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Molecular cloning and sequence analysis of 3-hydroxy-3-methylglutaryl-coenzyme A reductase from the human parasite *Schistosoma mansoni*

(cDNA cloning/schistosomiasis/active recombinant protein)

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ABSTRACT **cDNA clones encoding the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [(*S*)-mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34] from the human parasite *Schistosoma mansoni* have been isolated and characterized. The composite 3459 base pairs of cDNA sequence contains a 2844-base-pair open reading frame corresponding to a protein of 948 amino acids. The predicted *S. mansoni* HMG-CoA reductase protein contains a hydrophobic amino terminus consisting of seven potential transmembrane domains that are structurally conservative but are not identical in amino acid sequence with HMG-CoA reductases from other species. The hydrophilic carboxyl terminus of the *S. mansoni* HMG-CoA reductase protein, however, shares 48–52% sequence identity with the carboxyl termini of other HMG-CoA reductases in a region that contains the catalytic domain. When expressed as a fusion protein in *Escherichia coli*, the carboxyl-terminal domain of the schistosome protein exhibits HMG-CoA reductase enzyme activity.**

Three species of schistosomes (*Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum*) infect more than 200,000,000 people worldwide causing considerable morbidity and mortality. Adult male and female worms reside in the venules of mesenteries and produce a large number of eggs (300–3000 eggs per pair per day) that deposit in various body organs. The immune response of the human host to the eggs results in a granulomatous inflammation at specific tissue sites and is the major cause of pathology in schistosomiasis (1). Consequently, inhibition of egg production would reduce the pathology and associated morbidity and mortality.

Mevinolin (2) is a highly selective and potent inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [(*S*)-mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34], a transmembrane glycoprotein of the endoplasmic reticulum that catalyzes the conversion of HMG-CoA to mevalonate, an important step in the synthesis of cholesterol and nonsterol isoprenoid compounds such as dolichol, ubiquinone, and the isopentenyl group of transfer RNAs (3). When mice infected with *S. mansoni* are treated with mevinolin, egg production in adult worms drops, and the liver pathology associated with the infection is reduced (4). Treatment of adult worms in culture with mevinolin also results in a 70% inhibition of egg production, and this inhibition can be overcome by the addition of exogenous mevalonate (4). These experiments suggest the importance of isoprenoid compounds in schistosome egg formation.

As a first step in understanding the role of HMG-CoA reductase in schistosome egg formation, its regulatory properties, and its potential as a chemotherapeutic target, we have isolated a series of overlapping cDNA clones that encode this enzyme* and compared its deduced protein structure with other known HMG-CoA reductases. To confirm the identity of this sequence, a bacterial fusion protein was produced that demonstrated HMG-CoA reductase enzyme activity.

MATERIALS AND METHODS

Oligonucleotides. Antisense oligonucleotides A (5′-TTC IAT CCA ATT IAT IGC IGC IGG TTT TTT ATC IGT ACA ATA ATT-3′, where I indicates inosine, see Fig. 1*B*), B (5′-CAG CAA TAA CAG ATT TAC CAC GAC CAA GAA TTG TGT TTA TAG TAG CTG GC-3′, complementary to nucleotides +2100 to +2149 in Fig. 1*B*), and C (5′-CAC CAA TTC AAT TTC CAA CAA ATA GTA CTC-3′, complementary to nucleotides +421 to +450 in Fig. 1*B*) were synthesized to screen adult *S. mansoni* genomic and cDNA libraries and to construct an oligonucleotide-primed cDNA library, respectively.

Screening and Isolation of Genomic and cDNA Clones. An adult *S. mansoni* EMBL 3 genomic library (5) was screened with oligonucleotide A as described (5, 6), except that prehybridization and hybridization were done at 42°C, and filters were washed at 37°C. An adult *S. mansoni* λgt11 (5) library was screened with oligonucleotide B and random primer-labeled cDNA fragments, as described (5–7).

An oligonucleotide-primed λgt10 cDNA library was constructed (8) by using the antisense oligonucleotide C and adult schistosome poly(A)$^+$ RNA (5). The cDNA synthesis, vector, and packaging components used commercially available reagents from Invitrogen (San Diego, CA) and Stratagene.

cDNA and genomic fragments were subcloned into the pBSm13+ vector (Stratagene) and sequenced by the dideoxynucleotide chain-termination method (9). Synthetic oligonucleotides were used as primers to sequence the subcloned DNA fragments. Both DNA strands for the coding and untranslated regions were completely sequenced.

Fusion Protein Construction and Enzymatic Assay. The terminal 522 amino acids, containing an extra glycine at the amino terminus derived from the pBluescript SK+ (Stratagene) vector's polylinker, were subcloned as a *Sal* I/*Eco*RI DNA fragment (corresponding to nucleotides +1279 to +3048 in Fig. 1*B*) into the corresponding sites in pATH3 (a *trpE*-encoded protein expression vector) (10), and introduced into *Escherichia coli* strain RR1 (10). The induced fusion protein was soluble in an extract prepared in a Hepes buffer (160 mM Hepes/200 mM KCl/0.5 mM phenylmethylsulfonyl fluoride, pH 7.5). Activity of HMG-CoA reductase was determined as

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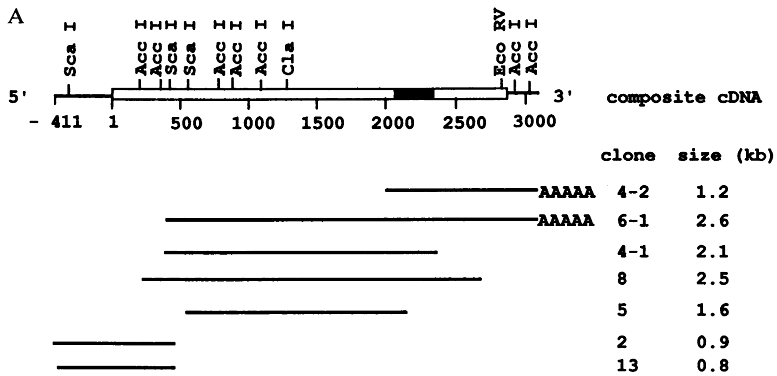
Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; TrpE, protein encoded by *trpE*.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M27294).

A



B

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GTTTCATATTCTCAATGTTCATTCTTCTGTATCATCATTTTAGTATCAACTGGATTTGAACTGTTCGCAATTATTCCCATTTACTTACAGGAAAAATTGAGTATTCGTAGATTAGGCAAC - 52
GTTTTGCTGCTTGACAATTTACCTGTAGGCTTTATATTCTGAGGTATAAAT                                                                     - 1
                                                    +1  ATGCTCAAAATACTAAATACTGTACTGTTATTTTTCGATTGTTTTTCAACTGGAACCTTTTTCGTGCTC   69
                                                        MetLeuLysIleLeuAsnThrValLeuLeuPhePheAspCysPheSerThrGlyThrPhePheValLeu   23

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CysSerLeuSerSerValLeuPheValProTyrThrSerPheAlaIlePheLeuLeuSerThrSerValPheLeuLeuPheSerAspLeuSerValPhePheIleValLeuGluTyrTyr  143

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CAAACTTTAAAATCAGCTTTCGATAAAACTAGCGCACATGTAAACTTATTATCAGTTTTTGCTTGTCCAGCTGGTCGATATATTCACATTCGATTTGCTGCACGTACAGGTGATGCTATG 1989
GlnThrLeuLysSerAlaPheAspLysThrSerAlaHisValAsnLeuLeuSerValPheAlaCysProAlaGlyArgTyrIleHisIleArgPheAlaAlaArgThrGlyAspAlaMet  663

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SerHisTyrThrMetEnd                                                                                                       948
CATTTTTAGATGTTTACTTCATTATCCCATGTTTTTTTCCAATTTCTGTTTCCGTACGTTCGATAGAAATAAACAAAGTATACTTCAAAAAAAAAAAAA                     3048
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FIG. 1. (*Legend appears at the bottom of the opposite page.*)

described by Shapiro *et al.* (11). [^{14}C]Mevalonate was a gift from J. Bennett (Michigan State University).

RESULTS

Isolation of cDNAs Encoding *Schistosoma* HMG-CoA Reductase and Nucleotide Sequence Analysis. The strategy to isolate cDNAs encoding *S. mansoni* HMG-CoA reductase consisted of constructing oligonucleotide A probe that encodes a protein sequence of 15 amino acid residues (Asn-Tyr-Cys-Thr-Asp-Lys-Lys-Pro-Ala-Ala-Ile-Asn-Trp-Ile-Glu, residues 685–699 in hamster) conserved in hamster, human, and yeast *HMG1*-encoded enzymes (12–15). Where clear preference for one codon was not evident from the codon usage derived from published schistosome cDNA sequences, an inosine residue was inserted. This oligonucleotide was used to screen ≈250,000 plaques from the adult genomic library. Two recombinant bacteriophage (1-1 and 1-3), which hybridized under low-stringency conditions, were characterized further. Restriction DNA fragments from these two genomic clones were subcloned and sequenced. Sequence analysis of genomic clone 1-1 showed that it lacked an open reading frame and had no homology to the hamster (12), human (13), or yeast *HMG1* (14) HMG-CoA reductase DNA sequences. However, the sequence from a portion of genomic clone 1-3 contained a putative 279-base-pair (bp) exon (Fig. 1*A*) that encodes 93 amino acids with 52% identity to amino acids 679–769 in the hamster HMG-CoA reductase protein.

To isolate cDNA clones encoding *S. mansoni* HMG-CoA reductase, an adult λgt11 cDNA library was screened with oligonucleotide B derived from the exon in genomic clone 1–3. Three cDNAs, 4-2, 4-1, and 6-1, were isolated and sequenced. Because these cDNAs did not encode the entire amino terminus, the 5′-end fragment of cDNA 4-1 [0.4-kilobase (kb) *Eco*RI–*Acc* I fragment] was used to rescreen the adult λgt11 library. cDNAs 8 and 5 (Fig. 1*A*) were isolated, and cDNA 8 extended only an additional 176 bp 5′ of cDNA 4-1. To obtain the remaining 5′ coding sequence, adult poly(A)$^+$ RNA was primed with oligonucleotide C (this oligonucleotide lies 206 bp from the 5′ end of cDNA 8) to construct a cDNA library in λgt10. The 5′-end fragment of cDNA 8 (0.2-kb *Eco*RI–*Sca* I fragment) was used to screen the oligonucleotide C-primed λgt10 library, and five cDNA clones were isolated, of which two (2 and 13) were sequenced (Fig. 1*A*). The composite nucleotide sequence, derived from the overlapping cDNA clones, and the inferred protein sequence are shown in Fig. 1*B*.

The composite cDNA sequence (Fig. 1*B*) is 3459 bp long with a 5′-untranslated sequence of 411 bp, an open reading frame of 2844 bp, and a 3′-untranslated sequence of 188 bp followed by a poly(A) tail. The methionine at position +1 was designated as the initiator methionine based on the following criteria: (*i*) the 5′-untranslated sequence contains six stop codons at positions −369, −351, −323, −321, −273, and −12; (*ii*) the hydrophobicity plot of the schistosome HMG-CoA reductase amino terminus predicts seven potential transmembrane domains, as reported for yeast HMG-CoA reductase isozyme 1 and other animal enzymes, when this methionine is designated +1; and (*iii*) the next methionine is located 81 amino acids downstream from the designated initiator methionine, and the use of this methionine as initiator eliminates two potential transmembrane domains. The predicted 2844-bp coding regions encodes a protein of 948 amino acids with a predicted molecular mass of 107 kDa. Northern (RNA) blot analysis on poly(A)$^+$ mRNA isolated from paired adult worms shows a major 3.6-kb and a minor 2.9-kb transcript (data not shown). The size of the larger transcript corresponds closely to the length of the composite cDNA.

Protein Structure of *S. mansoni*-HMG-CoA Reductase. A hydrophobicity plot of the *S. mansoni* HMG-CoA reductase protein (Fig. 2*A*) reveals a hydrophobic amino-terminal domain (residues 1–364) followed by a relatively hydrophilic carboxyl-terminal domain (residues 365–948). The schistosome HMG-CoA reductase amino-terminal domain shares no amino acid sequence homology with any of the known sequences for this enzyme, including hamster (12), human (13), yeast HMG-CoA reductase isozyme 1 (14), *Drosophila* (15), sea urchin (18), and *Arabidopsis* (19) amino termini. Despite the absence of amino acid sequence homology, the structural similarity between schistosome and hamster HMG-CoA reductases is clear when hydrophobicity profiles (16) for the two enzymes are compared (Fig. 2*A*). Based on the algorithm of Klein *et al.* (20), the schistosome hydrophobic amino terminus is predicted to contain seven potential transmembrane domains, and these are located at similar positions in the hamster hydrophobic plot. The amino termini of other species, except *Arabidopsis*, which has only one potential transmembrane domain, also have been predicted to transverse the membrane seven times. The only species that share amino acid sequence homology at their amino termini are hamster, human, *Drosophila*, and sea urchin HMG-CoA reductases with homology ranging from 38% identity between sea urchin and *Drosophila* to 98% identity between hamster and human (13).

The amino acid sequence homology between *S. mansoni* HMG-CoA reductase and that of other species is confined to amino acids 487–874 (Fig. 2*B*). In this 388-amino acid sequence, the schistosome protein shows 52%, 52%, 51%, 49%, 51%, and 48% identity with hamster, human, yeast HMG-CoA reductase type 1, *Drosophila*, sea urchin, and *Arabidopsis*, respectively. This highly conserved region, which encompasses most of the HMG-CoA reductase catalytic site (17), has been predicted to contain two regions of extended β structure (β1 and β2) in hamster and yeast HMG-CoA reductase type 1 enzymes (14, 17). These two regions correspond to amino acids 525–595 (β1) and 743–837 (β2) in the schistosome enzyme and are highly conserved across species (Fig. 2*B*). The β1 region is relatively hydrophobic and contains 3 of 5 conserved cysteines (residues 534, 535, and 578, with the other two cysteines at positions 696 and 787), 6 of 21 conserved glycines, and 3 of 6 conserved prolines. The β2 region is also relatively hydrophobic and contains 9 of 21 conserved glycines as well as 1 of the 2 conserved histidines at position 762 (the other conserved histidine is at position 869). The conserved histidine at residue 762 may correspond to a histidine residue implicated in catalysis (21).

Enzyme Activity of TrpE–Schistosome Fusion Protein. To investigate whether recombinant schistosome HMG-CoA reductase exhibits enzyme activity, a TrpE–HMG-CoA reductase fusion protein (TrpE being the enzyme encoded by *trpE*) was constructed containing the putative catalytic domain of the molecule extending from amino acids 427–948. Upon induction in *E. coli* a soluble fusion protein was

FIG. 1 (*on opposite page*). Overlapping cDNA clones and composite nucleotide and amino acid sequence for *S. mansoni* HMG-CoA reductase. (*A*) Overlapping cDNAs encoding schistosome HMG-CoA reductase were isolated from the adult λgt11 library (cDNAs 4-2, 6-1, 4-1, 8, and 5) and oligonucleotide C-primed adult λgt10 library (cDNAs 2 and 13). The coding region in the composite cDNA is schematically represented by the open rectangle, while the lines represent 5′- and 3′-untranslated regions. The solid rectangle in the composite cDNA represents the nucleotide sequence corresponding to the exon from genomic clone 1-3 (nucleotides +2022 to +2300 in Fig. 1*B*). (*B*) The 5′-untranslated nucleotide sequence extends from −1 to −411. The 2844 bp of coding sequence and the corresponding amino acid sequence start at +1. Nucleotides in genomic clone 1-3 that hybridized to oligonucleotide A are double-underlined.

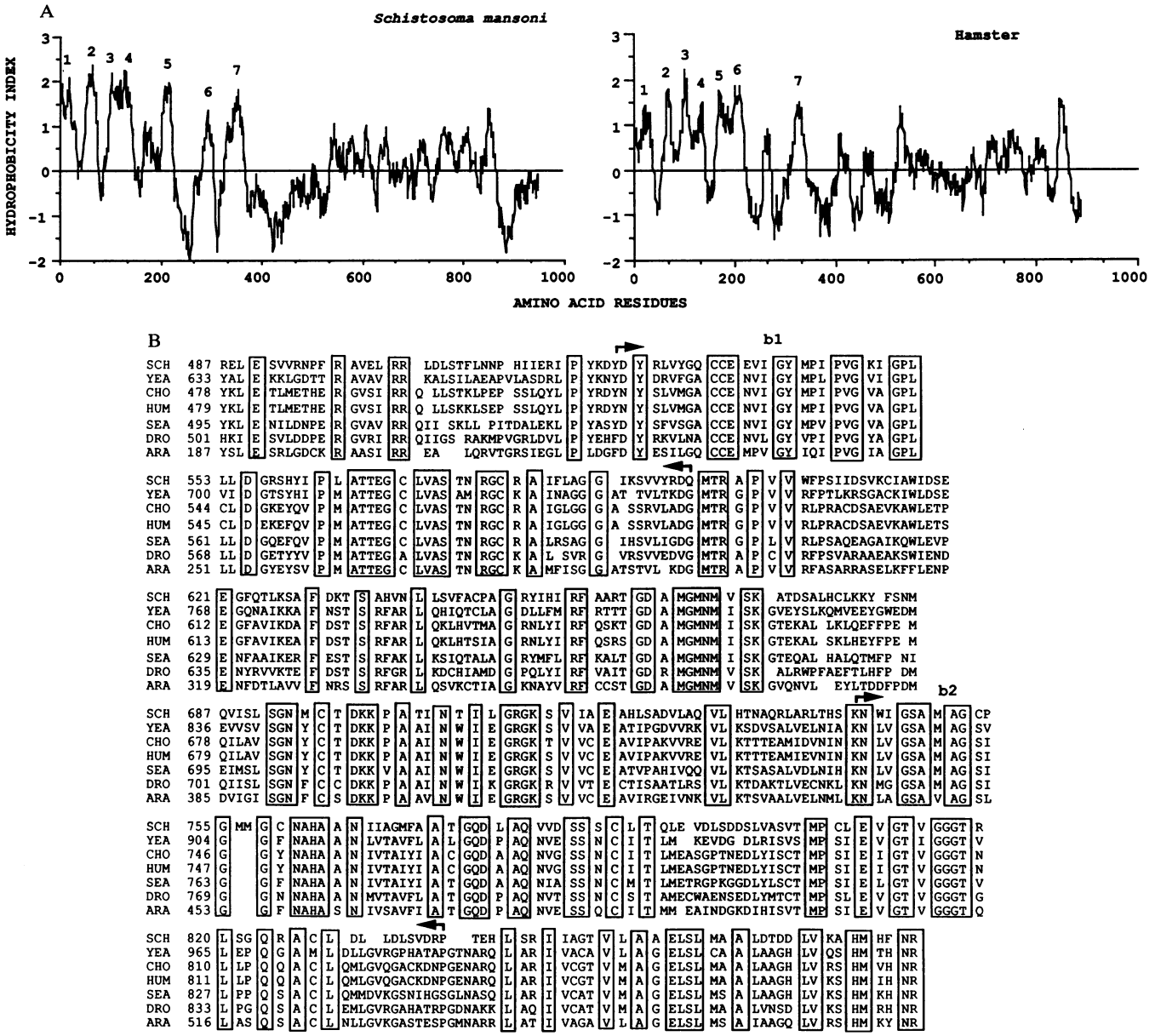

FIG. 2. Kyte and Doolittle (16) hydrophobicity plots and comparison of HMG-CoA reductase carboxyl termini among species. (*A*) The arithmetic averaging was performed over a window of 21 residues. Positive values indicate relatively hydrophobic regions, and negative values indicate relatively hydrophilic regions in the proteins. Potential transmembrane domains are labeled 1–7 on the schistosome and the hamster hydrophobicity plots. The potential transmembrane domains 1–7 in schistosome HMG-CoA reductase correspond to amino acid residues 9–25, 55–71, 96–112, 124–140, 207–223, 286–302, and 347–363, respectively. (*B*) Schistosome (SCH, residues 487–874), yeast HMG-CoA reductase isozyme 1 (YEA, residues 633–1025), hamster (CHO, residues 478–870), human (HUM, residues 479–871), sea urchin (SEA, residues 495–887), *Drosophila* (DRO, residues 501–893), and *Arabidopsis* (ARA, residues 187–576) were aligned for maximum homology. Boxed residues indicate regions of absolute identity across species. The extended β structures (b1 and b2) are predicted from modeling of the hamster and yeast enzymes (14, 17) and are indicated by enclosed arrows. Intelligenetics programs PEP and GENALIGN, located in the Bionet system, were used to calculate hydrophobicity values and perform the alignment.

identified at the expected molecular mass of 94 kDa (data not shown). When assayed for enzyme activity, the soluble bacterial extract containing the fusion protein catalyzed the conversion of [^{14}C]HMG-CoA to [^{14}C]mevalonate (Fig. 3). This conversion increased with increasing amounts of bacterial extract containing the fusion protein. No such enzymatic activity was seen with extracts from cells containing the *trpE* vector alone.

DISCUSSION

We have isolated overlapping cDNA clones and deduced, from the translated composite nucleotide sequence, a protein with structural and amino acid sequence homology to yeast HMG-CoA reductase isozyme 1, animal, and plant HMG-CoA reductases. When expressed as a fusion protein, the carboxyl terminus of the schistosome protein exhibits HMG-CoA reductase enzyme activity. With this structural and functional evidence we conclude that we have characterized cDNAs encoding the *S. mansoni* HMG-CoA reductase.

Despite the high structural conservation of the schistosome HMG-CoA reductase protein with other species, its gene and protein may be regulated differently from the mammalian enzymes. In mammalian cells mevalonate produced by HMG-CoA reductase serves as a precursor for the synthesis of cholesterol and a variety of nonsterol isoprenoid com-

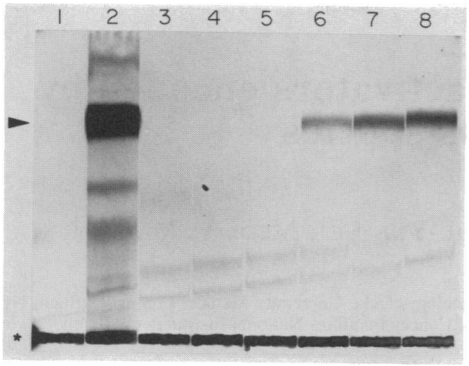

FIG. 3. Enzyme activity of the TrpE–schistosome HMG-CoA reductase (amino acids 427–948) fusion protein. Increasing amounts of soluble bacterial extracts from cells containing the *trpE* vector alone (lanes: 3, 10 μl; 4, 20 μl, and 5, 50μl) or *TrpE*–schistosome fusion protein (lanes: 6, 10 μl; 7, 20 μl; and 8, 50 μl) were assayed for conversion of [^{14}C]HMG-CoA to [^{14}C]mevalonate (11). Products of the reaction were acidified with HCl to convert [^{14}C]mevalonolactone and resolved on silica gel TLC plates in the benzene/acetone (1:1 vol/vol) solvent system (11). [^{14}C]Mevalonolactone was detected by exposing the Kodak XAR film to TLC plates overnight at 20°C. Lanes 1 and 2 were loaded with [^{14}C]HMG-CoA and [^{14}C]mevalonolactone, respectively. The asterisk indicates origin, and the arrowhead indicates [^{14}C]mevalonolactone ($R_f \approx 0.69$).

pounds (3). *S. mansoni*, like other parasitic and free-living platyhelminths, is incapable of *de novo* sterol biosynthesis (22). Polyisoprenoid lipids have been detected in *S. mansoni* (4, 23), and inhibition of their synthesis correlates with a decline in the production of eggs (4). These lipids have been implicated in schistosome glycoprotein biosynthesis and may be of particular importance to parasite eggs where glycoproteins are prevalent, many of which are antigenic (24, 25). The chemical structures and regulatory properties of these lipids have not been well characterized in *S. mansoni*. It is also unclear what effects, if any, host lipids and cholesterol have on this pathway. This may be of interest because lipoprotein receptors appear to be present on the surface of schistosomula of *S. mansoni* (26), and low density lipoprotein receptors and HMG-CoA reductase are known to be coregulated by cholesterol in mammals (27).

The availability of these cDNAs will permit the development of specific reagents to probe the role of this enzyme in parasite egg formation and to better define its regulatory properties. In addition, the production of recombinant enzyme will permit a more detailed evaluation of its catalytic properties and evaluation of this pathway as a potential target for chemotherapy.

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