CHARACTERIZATION OF SCHISTOSOME ANTIGENS RECOGNIZED BY PROTECTIVE ANTISERA



KFB 4266

THESIS

Submitted to The

Faculty of Science

Ain Shams University

In Partial Fulfillment of The

Requirements for The Degree of

MASTER OF SCIENCE

IN BIOCHEMISTRY

BY

ENAS ALY GAD EL KARIM

59910

Supervisors

Prof. Dr. MOHAMMED MOHAMMED ABDEL-FATTAH MALL Fattala

Professor of Biochemistry in the Biochemistry Dept.

Faculty of Science

Ain Shams University

Prof. Dr. AMR MAHMOUD KARIM

Professor of Biochemistry in the Biochemistry Dept.

Faculty of Science

Ain Shams University

The state of the s

1997



CHARACTERIZATION OF SCHISTOSOME ANTIGENS RECOGNIZED BY PROTECTIVE ANTISERA

THESIS

Submitted to The

Faculty of Science

Ain Shams University

In Partial Fulfillment of The

Requirements for The Degree of

MASTER OF SCIENCE

IN BIOCHEMISTRY

BY

ENAS ALY GAD EL KARIM

Supervisors

Prof. Dr. MOHAMMED MOHAMMED ABDEL-FATTAH

Professor of Biochemistry in the Biochemistry Dept.

Faculty of Science

Ain Shams University

Prof. Dr. AMR MAHMOUD KARIM

Professor of Biochemistry in the Biochemistry Dept.

Faculty of Science

Ain Shams University

1997

Scientific Board:

Prof. Dr. Mohammed Mohammed Abdel-Fattah

Professor of Biochemistry in the Biochemistry Department, Faculty of Science,

Ain Shams University.

Prof. Dr. Amr Mahmoud Karim

Professor of Biochemistry in the Biochemistry Department, Faculty of Science,

Ain Shams University.



ACKNOWLEDGMENT

I would like to express my gratefulness to Prof. Dr. Mohammed Abdel-Fattah, Biochemistry Dept., Ain Shams University, for his support and kind supervision for completing this work.

My great thanks to Prof. Dr. Amr Mohamoud Karim, Biochemistry Dept., Ain Shams University, for his suggestions, help, encouragement, supervision and efforts throughout the work.

I would also like to express my deepest and most sincere gratefulness to Dr. Marie D. Ricciardone, Molecular Biologist, NAMRU-3, who encouraged me to start the whole work, supervised supported and guided me and to whom I owe all what I have learned.

I would also like to express my deepest gratefulness to Dr. Beverly L. Mangold, Immunologist, NAMRU-3, for her supervision and support for completing this work.

My special thanks to Dr. David A. Dean, Immunologist, NAMRU-3, for approval to start the work and for his assistance and advice.

I would also like to thank Dr. Moustafa Mansour, Head of Basic Sciences Department, NAMRU-3, Dr. Karim Kamal, Head of Technical Support Branch, NAMRU-3, and Dr. Sam Lewis, Technical Support Branch, NAMRU-3, for their kindness, generosity, help and assistance.

I would also like to express my deepest and sincere gratefulness to Mrs. Iman Toni, Technical Support Branch, NAMRU-3, Dr. Nagwa Abdel Tawab, Immunologist, Vaccines and Sera Institute, for their help and assistance.



TABLE OF CONTENTS:

INTRODUC	FION:	
AIM OF WORK		
1. Schistosomiasis, An Overview		2
2. Control Measures		3
3. Schistosome Vaccine Immunity		4
4. Identification of Candidate Vaccine Antigens		8
4.1. Identification of antigens seen by resistant individuals		
4.2. Sc	oluble adult worm antigens	10
4.3. Ai	ntigens identified by sera from irradiated-cercariae	
im	nmunized mice	1
	4.3.1. The 38 kDa antigen	12
	4.3.2. The 200 kDa antigen	12
	4.3.3. The 18 kDa antigen	14
	4.3.4. Schistosoma mansoni Tropomyosin	14
4.4. Te	egumental surface membrane antigens of adult worms	s 16
	4.4.1. The 25 kDa antigen	17
	4.4.2. The 23 kDa antigen	18
	4.4.3. The 21.7 kDa antigen	20
4.5. A	ntigens recognized by protective monospecific	
an	tibodies	21
	4.5.1. Triose-Phosphate Isomerase (TPI: D-glycer-	
	aldehyde3-phosphate ketol-isomerase)	21
	4.5.2. Glutathione-S-Transferrases (GSTs)	23
MATERIAL	AND METHODS:	27
1. Molecular c	characterization of the gene recognized by protective	
antiserum		27
1.1. Immunoscreening of cDNA library		27
1.2. Preparation of Lambda-Lysate		28
1.3. Polymerase Chain Reaction (PCR)		28

1.3.1. Principle		
1.3.2. PCR Reaction		
1.3.3. Cleaning of the PCR fragment		
1.3.3.1. Removing excess primer DNA.		
1.3.3.2. Removing residual lambda arms by		
restriction enzyme digestion	32	
1.4. M13 cloning		
1.4.1. Principle		
1.4.2. Restriction enzyme analysis	34	
1.4.3. Preparation of vector and insert DNA		
1.4.3.1. Forward Orientation	37	
1.4.3.2. Reverse Orientation	38	
1.4.4. DNA purification	38	
1.4.4.1. DNA electrophoresis and purification		
from low melting point agarose gel	38	
1.4.4.2. Recovery of DNA from low gel		
temperature agarose	39	
1.4.5. Concentration Agarose gel of the vector and	insert	
DNA	41	
1.4.6. Preparation of competent bacterial cells	42	
1.4.7. Ligation	42	
1.4.8. Transformation	43	
1.4.9. Miniplasmid DNA preparation	43	
1.5. Single stranded nucleic acid sequencing	44	
1.5.1. Principle	44	
1.5.2. Template DNA preparation	46	
1.5.3. Labeling of template DNA with ³⁵ S-dATP	46	
1.5.4. Sequencing gel preparation	47	
1.5.5. Loading and running the sequencing gel	49	
1.5.6. Autoradiography	49	
1.5.7. Computer analysis of the DNA sequence	50	

	51
2. Nucleic acid hybridization	
2.1. Radioactive labeling of the nucleic acid probe	
2.2. Nucleic acids preparation	52
2.2.1. Total RNA extraction	52
2.2.2. Selection of Poly(A)* RNA	54
2.2.3. Genomic DNA extraction	56
2.3. Southern blot	
2.4. Northern blot	60
3. Vaccination trials: Assessment of the protective capacity of	
recombinant protein	61
3.1. Generation of recombinant lambda lysogens in	
Bacterial strain Y1089	61
3.1.1. Principle	61
3.1.2. Lysogeny	62
3.1.3. Preparation of crude lysate from a lambda	
lysogen	62
3.1.4. Sodium Dodecyl Sulfate-Polyacrylamide Gel	
Electrophoresis (SDS-PAGE) for separation of	of
recombinant β-galactosidase fusion protein	65
3.1.4.1. Principle and apparatus used	65
3.1.4.2. Assembling the glass plates	65
3.1.4.3. Separating gel	66
3.1.4.4. Stacking gel	66
3.1.4.5. The electrode (running) buffer	67
3.1.4.6. Running conditions	67
3.1.4.7. Gel staining	68
3.2. Antigen Purification	68
3.2.1. SDS-PAGE Electrophoretic separation of	
recombinant β-galactosidase fusion protein	68
3.2.2. Electroelution	70
3.2.3. Lyophilization	70

3.2.4. Protein content determination	71	
3.2.4.1. Lowry technique	71	
3.2.5. Electrophoretic transfer of the purified lambo	ła	
recombinant fusion protein separated by		
SDS-PAGE to nitrocellulose paper		
(Western blot)	71	
3.2.5.1. Principle	71	
3.2.5.2. Protein fractionation	72	
3.2.5.3. Gel and membrane preparation	73	
3.2.5.4. Transfer of β-galactosidase fusion		
protein	73	
3.2.5.5. Detection using enzyme-conjugated		
antibodies	74	
3.3. Adjuvant	75	
3.3.1. MPL™ +TDM Emulsion (Ribi adjuvant)	75	
3.4. Vaccination trials	75	
3.4.1. Immunization schedule	75	
3.4.2. Challenge infection	76	
3.4.3. Perfusion and assessment of protection	76	
3.5. Antigen identification in S. mansoni soluble adult worm	!	
extract	76	
3.5.1. Soluble Adult Worm Preparation (SAWP)	76	
3.5.2. Bradford technique	7 8	
3.5.3 Electrophoretic transfer of SAWP protein		
separated by SDS-PAGE to nitrocellulose		
paper (Western blot)	78	
RESULTS	80	
1. Molecular characterization of the gene recognized by protective		
antiserum	80	
1.1. Determination of insert sizes by PCR		
1.2. Restriction mapping of clone Sm46	80	

1.3. Cloning in M13	
1.4. Single stranded nucleotide sequence of clone Sm46	
1.4.1. Sequencing strategy	82
1.5. Computer analysis of the DNA sequence	92
2. Nucleic acid hybridization	
2.1. Southern blot analysis of Sm46	97
2.2. Northern blot analysis of Sm46	97
3. Vaccination trials	
3.1. Generation of lambda-recombinant lysogen in	
E. Coli Y1089	100
3.2. Antigen purification	100
3.3. Vaccination studies in mice	100
3.4. Western blot	105
DISCUSSION	
REFERENCES	
ABSTRACT.	
ENGLISH SUMMARY.	
ARABIC SUMMARY.	