceraldehydes-3-phosphate dehydrogenase
GPI	Glucose phosphate isomeras
G6P	Glucose-6-phosphate
gm	Gram
hr	Hour
Ig	Immunoglobulin
Il	Interleukin

IPTG	Isopropyl-β-thiogalactoside
Kb	Kilobase
KDa	Kilodalton
λDNA	lambda DNA
LB medium	Lauria-Bertani medium
LB ampicillin	Lauria-Bertani medium / ampicillin
M	Molar
µg	Microgram
µl	Microliter
mg	Milligram
mg/ml	Milligram/milliliter
Min	Minute
mRNA	Messenger RNA
NBT	Nitro blue tetrazolium
NCBI	National center for biotechnology information
NHMRC	National health and medical research council of Australia
OD	Optical density
ORF	Open reading frame
PAGE	Polyacrylamide gel electrophoresis
Pbs	Phosphate buffered saline
PCR	Polymerase chain reaction
PGI	Phospho glucose isomerase
PH	Hydrogen ion concentration
P mole	Picomole
RACE	Rapid amplification of cDNA ends
RNA	Ribonucleic acid
rpm	Rotations per minute
rGPI	Recombinant glucose phosphate isomerase
RT-PCR	Reverse transcription- polymerase chain reaction

S. hematobium	*Schistosoma hematobium*
S. japonicum	*Schistosoma japonicum*
S. mansoni	*Schistosoma mansoni*
SDS	Sodium dodecyle suphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
ssDNA	Single stranded DNA
SGN	Schistosoma genome network
SINEs	Short repetitive elements
SWAP	Soluble worm antigen protein
TAE	Tris acetate EDTA
Taq	*Thermus Aquaticus*
TBRI	Theodore Bilharz research institute
TE	Tris EDTA buffer
TEMED	N,N,N',N'-tetramethylenediamine
TPI	Triose phosphate isomerase
Tris	Tris [hydroxymethyl] aminoethane
UV	Ultraviolet
WHO	World health organization

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INTRODUCTION

Human infection with the parasitic helminthes of the family Schistosomatidae represents a significant segment of the global burden of illness. Members of this family are estimated to infect 200 – 300 million people and to be endemic in more than 70 countries **(Fulford, 1998 and Fenwick *et al.*, 2006).**

Drug treatment and other existing control measures have failed to eliminate the incidence of infection, morbidity and mortality due to schistosomiasis infection (**Mitchell *et al.*, 1999)**; therefore development of an effective vaccine is a research priority, to complement existing control measures (**Bergquist, 1995; Bergquist and Colly, 1998; Capron, 1998; Wilson and Coulson, 1998).**

There is good evidence that humans can acquire immunity against Schistosome **(Butterworth *et al.*, 1988)**. Furthermore, high levels of protective immunity have been achieved by using DNA-based vaccination **(Watts and Kennedy, 1999; Boyle and Robinson, 2000).**

The use of recombinant DNA technology and computer database have led to a greater understanding of the structure of many genes of the Schistosoma genome and the mechanisms involved in the regulation of gene expression **(Reis *et al.*, 1989 and Mutapi *et al.*, 2006)**. This knowledge is significant in that it provides the potential to formulate a rational pharmacological attack and to develop a vaccine for the treatment and control of schistosomiasis.

AIM OF THE WORK

The control of schistosomiasis is still an urgent task that requires improved diagnosis and treatment as well as effective prevention.

In spite of the development of active drugs, prevention of re-infection has remained a problem, requiring repeated drug applications after infection. There is a clear need for an effective vaccine inducing significant level of protection against the parasite.

One of the genes coding for a key enzyme in carbohydrate metabolism has been studied in details. *Schistosoma mansoni* glucose phosphate isomerase (SmGPI) was the gene of choice, because this parasite is heavily dependent on energy generated from glucose metabolism. SmGPI characteristics may be different from human GPI and could be a target enzyme for specific drugs or may be a candidate vaccine for schistosomiasis.

In this study, we report the isolation and characterization of the full-length coding sequence of *S. mansoni* GPI that extends the EST cDNA sequence than was previously reported. The cDNA sequence encodes the complete sequence of the gene has been isolated, expressed in *E. coli* cells, characterized and proposed as a candidate vaccine for schistosomiasis.

REVIEW OF LITERATURE

I- SCHISTOSOMIASIS

Schistosomiasis, also known as Bilharziasis, is a parasitic disease that leads to chronic ill health. It is the major health risk in the rural areas of central China and Egypt and continues to rank high in other developing countries **(Curtale *et al*., 2006)**.

Schistosomiasis has been recognized since the time of the Egyptian pharaohs. The worms responsible for the disease were eventually discovered in 1851 by Theodor Bilharz, a young German pathologist, from whom the disease took its original name, Bilharziasis.

The disease is indicated either by the presence of blood in urine or in the case of intestinal schistosomiasis, by initially atypical symptoms which can lead to serious complications involving the liver and spleen **(Tsuboi *et al*., 2006)**.

A World Health Organization expert committee **(WHO, Geneva, 2002)**, concluded that yearly deaths could be as high as 200,000, compared with 15,000 as had been reported before. An analysis of these discrepancies indicates that there is underestimation, making schistosomiasis second only to malaria among tropical diseases as a cause of morbidity.

II- SCHISTOSOMES:

Schistosomes are eukaryotic intravascular parasites that cause of schistosomiasis, a chronic and debilitating disease **(Engels *et al*., 2002)**.

Even though extensive research into the control of schistosomiasis has been ongoing for the past four decades, with some success, this disease remains an endemic problem in many regions worldwide **(Bergquist *et al.*, 2002; Todd *et al.*, 2002)**.

The main forms of human schistosomiasis are caused by five species of the flatworm, or blood flukes, known as schistosomes:

-*Schistosoma mansoni*: causes intestinal schistosomiasis and is prevalent in 52 countries and territories of Africa, Caribbean, the Eastern Mediterranean and South America **(Kabatereine *et al.*, 2006)**.

-*Schistosoma japonicum / Schistosoma mekongi:* cause intestinal schistosomiasis and are prevalent in 7 African countries and the Pacific region **(Blas *et al.*, 2006 and Chigusa *et al.*, 2006)**.

-*Schistosoma intercalatun*: is found in 10 African countries.

-*Schistosoma haematobium:* causes urinary schistosomiasis and affects 54 countries in Africa and the Eastern Mediterranean.

People are infected by contact with water used in normal daily activities such as personal domestic hygiene and swimming, or by professional activities such as fishing, rice cultivation and irrigation **(Steinmann *et al.*, 2006).**

Due to lack of information or insufficient attention to hygiene, infected individuals may contaminate their water supply with feces or urine. The eggs of the Schistosomes in the excreta of an infected person open on contact with water and release a parasite, the miracidium. To survive, this motile form must find a fresh water snail.

Once it has found its snail host, the miracidium divides, producing thousands of new parasites; cercariae. The cercariae are then excreted by the snail into the surrounding water. They can penetrate an individual's skin within a few seconds, continuing their biological cycle once they have made their way to the victim's vessels. Within 30 to 45 days, the parasite is transformed into a long worm that is either male or female. The female lays from 200 to 2000 eggs per day over an average of 5 years, according to the species.

In case of intestinal schistosomiasis, the worms reside in the blood vessels lining the intestine. In urinary schistosomiasis, they live in the blood vessels of the bladder.

Only about a half of the eggs are excreted in the feces (intestinal Schistosomiasis), or in the urine (urinary Schistosomiasis). The rest stay in the body, damaging other vital organs. It is the eggs and not the worm itself which cause damage to the intestine, the bladder and other organs.

Chronic infection with schistosomiasis has been clearly associated with the development of bladder cancer and infestation is associated with a high incidence of colorectal cancer in endemic populations **(Madbouly *et al*., 2006).**

Although infection of the hepatic and urogenital systems commonly occurs, central nervous system involvement is rare **(Kim *et al*., 2006).** When presenting in the spinal cord, schistosomiasis can be difficult to diagnose because it can present as a mass lesion or a transverse mellitus.

Currently available methods for the diagnosis of human schistosomiasis often lack enough sensitivity and specificity. Recently, **Sandoval *et al*., (2006)** developed more specific and sensitive diagnostic methods, based on the polymerase chain reaction (PCR) technique.

III- SCHISTOSOMIASIS CONTROL

Schistosomiasis control is far more effective when placed in the context of a general health system. The integration process is slow, but this "horizontal" approach is now becoming an integral part of health care at village level. Schistosomiasis prevention and control measures should be implemented before dam construction work begins. Control approaches for each form of schistosomiasis varies and must be adapted to the epidemiological situation, available financial resources and the particular local culture **(Engels *et al*., 2002).** This strategy has produced excellent results; in some regions it has met the planned objectives within 2 years. It is nevertheless essential to plan surveillance and maintenance over periods of 10 to 20 years **(Capron *et al*., 2001).**

Campaigns in the Egyptian mass media have proved particularly successful in increasing awareness of the need for diagnosis and treatment **(El-Khoby *et al*., 2000).** Health education on schistosomiasis has greater importance than ever before. The introduction into schools of diagnosis and treatment has made children and parents much more aware of the problem connected with disease. School teachers and local health workers are effective in explaining the role played by people in the transmission of schistosomiasis **(Satayathum *et al*., 2006).**

- SCHISTOSOMIASIS TREATMENT

Until 1970s, treatment of schistosomiasis was nearly as dangerous as the disease itself. Modern treatment is effective and without risk.

Three new drugs have revolutionized treatment:

1. Metrifonate -effective for the treatment of urinary Schistosomiasis.
2. Oxamniquine -used exclusively to treat intestinal Schistosomiasis in Africa and South America **(Chan *et al.*, 1997).**
3. Praziquantel- effective in the treatment of all forms of Schistosomes, with virtually no side effects (**Cioli, 2000 and Chigusa *et al*., 2006).**

To be effective, Schistosomiasis control strategies should be adapted to the local epidemiological situation. Caution must be taken when destroying freshwater snails using chemicals, particularly in terms of impact on the environment.

III.2- SCHISTOSOMIASIS VACCINE:

Control programs based on chemotherapy are complicated by the rapidity and frequency of reinfection and the difficulties and expense involved in maintaining these programs over a long term **(Butterworth, 1992).** The possibility that the parasite may develop drug resistance is a concern that also needs to be addressed **(Bennett *et al.*, 1997; Glading *et al.*, 2002)**.

In regions of Egypt and Kenya where there has been heavy exposure to Praziquantel, there are reports of *S. mansoni* and *S. haematobium* infections that are not responsive to multiple courses of treatment. There is some laboratory evidence suggesting that these drug-