Characterization of a Stable Form of Tryptophan Hydroxylase from the Human Parasite Schistosoma mansoni*

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A cDNA (Schistosoma mansoni tryptophan hydroxylase; SmTPH) encoding a protein homologous to tryptophan hydroxylase, the enzyme that catalyzes the rate-limiting step in the biosynthesis of serotonin, was cloned from the human parasite Schistosoma mansoni. Bacterial expression of SmTPH as a histidine fusion protein produced soluble active enzyme, which was purified to apparent homogeneity and a final specific activity of 0.17 µmol/min/mg of protein. The purified enzyme was found to be a tetramer of approximately 240 kDa with a subunit size of 58 kDa. Several of the biochemical and kinetic properties of SmTPH were similar to those of mammalian tryptophan hydroxylase. Unlike the mammalian enzyme, however, SmTPH was found to be stable at 37 °C, its t½ being nearly 23 times higher than that of a similarly expressed rabbit tryptophan hydroxylase. A semiquantitative reverse transcription polymerase chain reaction showed that the level of SmTPH mRNA in a larval stage of the parasite ( cercaria) is 2.5 times higher than in adult S. mansoni, suggesting possible differences in the level of enzyme expression between the two developmental stages. This study demonstrates for the first time the presence of a functional tryptophan hydroxylase in a parasitic helmint and further suggests that the parasites are capable of synthesizing serotonin endogenously.

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­ The abbreviations used are: TPH, tryptophan hydroxylase; SmTPH, S. mansoni TPH; PCR, polymerase chain reaction; RT-PCR, reverse transcription PCR; TH, tyrosine hydroxylase; PAH, phenylalanine hydroxylase; bp, base pair(s); 5-HT, 5-hydroxytryptophan; 5-HTP, 5-hydroxytryptamine; BH4, 6R-5,6,7,8-tetrahydrobiopterin; SL, splined leader.

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EXPERIMENTAL PROCEDURES

Chemicals—L-Tryptophan, 5-HTP, 5-HT, N-acetyl-5-HT, melatonin, p-chlorophenylalanine, isopropyl-β-thiogalactoside, dithiothreitol, dihydropteridine reductase, NADH, glycerol, and Sephacryl 200HR were purchased from Sigma. Tween 20, ferrous ammonium sulfate, and

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activated charcoal were from Fisher. [5-3H]-L-Tryptophan was from Amersham Pharmacia Biotech. (65)-F-6,7,8-tetrahydrobiopterin (BH4) and dopamine were from Research Biochemicals International. Aprotinin, leupeptin, phenylmethylsulfonyl fluoride, and catalase were from Roche Molecular Biochemicals. All other chemicals were of the highest purity available from commercial sources.

*S. mansoni*—A Puerto Rican strain of *S. mansoni* was maintained as described previously (40). Crude adult worm tissue extracts were prepared and used directly for TPH activity measurements as described earlier (40). Total RNA was extracted from *S. mansoni* using the TRIzol reagent (Life Technologies, Inc.). Poly(A+) RNA from adult *S. mansoni* was transcribed from total RNA using oligo(dT)-cellulose columns (Amersham Pharmacia Biotech).

Cloning of Full-length SmTPH—A partial *S. mansoni* cDNA sequence (576 bp) homologous to other TPH sequences was isolated by homology RT-PCR. Oligonucleotide primers were synthesized based on a predicted genomic TPH sequence from the free-living nematode *Caenorhabditis elegans* (cosmid ZK1290, GenBank accession no. U22130) and used for PCR amplification of adult *S. mansoni* oligo(dT) reverse transcribed cDNA. The sense and antisense primers targeted a region of the predicted catalytic domain that is highly conserved among all aromatic amino acid hydroxylases (see Fig. 3). Primer sequences and cycling parameters as above) using the same sense primer (SmSL)

For purification of recombinant SmTPH, cell pellets from 100 ml of induced bacterial cultures were thawed in 4 ml of 20 mM phosphate buffer (pH 7.4) containing 0.5 mM NaCl, 0.2% Tween 20, 5% glycerol, 10 mM imidazole, and a mixture of protease inhibitors (1 mM phenylmethylsulfonyl fluoride and 50 μg/ml each leupeptin and aprotinin). To purify the expressed SmTPH, a procedure similar to that described above but with two cycles of rapid freeze-thawing followed by sonication on ice (seven pulses of 15 s separated by intervals of 30 s) using a vibra cell sonicator (Sonics and Material, Danbury, CT) set at 20% maximal power. Cell lysates were centrifuged at 12,000 × g for 15 min and 4 °C. The resulting pellet was resuspended in 2.5 ml of the same buffer and subjected to tissue extracts as above. All supernatant fractions were pooled and used for direct enzymatic assays and for subsequent purification of the expressed enzyme.

Recombinant SmTPH was purified by immobilized metal (nickel) affinity chromatography (44) using the HiTrap kit (Amersham Pharmacia Biotech) for purification of histidine-tagged proteins, as described previously (40). Excess imidazole was removed by gel filtration through a Sephadex G-25 column (PD-10; Amersham Pharmacia Biotech), and the purified enzyme was in 50 mM HEPES (pH 7.5) containing 0.2 mM NaCl, 10% glycerol, 0.05% Tween 20, and 1 mM dithiothreitol. Purified enzyme preparations (0.2 mg/ml) were stable for at least 4 days at 4 °C and could be stored at −80 °C for at least 1 month with no significant loss in activity.

**TPH Stability Assay**—The stability of SmTPH was assessed in comparison with that of recombinant rabbit brain TPH similarly expressed in E. coli. In preparation for these experiments, the complete coding sequence of rabbit brain TPH cDNA (13) was subcloned into pET15b and expressed as a histidine-tagged protein in BL21(DE3)pLysS E. coli. Bacterial cells expressing rabbit TPH or SmTPH were lysed, deproteinized using 10 mM of scintillation mixture (ICN). Enzyme activity data were analyzed using Lineweaver-Burk plots or by computer-assembled, nonlinear curve fitting to the Michaelis-Menten model. All kinetic parameters (Km and apparent Kp (S0.5)) were determined using the program Enzyme Kinetics (version 1.6; DogStar Software) and were obtained from three to two independent experiments, each performed in duplicate (see Figs. 1 and 2). For for purification of recombinant SmTPH, cell pellets from 100 ml of induced bacterial cultures were thawed in 4 ml of 20 mM phosphate buffer (pH 7.4) containing 0.5 mM NaCl, 0.2% Tween 20, 5% glycerol, 10 mM imidazole, and a mixture of protease inhibitors (1 mM phenylmethylsulfonyl fluoride and 50 μg/ml each leupeptin and aprotinin). To purify the expressed SmTPH, a procedure similar to that described above but with two cycles of rapid freeze-thawing followed by sonication on ice (seven pulses of 15 s separated by intervals of 30 s) using a vibra cell sonicator (Sonics and Material, Danbury, CT) set at 20% maximal power. Cell lysates were centrifuged at 12,000 × g for 15 min and 4 °C. The resulting pellet was resuspended in 2.5 ml of the same buffer and subjected to tissue extracts as above. All supernatant fractions were pooled and used for direct enzymatic assays and for subsequent purification of the expressed enzyme.

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ethidium bromide-stained RT-PCR products were performed with the NIH Image program version 1.61 (Bethesda, MA).

Other Methods—Size exclusion chromatography of purified SmTPH was performed on a Sephadryl-200HR gel filtration column (10-mm inner diameter × 50 cm; Bio-Rad), as described previously (27). Protein concentrations were measured by the method of Bradford (50), using the Bio-Rad protein assay kit and bovine serum albumin as a standard. Reducing SDS-polyacrylamide gel electrophoresis was performed according to the method of Laemmli (51) using precast 10% acrylamide gels from Novex, Inc. For Western blot analysis of SmTPH, aliquots of purified enzyme (0.5–1 μg) were electrophoresed as above, transferred onto nitrocellulose (52), and reacted with a sheep polyclonal antibody (1:500 dilution) raised against rabbit TPH (Chemicon International) followed by a peroxidase-conjugated rabbit anti-sheep IgG (Pierce) as the secondary antibody (1:1000 dilution).

RESULTS

Cloning of the Full-length SmTPH cDNA and Protein Sequence Analyses—A partial TPH sequence was first obtained by RT-PCR using oligo(dT) reverse-transcribed S. mansoni cDNA and C. elegans primers (40) that targeted a region conserved among all aromatic amino acid hydroxylases. A 576-bp product was sequenced and found to have high homology with TPH sequences from other species. The missing 5’- and 3’-ends were subsequently obtained by an anchored PCR-based strategy. The 5’-end was amplified in a RT-PCR reaction that targeted the conserved SL sequence of S. mansoni (40, 41). A 741-bp product corresponding to the 5’-end of TPH was sequenced and found to carry a complete S. mansoni SL sequence (nucleotides 9–36 of the SL sequence; see Fig. 1) including the last four nucleotides, which were not part of the SmSL primer used in the anchored PCR reaction. This finding suggests that SmTPH is trans-spliced at the 5’-end to the SmSL SL, just as described previously for several other S. mansoni cDNAs (40–42). The 3’-end of SmTPH was PCR-amplified directly from a S. mansoni cDNA plasmid library, using TPH-specific and vector-derivated primers (see Fig. 1). The resulting product (1000 bp) contains a potential polyadenylation sequence (AATAAA) (53) upstream of a poly(A) tail and thus is presumed to represent the 3’-end of the full-length transcript. Fig. 1 shows the nucleotide and predicted amino acid sequence of SmTPH. The composite cDNA reveals a single open reading frame of 1494 bp encoding a predicted protein of 497 amino acids with a calculated molecular mass of 58 kDa. Protein sequence analysis revealed the presence of two consensus sites (Ser151 and Thr213) for phosphorylation by the Ca 2

The conserved catalytic domain of TPH (15, 16, 21, 24) consists of a leucine heptad repeat interspersed by a 4,3-hydrophobic repeat (26, 27) (Fig. 1). Potential intersubunit binding domain (SmTPH Leu462–Ile480), of approximately 40 amino acids. The latter region contains a potential tetramerization domain. The potential polyadenylation consensus sequence is underlined, and the stop codon (TAG) is indicated by an asterisk.

| a | c | d | e | f | g |
|---|---|---|---|---|---|
| S. mansoni Tryptophan Hydroxylase | 974 | 982 | 990 | 998 | 1006 |
| | 575 | 583 | 591 | 600 | 608 |
| | 221 | 229 | 237 | 245 | 253 |
| | 1088 | 1096 | 1104 | 1112 | 1120 |
| | 1224 | 1232 | 1240 | 1248 | 1256 |
| | 292 | 300 | 308 | 316 | 324 |
| | 1352 | 1360 | 1368 | 1376 | 1384 |
| | 1436 | 1444 | 1452 | 1460 | 1468 |
| | 1532 | 1540 | 1548 | 1556 | 1564 |
| | 1632 | 1640 | 1648 | 1656 | 1664 |
| | 1736 | 1744 | 1752 | 1760 | 1768 |
| | 575 | 583 | 591 | 600 | 608 |
| | 472 | 480 | 488 | 496 | 504 |
| | 1638 | 1646 | 1654 | 1662 | 1670 |
| | 1764 | 1772 | 1780 | 1788 | 1796 |

FIG. 1. Complete cDNA and predicted amino acid sequence of SmTPH. Overlined sequences represent sense (SmSL, S, C, and D) and antisense primers (A, B, E, and F) used in RT-PCR and cloning procedures. The conserved S. mansoni SL sequence is shown in italics at the beginning of the cDNA sequence. Putative Cα-calmodulin-dependent protein kinase type II phosphorylation sites (boxed) and a consensus leucine zipper motif (SmTPH amino acid positions 358–377). BLAST analysis (54) of the predicted protein sequence indicated that SmTPH is highly homologous to tryptophan hydroxylase from other species. The dendrogram in Fig. 2 shows that SmTPH is more related to TPH sequences than to those of the other two aromatic amino acid hydroxylases, TH and PAH. Based on pairwise CLUSTAL protein alignments (55), SmTPH shares high amino acid sequence homology (65–67%) with vertebrate TPH sequences (13, 18–23). In contrast, there is considerably less homology (57%) with the only other invertebrate sequence available, a Drosophila enzyme that has been described as a TPH/PAH hybrid (56). Structurally, the Drosophila enzyme appears to be more closely related to PAH than any of the TPH sequences.
of six histidines followed by a thrombin cleavage site, added an extra 20 amino acids (~2 kDa) to the amino terminus of SmTPH.

Induction of SmTPH-transformed BL21(DE3)pLysS cells produced large amounts of active TPH. Western blot analysis of soluble bacterial protein extracts identified a predominant band of the expected size that reacted with a rabbit anti-TPH antibody and lysates prepared from E. coli transformed with pET15b vector containing no insert (Fig. 4). The average specific activity of SmTPH in crude bacterial extracts was 3.5 ± 0.5 nmol/min/mg of protein with about 80% of total enzyme activity being recovered in the soluble fraction. The remaining activity was retained in the pellet in the form of inclusion bodies.

Purification and Macromolecular Structure of SmTPH—SmTPH was purified by nickel affinity chromatography as an N-terminal histidine fusion tag. Based on specific activity measurements, SmTPH was purified about 45-fold to a final specific activity of 170 ± 5.0 nmol/min/mg of protein. The yield from 100 ml of induced bacterial culture was 0.66–0.8 mg of purified SmTPH. Coomassie Blue staining and densitometric analysis of the purified protein showed a predominant Western positive band of ~60 kDa, which was consistent with the expected size of the SmTPH monomer (Fig. 4). The purified enzyme was subjected to size exclusion chromatography to determine the oligomeric organization of the protein. Calculations of the molecular weight from a standard V/V plot suggest that SmTPH exists as a tetramer with approximate molecular mass of ~240 kDa (Fig. 5).

Kinetic Properties of Purified SmTPH—All three aromatic amino acid hydroxylases display an absolute requirement for tetrahydrobiopterin as a cofactor (2). Fig. 6A is a representative BH4 saturation curve showing that the activity of the purified SmTPH is also dependent on its cofactor BH4. Removing the cofactor abolished all enzymatic activity. The Km for BH4 was measured by varying the concentration of BH4 (2.5–200 µM) and keeping the concentration of the substrate, tryptophan, constant at 100 µM. Purified SmTPH displayed a Km for BH4 of 6.7 ± 0.7 µM (mean ± S.E.) and a Vmax value of 163 ± 13 nmol/min/mg of protein (mean ± S.E.).

The Km for the substrate was investigated by varying tryptophan concentration (1–250 µM) while fixing the BH4 concentration at 200 µM (Fig. 6B). SmTPH activity was inhibited at high concentrations of tryptophan (>100 µM), in a similar fashion to what was previously reported for mammalian TPH (6, 15, 17, 60). Since an accurate Km determination for tryptophan was not possible due to substrate inhibition, an apparent Km (S0.5) was estimated at (mean ± S.E.) 22 ± 2.0 µM.

Several known inhibitors of mammalian TPH, including p-chlorophenylalanine, dopamine, and the product of tryptophan hydroxylase, 5-HTP (11, 13, 61–66), all caused inhibition of purified SmTPH (Table 1). The parasite enzyme was not sensitive to feedback inhibition by 5-HT or by the products of 5-HT metabolism, N-acetyl-5-HT, and melatonin, even at high concentrations of 0.1 mM (Table 1). The inhibition by dopamine was found to be predominantly competitive with respect to the cofactor, BH4 (Fig. 7A). In the case of 5-HTP, the inhibition was predominantly noncompetitive with respect to tryptophan. The addition of 50 µM 5-HTP caused a marked 2–3-fold decrease in Vmax with very little change in the apparent Km for the substrate (Fig. 7B).

Low levels of catechols, in particular dopamine, have been shown to cause a sustained time-dependent inhibition of mammalian forms of tyrosine hydroxylase (67–69). To determine if SmTPH is similarly sensitive to this form of dopamine inhibition, aliquots of the purified enzyme (2.8 µM) were preincubated with a stoichiometric amount of dopamine for 2, 4, 6, 10, and 15 min at 30 °C and then assayed for TPH activity. No change in SmTPH activity was detected compared with a control sample incubated for the same length of time in the absence of dopamine (data not shown).

Stability of SmTPH—The stability of SmTPH was compared with that of rabbit brain TPH (13) that was similarly subcloned into pET15b and expressed in E. coli BL21(DE3)pLysS strain as a histidine-tagged fusion protein. Initial attempts to purify the rabbit enzyme by nickel chelation chromatography yielded a very unstable enzyme (t1/2 < 10 min at 37 °C) with low specific activity. Therefore, the comparison between SmTPH and the rabbit enzyme was carried out with crude bacterial lysates, which had similar specific activities of 3.3 nmol/min/mg of protein for SmTPH and 1.49 nmol/min/mg of protein for rabbit TPH, respectively. The latter value is essentially identical to what was previously reported for similar preparations of this enzyme (13). Aliquots of crude SmTPH or rabbit TPH containing the same amount of protein (~100 µg) were preincubated at 37 °C for up to 80 min and then assayed for TPH activity. As can be seen in Fig. 8, SmTPH activity remained virtually unchanged over the incubation period, with an estimated half-life (t1/2) of ~21 h. In contrast, the rabbit brain TPH displayed a significantly shorter t1/2 of 54 min. This latter value is comparable with the previously reported t1/2 for recombinant rabbit TPH (25). The same experiment was repeated with aliquots (1.75 µg) of purified SmTPH. The results (data not shown) produced an estimated t1/2 value for the pure enzyme of 99 min at 37 °C.

TPH Activity in Crude S. mansoni Extracts—Crude tissue extracts of adult S. mansoni were prepared and tested directly for TPH enzymatic activity as described above. When compared with a boiled enzyme control, the specific activity level of the native S. mansoni TPH was ~0.02–0.04 nmol/min/mg of protein (data not shown). This level of activity is similar to that of mammalian TPH.
reported earlier for native TPH measured in rabbit brain extracts (13). When BH4 was omitted from the assay mixture, no detectable levels of activity were obtained from the worm extracts. This illustrates that the native S. mansoni TPH has the same absolute requirement for the biopterin cofactor as the recombinant SmTPH.

**SmTPH Developmental Expression in S. mansoni**—The expression of SmTPH was examined by semiquantitative RT-PCR in two different developmental stages of S. mansoni, cercaria and adults. Expression levels were standardized by comparison with a constitutively expressed control gene from S. mansoni, α-tubulin (47, 48). Fig. 9 indicates that the SmTPH expression level is approximately 2.5-fold higher in the cercarial stage than in the adult stage of S. mansoni. No α-tubulin PCR products were detected in a control reaction that lacked reverse transcriptase, thus ruling out the possibility of genomic DNA contamination.

**DISCUSSION**

This study describes the cloning and functional characterization of tryptophan hydroxylase from a lower invertebrate, the parasitic platyhelminth S. mansoni. This is the second member...
of the aromatic amino acid hydroxylase family identified in *S. mansoni*; we recently cloned a functional form of TH from this same parasite (40). The finding of these enzymes in such a primitive invertebrate raises interesting questions about the evolution of the three hydroxylases. There is general agreement that the three enzymes are derived from a common ancestral gene through a series of two gene duplications, the first of which gave rise to TH, whereas the second separated TPH from PAH (18, 70, 71). It has been suggested that the divergence of TPH and PAH occurred late in evolution, possibly after the emergence of arthropods (71). However, as pointed out by Boularand *et al.* (72), recent genome sequencing data have identified distinct predicted genomic sequences for all three enzymes in the nematode, *C. elegans*, suggesting that the two gene duplications occurred earlier than was previously thought. The finding of TPH and TH in *S. mansoni* strengthens this point and further suggests that the divergence of the aromatic amino acid hydroxylases may have occurred before the emergence of platyhelminths.

An alignment of SmTPH with other TPH sequences identified a core of high amino acid sequence identity in the middle to C-terminal region of the enzyme. This stretch of conserved sequence corresponds roughly to the previously defined catalytic domain of TPH (1) and includes several distinctive motifs, including the predicted iron binding site (57, 58) and a putative biopterin binding domain (59). The N-terminal region, on the other hand, shows little sequence conservation across the different species of the enzyme and is particularly divergent in SmTPH, with identity scores of 14–17% when compared with cognate mammalian sequences (see Fig. 3). The divergence at the N-terminal end is consistent with the notion that this region lies outside the catalytic domain and may serve a regulatory function (15, 21, 24, 25, 28, 65), just as shown for other aromatic amino acid hydroxylases (2, 3). The present identification of a putative phosphorylation site for Ca$^{2+}$/calmodulin-
dependent protein kinase II in this N-terminal region of SmTPH (Thr^{130}) gives credence to this notion. At the C-terminal end, the homologous parasite sequence extends to position 455 (rabbit TPH position 417), which places a tentative C-terminal boundary for the catalytic domain nearly 40 residues prior to the C terminus. This region shows very little sequence conservation among the different TPH species except for a common absolute requirement for the reduced pterin cofactor, BH$_4$.

The $K_m$ for BH$_4$ was 4–7-fold less than what was previously reported for purified mammalian brain TPH tagged to glutathione S-transferase (16) or maltose-binding protein (15). It is unknown if this discrepancy is due to the presence of large fusion tags on the two mammalian enzymes, which may have influenced the kinetic determination, or whether the parasite hydroxylase has a significantly higher affinity for the cofactor. With respect to the substrate, tryptophan, SmTPH exhibited a typical kinetic profile, with characteristic substrate inhibition at tryptophan concentrations above 100 $\mu$M, similar to what was described previously for mammalian forms of the enzyme (6, 15). Additional characterization of the parasite hydroxylase showed that the enzyme is sensitive to inhibition by the classic TPH inhibitor, p-chlorophenylalanine, as well as the immediate product of the reaction, 5-HTP, but not serotonin or its metabolites, N-acetylserotonin and melatonin. The lack of inhibition by serotonin was reported previously in crude brain extracts of native TPH (11). It is noteworthy that the inhibition of SmTPH by 5-HTP did not follow classical competitive kinetics, as might have been expected from standard product inhibition. Instead, 5-HTP inhibition (as a function of tryptophan) showed mixed, predominantly noncompetitive characteristics, with $V_{max}$ decreasing nearly 3-fold and the apparent $K_m$ increasing by about 50%. The significance of this inhibition profile is unclear, nor is it known if it is unique to the parasite. All available data on product inhibition of mammalian TPH stem from preparations of crude native enzyme (5, 11), which is not directly comparable with the purified enzyme preparations used in this study. Additional research on the role of 5-HTP in TPH regulation is needed.

Previous studies have shown that TPH is susceptible to inhibition by catechol products of the TH reaction, in particular dopamine (62–66). Inhibition by dopamine is thought to be biologically relevant in regions of the nervous system where serotonergic and catecholaminergic neurons may interact. A large body of evidence on dopamine inhibition of TPH shows the existence of two major mechanisms of hydroxylase inhibition, a time-dependent sustained inhibition seen at low (stoichiomet-
Semiquantitative RT-PCR was performed on total RNA extracted from two different developmental stages of *S. mansoni* (cercaria and adult), as described under “Experimental Procedures.” The RT-PCR reactions in both developmental stages were standardized by simultaneous amplification of an internal control house keeping gene from *E. coli* (α-tubulin). The SmTPH PCR product and that of the α-tubulin control are shown in the top panel. A negative PCR control was done using *S. mansoni* α-tubulin primers on total RNA samples subjected to a mock RT reaction (-RT). The PCR products were analyzed on a 1.2% agarose gel containing ethidium bromide followed by densitometric analysis. The lower panel shows a bar graph displaying the relative optical density (ROD = optical density of SmTPH PCR product/optical density of α-tubulin control) obtained from adult and cercaria *S. mansoni*. Results are the mean ± S.E. of three independent RT-PCR experiments, each done in duplicate. Unpaired *t* test showed that the ROD differences between the adults and cercaria are statistically significant (*p* = 0.015).

Biochemical studies of TPH have been hindered by the difficulty in obtaining large amounts of purified active enzyme that is suitable for characterization. Even with the advent of molecular biology techniques, researchers have found that recombinant mammalian TPH overexpressed in *E. coli* tends to form inactive inclusion bodies (13, 14), unless it is expressed with large fusion partners (15, 16) and also becomes unstable upon purification. In contrast to mammalian TPH, however, the parasite enzyme was expressed as a soluble protein, which could be purified and was both active and stable. The specific activity of SmTPH was 2–13-fold higher than values reported for purified forms of recombinant mammalian TPH (15–17). In addition, results presented here showed that the half-life of a crude SmTPH extract was about 23 times longer than that of a similar preparation of rabbit TPH also expressed in *E. coli*. After purification, the rabbit enzyme lost activity very rapidly, whereas SmTPH remained relatively stable, with a half-life at 37 °C of 99 min, and could be stored frozen with virtually no loss of activity. The reason for this dramatic difference in enzyme stability is unknown. Recent evidence has suggested that the notorious instability of mammalian TPH (10, 11, 14, 25, 63) may be associated, at least in part, with the enzyme’s regulatory domain (25, 60), roughly the same region that is least conserved in the parasite. This raises the interesting possibility that the stability of SmTPH is related to its distinctive N-terminal domain, which may stabilize activity more effectively than the cognate region of mammalian TPH. A stabilizing effect of the N-terminal regulatory domain on enzyme activity has been reported for the related hydroxylase, TH (25).

The finding of TPH in *S. mansoni* has clarified a long-standing question of how this parasite obtains its serotonin. Just as other parasitic worms, *S. mansoni* has high levels of serotonin within its nervous system and is well known to rely heavily upon serotonin for a wide range of essential activities, among them the regulation of motility and carbohydrate metabolism. Earlier difficulties in identifying TPH activity in tissue extracts of *S. mansoni* led to the generalized belief that the parasite lacked the enzymatic capacity to synthesize serotonin endogenously and thus relied on the human host for a supply of the neurotransmitter. By cloning an active form of TPH from the parasite and also demonstrating TPH activity in crude worm extracts, the present study has shown clearly that the enzyme is present and active in *S. mansoni*. The earlier negative results were probably due to the paucity of the enzyme in the worm combined with the low sensitivity of the assay (74), both of which would limit the ability to detect TPH activity in the crude worm extracts. It should be noted, however, that the present results do not rule out the possibility that the parasite may recruit some exogenous serotonin from the host, possibly through a tegumental carrier (32, 33, 36), in addition to synthesizing the neurotransmitter endogenously. In this respect, it is interesting that the levels of SmTPH mRNA in the adult worm, which is strictly parasitic, are nearly 2.5 times lower that in a free living larval stage (cercaria). Although the difference is small, it is nonetheless surprising in light of the greater development of the serotonergic nervous system in the adult compared with the larva. The possibility exists that the adults rely both on endogenous synthesis and exogenous intake of serotonin, whereas the free living stage, which must rely entirely on biosynthetic activity, has proportionally higher levels of TPH. The significance of these results for the development of the parasite and its survival in the host is currently under investigation.

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