A 14-kDa Schistosoma mansoni Polypeptide Is Homologous to a Gene Family of Fatty Acid Binding Proteins*

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The complete nucleotide sequence encoding a Schistosoma mansoni protein termed Sm14 was determined from cDNA clones propagated in bacteriophage λgt11 in Escherichia coli. The 14.8-kDa protein bears significant homologies with a family of related polypeptides which bind hydrophobic ligands. Members of this group of cytosolic proteins were originally identified based on their affinity for long chain fatty acids. The purified recombinant protein exhibited an affinity to fatty acids, in contrast to a mutant lacking 16 N-terminal amino acids. Immunofluorescence experiments show that tubercles, which are structures located on the dorsal surface of adult male schistosome and known to contain lipids, are stained using antibodies raised to the α-galactosidase fusion protein. A regular staining pattern is also evident in the muscle layers of the body of the parasite. As the schistosome cannot synthesize fatty acids de novo and is dependent on the uptake of lipids from serum, the available data support a role for Sm14 in the transport of fatty acids.

It has been shown that one third of the total dry weight of the schistosome consists of lipids (1, 2). Much of these lipids is associated with the tegumental membrane, which is made up of two trilaminar layers. There is a 325% increase in the area of the surface during the first 3 days in the development in the vertebrate host (3). The schistosome lacks pathways for sterol and long chain fatty acid synthesis (4), and essential lipids are therefore derived from the host's blood. Ultrastructural demonstration of lipoprotein binding to the surface was confirmed independently by fluorescence and immunoelectron microscopy using antiserum to human apolipoprotein B, the major component of low density lipid particles (5). Several studies have also identified potential receptors for serum lipoproteins (6–9). Additionally, selective uptake and incorporation of fatty acids into the tegumental membrane have been described (10, 11).

We have raised a polyclonal antiserum to the constituents of an adult Schistosoma mansoni extract, prepared by incubating the parasite in phosphate-buffered saline. The extract was previously shown to confer resistance to S. mansoni infections in experimental animals (12). As part of an investigation to identify the constituents of the adult schistosome extract, we used the antiserum to screen an S. mansoni cDNA library and have isolated several cDNA clones. In this report, we describe the identification of a protein homologous to members of a family of lipid binding proteins, which include fatty acid binding proteins (13, 14), adipocyte P2 (15) and myelin P2 (16, 17) proteins, mammary-derived growth inhibitor (18), and cellular retinol/retinoic acid binding proteins (19). This group of proteins shows a close similarity in molecular size (14–16 kDa), amino acid composition, and in some cases, an affinity to lipids. They appear to play a central role in the uptake, transport, and compartmentalization of lipids (reviewed in Refs. 20–22).

On the basis of sequence homology with lipid binding proteins, we postulated a possible role for the S. mansoni protein as a lipid carrier. The protein expressed in E. coli was analyzed for its fatty acid binding properties. Our data demonstrate its ability to bind fatty acids, in contrast to an N-terminal deletion mutant, which exhibited no appreciable affinity for fatty acids. This study is a first step in gaining an insight into the molecular mechanisms that regulate lipid-protein interaction in the schistosome, with particular emphasis on the expression of a schistosome gene responsible for the binding of lipids.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—The Escherichia coli expression vector pDS56/RBSII, 6xHis (42) was generously provided by D. Stüber (Hoffmann-La Roche Ltd., Basel). All pDS plasmid constructions were maintained in E. coli SG13009 containing repressor plasmid pREP4 (53).

Production of Antibodies to a Schistosome Extract—The extract was prepared by incubating live S. mansoni adult worms in phosphate-buffered saline for 2 h at room temperature and collecting the supernatant after centrifugation at 10,000 g × 4 h at 4 °C (12). Male New Zealand rabbits were given two footpad injections each of 0.6 mg of extract in Freund's complete adjuvant, administered 1 week apart, followed by an intraperitoneal booster. Final blood samples were taken after 6 weeks.

Immunoscreening—An S. mansoni cDNA expression library was constructed starting from poly(A)* mRNA from adult male and female worms as described previously (23). Antiserum to the schistosome extract was used at a dilution of 1:500 to screen 2 × 10^6 recombinant phages.

DNA Sequencing—Nucleotide sequencing was performed by the dideoxynucleotide chain termination method of Sanger (26). DNA sequences were processed using the program of Osterberg (54) and the proposed amino acid sequences analyzed in the National Biomedical Research Foundation database.
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FIG. 1. Construction of S. mansoni expression vectors. Details are provided under “Experimental Procedures.” The stippled shading shown in pDS66/RBSII, 6xHis represents the regulatable promoter/operator element and the ribosome binding site on the plasmid, and the black box represents the transcriptional terminator. cat is a relic of an old construction.

RNA isolation and Northern Blot Hybridization—Total RNA from adult worms was isolated using the guanidinium/cesium chloride centrifugation method, as previously described (56). RNA samples (2 μg) were heat-denatured in 50% formamide, and electrophoresed through 1.3% agarose gels containing formaldehyde. RNA was transferred to nylon membranes and probed with the cDNA insert labeled using random primers.

Construction of Expression Vector Containing Sm14 cDNA—The expression vectors were constructed as outlined in Fig. 1. The 600-bp schistosome cDNA insert Sm14-1 was subcloned into plasmid pEMBL8+/16 (M. Nassal, Zentrum für Molekulare Biologie Heidelberg) via the NcoI restriction sites on the adaptor sequences (23). The resulting construct pEMBL8+/16-Sml4-1 was digested with SpeI. Following purification using the Geneclean Kit (Bio 101 Inc., La Jolla, CA), the linearized DNA was incubated in 60 μl of 20 mM Tris-HCl (pH 8.0), 12 mM MgCl₂, 1 mM EDTA, 0.1 M NaCl, and 10 units of Bal31 at 32 °C, as previously described (56). Aliquots of 15 μl were removed at 30-s intervals, and the reaction was stopped with 25 μl of 0.1 M EDTA. The rate of exonuclease degradation was analyzed by electrophoresis on agarose gels (5-μl portions). DNA samples digested for 30 s and 1 min were pooled and incubated with E. coli polymerase I and dNTPs to remove 5' and 3' protruding ends. Deletion derivatives of the Sm14-1 cDNA fragment were recovered from SpeI- and Bal31-treated pEMBL8+/16-Sm14-1 by digestion with HindIII. Purified fragments deleted at their 5' noncoding region and partially at the 5' coding sequence (approximately 600 bp in size) were ligated to pDS66/RBSII, 6xHis plasmid, digested with HindIII and HindII. Clones were selected for the presence of HindIII and BarnHI restriction sites. The transition from pDS sequences to the schistosome cDNA sequence was determined by the dideoxy chain termination method.

Expression and Purification of Recombinant Proteins—The host strain SG13009 containing either the expression plasmid pDS-Sm14 or pDS-ΔSm14 was grown in 200 ml of ST1 medium containing 100 μg/ml of ampicillin and 25 μg/ml of kanamycin at 37 °C to an A₆₀₀ of 0.7 (43). Expression was induced by adding isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 2 mM. Following an additional incubation of 5 h at 37 °C, bacteria were harvested by centrifugation and stored at −20 °C.

Frozen cells were allowed to thaw for 15 min. 20 ml of buffer A (6 M guanidine HCl in 0.1 M Na₂HPO₄, pH 8.0) were added and the cells were stirred for 1 h at room temperature. After centrifugation for 10 min at 10,000 × g, the supernatant was pumped at 10 ml/h onto a column (1.5 cm in diameter) containing 3 ml of nitricolic acid-resin charged with nickel (44, 45). The column was washed sequentially at the same flow rate with 10 ml of buffer A, 4 ml of buffer B (8 M urea, 0.1 M NaH₂PO₄, 0.01 M Tris, pH 8.0), and 6 ml of buffer C (buffer B, pH 6.3). The (His)₅ fusion proteins were eluted with 9 ml of buffer D (buffer B, pH 5.9) and 4 ml of buffer E (buffer B, pH 4.5). The column was washed with 5 ml of 6 M guanidine HCl in 0.2 M acetic acid, pH 2.7, and equilibrated with 10 ml of buffer A before re-use. Fractions of 5 ml were collected while loading the extract and washing with buffers A and B, thereafter fraction size was reduced to 1 ml. Proteins (10-μl aliquots) were analyzed on 17.5% SDS-polyacrylamide gels.

Before assaying for fatty acid binding, fractions containing the (His)₅ fusion proteins were pooled and dialyzed against 2 M urea in 10 mM potassium phosphate (pH 7.4) containing 5 mM 2-mercaptoethanol and 0.02% sodium azide. Urea was completely removed upon further dialysis against 10 mM potassium phosphate (pH 7.4), 5 mM 2-mercaptoethanol, and 0.02% sodium azide. Fusion proteins were

1 The abbreviations used are: bp, base pair(s); FABP, fatty acid binding protein; SDS, sodium dodecyl sulfate.
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stored at 4 °C at a concentration of 80 µg/ml in the above buffer containing 5% glycerol. Protein concentration was determined by the Bradford method using bovine serum albumin as a standard.

Chloroform-n-hexane acetyltransferase fused to (His)6 at its N terminus, referred to as (His)6-CAT derived from a related pDS plasmid, pDS76/RB14, was kindly provided by M. Müller (Zentralinstitut für Molekularbiologie Heidelberg) and used as a negative control in fatty acid binding assays.

Measurements of Fatty Acid Binding—A modification of the method as described by Glatz and Veerakamp (46) was used to determine the affinity of fatty acid binding to the purified E. coli-derived schistosomal proteins. Binding assays were performed in a solution of 10 mM potassium phosphate (pH 7.4), 0.1–20 µg of schistosomal FABP homologue or bovine serum albumin as a positive control or a heterologous (His)6 fusion protein, (His)6-CAT, as a negative control, and the appropriate volume of an aqueous solution of fatty acids (final concentration varying from 0.1 to 10 µM) in a total volume of 1 ml. After a 5-min incubation at 37 °C, the tubes were chilled on ice for 10 min. Unbound fatty acids were removed from the solution by adding 200 µl of a 50% (v/v) suspension of Lipidex 1000 (Canberra-Packard, Frankfurt, Germany) in 10 mM potassium phosphate. Following a 10-min incubation at 0 °C, the assay tubes were centrifuged at 10,000 × g for 3 min at 4 °C. 400-µl aliquots of the supernatants were removed and subjected to scintillation counting using a Packard counter. Blank assays in which no protein was added were performed in parallel for each fatty acid concentration. The amount of radioactivity measured in the supernatant of the blank assay was subtracted from that determined in 400-µl aliquots of FABP-containing assay supernatants. Fatty acid binding was expressed as picomoles of fatty acid per pmol of protein.

Immunoassay—Sections of S. mansoni adult worms were incubated with anti-β-gal-Sm14 antisera at various dilutions for 30 min. Following several washings in phosphate-buffered saline, fluorescein isothiocyanate-conjugated anti-rabbit antisera was added for an additional 30 min. The sections were then washed and examined under the fluorescent microscope. Normal rabbit serum served as negative control.

RESULTS

Identification of Cloned cDNA Sequence—Antiserum produced in rabbits immunized with the schistosome extract (12) was used to screen our adult S. mansoni cDNA library (25). A clone designated Sm14 was plaque-purified after three rounds of immunoscreening. The recombinant phage was lysogenized in E. coli Y1089 and induced to express a β-galactosidase-Sm14 fusion protein of 122 kDa (data not shown). The protein was purified by preparative SDS-polyacrylamide gel electrophoresis, and antibodies to the fusion protein were raised in a rabbit, according to the method previously described (24). In a Western blot analysis of total schistosome extract, transferred to nitrocellulose filter and stained with amido black (Fig. 2, lane 1), anti-β-gal-Sm14 fusion protein reacted predominantly with a polypeptide of 14 kDa (lane 2, arrow). The antiserum appeared to recognize another protein around 50 kDa. Since the preimmune serum was also found to react weakly with the 50-kDa protein, this reaction can be regarded as nonspecific (lane 3).

Nucleotide Sequence of Cloned Sm14—The 350-bp EcoRI insert of Sm14 was subcloned into a vector pSP65 (25), from which sequences of both cDNA strands were determined. Two unique primers, SP6 and M13+, constructed to regions flanking the schistosome cDNA inserted in the polynucleotide sequence of pSP65, were used. Sequencing protocols followed the dideoxynucleotide chain termination method as described by Sanger et al. (26). From an initial analysis, we observed a stop codon situated approximately 150 nucleotides from the 5′ end of the sequence, followed by a long 3′-untranslated region, which contained a polyadenylation site and a stretch of poly(A)s, representing presumably the poly(A)tail. These results indicate the need to isolate further clones carrying additional nucleotides in the 5′ region.

An Sm14 EcoRI insert was used as a probe to isolate a further clone from our Agt10 library (23). A second clone, termed Sm14-1, with an internal EcoRI restriction site, was found to carry an N-terminal extension of Sm14 of approximately 300 bp. For subsequent sequence analysis, this 600-bp cDNA fragment was subcloned into plasmid pEMBL8*16 via the Ncol sites of the adaptor sequences.

The complete nucleotide sequence is compiled in Fig. 3A. An open reading frame begins at the initiation triplet ATG positioned at nucleotides 123–125. The coding region comprises 399 nucleotides, ending at position 521. The protein of 133 amino acid residues has a molecular mass of 14,847 kDa, calculated on the basis of the sequence. The coding region was bounded by a 148-nucleotide-long 5′-noncoding region (present in clone Sm14-1) and a 3′-untranslated region of 196 nucleotides (present in clones Sm14 and Sm14-1).

Amino Acid Sequence of Sm14-1—The amino acid sequence deduced from the nucleotide sequence of cDNA Sm14-1 was compared to the National Biological Research Foundation database. Amino acid comparison of the schistosome sequence with members of the gene family of lipid binding proteins is shown in Fig. 3B. The most significant homology (42%) was found with heart fatty acid binding protein (H-FABP (27–31)) and myelin P2 (mP2) of peripheral nerves (16, 17). The deduced amino acid sequence could also be aligned with bovine mammary-derived growth inhibitor (42% (18)), even though this putative growth regulating factor has not been established conclusively as being a member of the FABP gene family. In addition, homologies were demonstrated with other members, including murine adipocyte P2 protein (αP2, 41.1% (15)), murine adipocyte lipid binding protein (41.1% (32)), rat cellular retinol binding protein II (34.4% (19, 33–35)), rat cellular retinol binding protein I (28.5% (19, 36)), cellular retinoid acid binding protein from rat liver (33.1% (37)), liver fatty acid binding proteins from human and rat (29.2% (38–40)), and human intestinal fatty acid binding protein (27.7% (41)).

The sequence homologies suggest that the schistosome protein has some structural similarities in common with known lipid binding proteins and raises the possibility that the schistosome homologue has similar domains required for protein-lipid interaction.

Expression of Sm14-1 cDNA in the pDS56/RBS1, 6xHis Vector—In order to express the putative schistosomal FABP in E. coli, we chose the pDS vector system which expresses recombinant proteins with an N-terminal extension of 6 histidine residues (42, 43). (His)6 fusion proteins can be easily purified by affinity to immobilized metal-chelate resins. Recombinant proteins carry seven additional N-terminal amino acids as derived from the vector sequence (Fig. 4).
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FIG. 3. A, nucleotide and inferred amino acid sequences of the complete Sm14 gene including the 5’- and 3’-flanking regions. The arrow positioned at nucleotide 327 corresponds to the start of the original 350-bp EcoRI cDNA insert, used for isolating the full-length clone. The amino acid sequence is specified by an open reading frame of 133 amino acids. B, alignment of the deduced amino acid sequence of Sm14 with members of the gene family of lipid binding proteins. Sequences of adipocyte P2 (aP2), adipocyte lipid binding protein (ALBP), myelin P2 (mP2), heart FABP (H-FABP), mammary-derived growth inhibitor (MDGI), S. mansoni Sm14, cellular retinol binding proteins I and II (CRBP I and II), cellular retinoic acid binding protein (CRABP), intestinal and liver FABPs (I- and L-FABPs, respectively) are compared. Identical amino acids found in at least five of the sequences are indicated in boldface.

FIG. 4. Transition from pDS vector to S. mansoni sequence. Top line, RBS refers to the ribosome binding site on the expression vector. The stippled box represents the nucleotides encoding 6 histidine residues. S. mansoni-specific nucleotides are shown in the white box. Bottom line, the first 13 amino acids are deduced from the nucleotide sequence of pDS56/RBSII, 6xHis. Schistosome-specific amino acids are in boldface and numbered. Δ, a deletion of the first 16 amino acids in the schistosome sequence.
As a result of a deletion of the 5'-noncoding region, the protein as derived from plasmid pDS-Sm14 comprises the (His)$_6$ cluster fused to the schistosome initiator methionine (Fig. 4) and has a molecular mass of 16.5 kDa. The mutant recombinant protein derived from pDS-DsSm14 has an approximate molecular mass of 14.7 kDa and differs from the above full-length recombinant protein in that 16 of the N-terminal-most amino acids have been deleted (Fig. 4).

Expression and purification of the recombinant proteins were carried out as described under “Experimental Procedures.” Fractions collected during the purification steps, as shown for Sm14, were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 5). After disruption of the cells and removal of cell debris, the supernatant was subjected to nickel-chelate affinity chromatography. The column was washed with buffers A and B and proteins eluted with buffers C, D, and E. The recombinant (His)$_6$-Sm14 fusion protein purified to >95% homogeneity was present in fractions eluted with buffer E at pH 4.5. After complete removal of urea via stepwise dialysis against 10 mM potassium phosphate buffer (pH 7.4) containing decreasing concentrations of urea, the recombinant proteins were analyzed in fatty acid binding assays.

**Fatty Acid Binding Assays**—Using the Lipidex assay of Glatz and Veerkamp (46), the purified recombinant proteins were tested for their capacity to bind fatty acids, and compared with albumin and an E. coli-derived heterologous (His)$_6$-CAT fusion protein (47). Multiple assays were performed for varying protein concentrations (0.1–20 μg) at varying fatty acid concentrations (0.1–10μM) for both palmitate and linolenate. The results of the binding assays as performed for palmitic acid at a final concentration of 1 mM are shown (Fig. 6A). Serum albumin, a known transport protein for organic compounds including free fatty acids (48), served as a positive control. Strong fatty acid binding by albumin was observed under our assay conditions. Fatty acid binding was also demonstrated for recombinant Sm14. From the binding assay data of the schistosomal FABP, we estimated that 0.2 mol of fatty acid/mol of protein, which correlated to one-tenth to one-fifth of the values reported for other FABPs (49, 50). Recombinant ∆Sm14, from which the first 16 amino acids were deleted, was found to bind palmitic acid with an even lower capacity (0.05 mol of fatty acid/mol of protein), suggesting that the truncated protein is incorrectly folded and hence incapable of binding. Alternatively, the N-terminal domain is directly involved in fatty acid binding.

The binding property of a nonrelated protein chloramphenicol acetyltransferase also comprising 6 tandem histidine residues at its N terminus, (His)$_6$-CAT, was tested in parallel. This protein was chosen as a control to determine the influence of the histidine residues on fatty acid binding. As expected, this protein showed no capacity to bind palmitic acid, indicating that affinity to the fatty acid was not due to the presence of the histidine extension. This result confirms the specificity of the interaction of recombinant Sm14 with palmitic acid.

The E. coli-derived recombinant Sm14 also showed an ability to bind linolenic acid (Fig. 6B). High affinity of serum albumin, which was used as a positive control, was demonstrated. On the other hand, binding was abolished in the presence of the truncated protein ∆Sm14 as well as the heterologous (His)$_6$-CAT fusion.

**Hybridization of Cloned Sm14 cDNA to Schistosome RNA**—To determine the size of the mRNA of the schistosome homologue, we performed Northern blot hybridization experiments using total RNA extracted from adult worms. The RNA was separated by electrophoresis through formaldehyde-agarose gels and transferred to a nitrocellulose filter. The 350-bp insert was labeled by random primers and hybridized.
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to the immobilized RNA on the filter. Based on its migration in a denaturing gel system, we estimated a 850-bp transcript (Fig. 7). This finding is consistent with message lengths reported for other FABPs. mRNA of 600–700 nucleotides has been described to be present in liver, using FABP cDNA as probe (39), and a 850–900-nucleotide-long mRNA for mature cytoplasmic intestinal FABP has been reported (51).

Immunofluorescence—Given that the degree of sequence homology with both FABPs and P2 is identical, we sought to determine the location of Sm14 in the schistosome. We examined the location of Sm14 expressed in the parasite, by carrying out immunocytological staining of frozen sections of adult parasites using antiserum to the β-galactosidase fusion protein. Specific binding was visualized by fluorescein isothiocyanate-conjugated anti-rabbit antibodies.

While the tegument and the muscle layers remained unstained, fluorescence was observed inside the tubercles, which are structures localized on the dorsal surface of the male parasite (Fig. 8, A and B, arrowhead). The reactivity appeared to be associated with the lipid globules which characterize the core structure of the tubercle. In addition, discrete staining was also evident in structures traversing the muscle layers (arrow); these might possibly be the connections between the subtegumental cells and the tegument. It is likely that what in effect is staining would be the membranous inclusion bodies known to comprise phospholipids, which are synthesized in the subtegumental cells and pass up the connections into the tegument to form the double outer membrane. It is known that the schistosome cannot synthesize sterols and fatty acids de novo, and must rely on uptake from the host. Thus, the location of Sm14 in the tubercles and possibly the membranous inclusion bodies in subtegumental cells argues for a role as a lipid carrier. Furthermore, some regular staining pattern was also implied in the body of the parasite. The reason why the immunoreactivity is present in the parenchyma is unknown. We are currently attempting to define its origin by immunogold labeling.

By using preimmune serum, the fluorescence pattern was reduced to background, showing the specificity of the above reactions (Fig. 8C).

DISCUSSION

We have determined the complete nucleotide sequence of a clone identified from our adult S. mansoni cDNA library, using an antiserum raised against a schistosome extract. The cDNA specifies the translation of a 133-amino acid polypeptide with a calculated molecular mass of 14.8 kDa. The deduced amino acid sequence shows significant homology to a family of proteins, whose members include heart, liver, and intestinal FABPs, mammary-derived growth inhibitor, the cellular retinol and retinoic acid binding proteins, as well as adipocyte P2 protein and myelin P2 protein of peripheral nerves. These proteins are abundantly expressed in a highly tissue-specific manner. Although their precise physiological roles have not yet been defined, they are believed to be part of a multifunctional cytosolic transport system, participating in uptake, intracellular transport, and metabolism of lipids (22).

We expressed the complete Sm14 protein by extending its N terminus by six histidines for rapid purification through metal-chelate affinity chromatography and compared its binding capacity with a deletion mutant lacking the first 16 amino acids. The purified full-length protein, in common with other lipid binding proteins, showed an appreciable affinity to palmitic and linolenic acids despite the fact that the estimated binding capacity was substantially lower than that reported for other FABPs. Competition of binding by endogenous fatty acids could be one reason for the observed poor binding, even though it has been shown that competition by endogenous fatty acids is absent at high fatty acid concentrations (46). Second, we have considered the possibility that exposure of the protein to urea and acidic pH may cause denaturation and irreversible precipitation of the protein, and this would therefore make comparisons of stoichiometry with other FABPs difficult.

Clearly, further studies will be necessary to examine the
nature of association of fatty acids and the schistosome FABP homologue. This will include an analysis of endogenous fatty acids associated with the recombinant proteins purified from E. coli and comparison of the effect of delipidation prior to initiating the binding studies, as well as the use of other agents for solubilization of fusion proteins in the purification to ensure stability and maintenance of the native state of the proteins. In addition, we will determine the binding specificities to different fatty acids. Further investigation on the involvement of the N-terminal segments in lipid binding will be carried out by functional studies using model peptides constructed to specific domains or by introduction of site-directed mutations into the corresponding cDNA clones.

This group of proteins, including Sm14, does not seem to be related to other lipid binding proteins such as the apolipoproteins and serum albumins. Upon comparison of their nucleotide and inferred amino acid sequences, no evidence of sequence similarities with each other was found. It was therefore concluded that this group of 14–16-kDa lipid binding proteins has evolved a different structural basis for the binding of lipids from the much larger apolipoproteins and serum albumin (39).

It is still unclear whether Sm14 has a biological function associated with the schistosome nervous system, in view of a sequence homology between Sm14 and myelin P2 protein of the peripheral nerves. It is surprising that the heart FABP shows higher sequence homology with myelin P2 than with intestinal liver FABP (35). Interestingly, however, is the fact that the P2 protein can be readily extracted from intact myelin by aqueous solvents, showing that it does not interact strongly with the myelin bilayer, and a role as a lipid carrier has been suggested (15,52). Such a basic cellular function for P2 may explain why the schistosome, which does not contain myelin, possesses a closely related gene.

The schistosome protein appears to be in close association with the tubercles, which are structures located on the dorsal surface of the adult male parasite. It remains to be clarified which structures are related to the regular patches of stain in the muscle layers, as well as the immunoreactivity present in the parenchyma. Immediate experiments will therefore aim at localizing the S. mansoni protein at the electron microscopic level.

The identification of a FABP homologue in the schistosome is significant in so far as the parasite is unable to synthesize long chain fatty acids and cholesterol de novo and depends largely on the acquisition and utilization of host molecules in order to survive in the host. In light of a recent study which provides the first measurements of the rate of incorporation of palmitate and oleate into neutral lipids and phospholipids of the schistosomula (11), we can begin to define the effects of specific antibodies on the incorporation of fatty acids, also in competition assays, as one possibility for determining the involvement of the schistosomal protein in lipid metabolism.

Interestingly, this cDNA was identified from our S. mansoni expression library using an antiseraum raised against an extract of adult worms incubated in phosphate-buffered saline. In view of the fact that this extract was previously shown to be protective in mice upon challenge with infectious cercariae, it will be desirable to evaluate which role Sm14 plays in the protein mixture in conferring immunity to experimental animals and whether it would be a relevant antigen for effective immunological attack on the parasite.

Acknowledgments—We thank Drs. Diane McLaren, Peter Skuce, Michael Müller, Dietrich Stöber, and Ewald Beck for valuable advice and discussions.

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