Functional Domains of Human Tryptophan Hydroxylase 2 (hTPH2)

Nurgul Carkaci-Salli‡, John M. Flanagan§, Matthew K. Martz†, Ugur Salli‡, Diego J. Walther‡, Michael Bader¶, and Kent E. Vrana††

From the ‡Department of Pharmacology and §Department of Biochemistry and Molecular Biology, Penn State College of Medicine, Hershey, Pennsylvania 17033-2360, the ¶Max Planck Institute for Molecular Genetics, Department for Human Genetics, 14195 Berlin, Germany, and the ††Max Delbrück Center for Molecular Medicine, 13125 Berlin, Germany

Tryptophan hydroxylase (TPH; EC 1.14.16.4), a tetrahydropterin (BH₄)-dependent amino acid hydroxylase, is the key regulator of serotonin (5-hydroxytryptamine) biosynthesis. Utilizing BH₄ and O₂ as co-substrates and Fe²⁺ as a cofactor, TPH hydroxylates tryptophan to 5-hydroxytryptophan (Fig. 1). Subsequent decarboxylation of 5-hydroxytryptophan by amino acid decarboxylase generates serotonin (1–3). This essential monoamine has been found in a variety of tissues and implicated in a wide range of physiological functions. In the central nervous system, serotonin is synthesized primarily in the dorsal raphe nucleus and acts as a neurotransmitter; in the pineal gland, it serves as a precursor for melatonin biosynthesis (4). In the periphery, serotonin constricts large blood vessels and regulates platelet adhesion (5–7). Serotonin, produced by the enterochromaffin cells of the intestinal system, initiates peristaltic and secretory reflexes (8). Altered activity of serotonin is associated with various disorders such as depression, impulsive behaviors, aggression, suicide, drug abuse and alcoholism, sleep disorders, gastrointestinal diseases (such as irritable bowel syndrome), and cardiovascular dysfunction leading to heart failure (9).

Recently, Walther et al. (10) discovered the gene responsible for nervous system TPH (TPH2). Unlike TPH1 now known to be the peripheral enzyme (11), TPH2 is neuron-specific and expressed predominantly in serotonergic neurons of the raphe nuclei (10, 12) and in the peripheral myenteric neurons in the gut (13). Human TPH1 and TPH2 display 72% sequence homology and have high sequence identity within the COOH-terminal catalytic domain. The chromosomal location of these sister enzymes differ; human TPH1 is located on chromosome 11, while TPH2 is on chromosome 12.

Full-length human TPH1 has been expressed and purified from *Escherichia coli* and *Pichia pastoris* (14). However, TPH1 is a notoriously unstable enzyme (15), and formation of insoluble inclusion bodies has limited its purification in large quantities from *E. coli* (14, 16). While there are a number of important and informative crystal structures for the related enzymes phenylalanine hydroxylase and tyrosine hydroxylase, there is single reported structure for TPH1 from Stevens and colleagues (17). This crystal structure, hypothetical models based on other hydroxylase structures (18, 19), and NMR studies (20) provide insight into the active site of TPH1 and the respective binding sites for substrates.

The recent discovery and cloning of TPH2 offers new opportunities for understanding serotonin physiology. TPH2 has a divergent NH₂-terminal regulatory domain, which shows more homology to that of TH (16). Chimerical tyrosine/tryptophan hydroxylase constructs have suggested that the tyrosine hydroxylase regulatory domain serves to stabilize the enzyme (21); therefore, recombinant TPH2 may exhibit more stability compared with TPH1. Additionally, a number of single nucleotide polymorphisms have already been identified in the TPH2 gene that are associated with mental health disorders (22–26).
Human TPH2 Domain Structure

![Tryptophan hydroxylase catalyzes the rate-limiting step in serotonin (5-hydroxytryptamine) biosynthesis using tetrahydrobiopterin (BH4) and dioxygen co-substrates. The subsequent and final reaction in serotonin biosynthesis is catalyzed by the aromatic amino acid decarboxylase (AAADC).](image)

Therefore, it is a priority to characterize structure/function relationships within this enzyme to better understand its regulation and role in human health and disease. Indeed, a report from the Haavik laboratory describes expression of human TPH2 (27), while a recent report from Kuhn and colleagues describes the expression of recombinant mouse TPH2 (mTPH2) and the functional characterization of a naturally occurring coding region polymorphism (28).

In this study, we describe expression, purification, and characterization of recombinant hTPH2 and truncation mutants from E. coli using a novel autoinduction procedure (29).

**EXPERIMENTAL PROCEDURES**

**Bacterial Expression and Purification of hTPH2**—The bacterial expression construct for hTPH2 was constructed in two steps. First, the internal Ndel and BamHI sites were removed through the introduction of silent mutations (using the QuikChange™ site-directed mutagenesis kit; Stratagene, La Jolla, CA). To remove the internal BamHI site the following primers were used: 5′-CATGTTCCACTATTGCCTGAAGTTGGCTCA-3′ (forward) and 5′-AATCGGACAACTTAGGACCCGCAGAGTGAACATG3′ (reverse). To remove the internal Ndel site, the following primers were used: 5′-GCCACACACTGGGACAGCAGGCTACTTCCCC3′ (forward) and 5′-AAAGGAGCTCTGCTCATGTGCGGACAGTTGC-CCTTC-3′ (reverse). These changes were constructed through mutagenesis using the QuikChange side-directed mutagenesis kit. Second, an Ndel site was introduced at the start codon and a BamHI site after the TAA stop codon. (The primers were 5′-AAGGAGCTCTGCTCATGTGCGGACAGTTGC-CCTTC-3′ (forward) and 5′-AAAGGAGCTCTGCTCATGTGCGGACAGTTGC-CCTTC-3′ (reverse). These changes were constructed through mutagenesis using the QuikChange side-directed mutagenesis kit. An internal Ndel site was then introduced at the start codon and a BamHI site after the TAA stop codon. (The primers were 5′-GCCACACACTGGGACAGCAGGCTACTTCCCC3′ (forward) and 5′-AAAGGAGCTCTGCTCATGTGCGGACAGTTGC-CCTTC-3′ (reverse). These changes were constructed through mutagenesis using the QuikChange side-directed mutagenesis kit.)

The inactive construct was then expressed in BL21-CodonPlus(DE3)-RIL (E. coli) cells (Stratagene) in ZYP-5052 autoinduction medium plus 50 μM l-tryptophan. Grown at low temperatures (15 °C), these cells divide until they exhaust their glucose source and then relyably induce recombinant protein expression at very high titers (see Figs. 2 and 3) (29).

TPH2-positive colonies were grown in ZYP-5052 autoinduction medium at 15 °C in an orbital shaker at 300 rpm. The cultures were sampled every 3 h between 33 and 51 h of culture, and their optical densities were measured at 600 nm. In parallel, hTPH2 enzyme activity was monitored from aliquots removed during the time course of enzyme induction. Following induction, E. coli were harvested by low speed centrifugation. The cell pellet was lysed with BugBuster® amine-free with benzonase and rLysosome (EMD Biosciences, Inc., Madison, WI) in the presence of EDTA-free protease inhibitor mixture (Complete EDTA-free; Roche Diagnostics GmbH). The lysate was then centrifuged at high speed (40,000 × g) to obtain soluble proteins. The clarified lysate was subjected to a rapid one-step metal chelate affinity chromatography procedure using either a 5- or 1-ml packed HiTrap nickel column (Amersham Biosciences) and an Akta FPLC (Amersham Biosciences) work station at 4 °C. Columns were first equilibrated with 25 mM imidazole and His-tagged protein buffer. Recombinant protein was then eluted with a phosphate buffer containing 500 mM imidazole and 1 mM dithiothreitol. Eluted proteins were then dialyzed using a Slide-A-Lyzer dialysis cassette with a 10,000 molecular weight cut-off (Amersham Biosciences) while undergoing buffer exchange into storage buffer containing 10% glycerol at 4 °C. Attempts to cleave the hexa-His fusion peptide (26 amino acids) were unsuccessful as (a) the enzyme is highly unstable and lost activity during the digestion, and (b) the recombinant hTPH2 fusion is a poor substrate for TEV protease resulting in incomplete digestion even when digestion was carried out with 1:20 ratio of TEV to hTPH2. In these preliminary experiments, treatment of the soluble protein with TEV protease produced less than 50% cleavage. More importantly, the full-length enzyme was inactive following the incubation. Therefore, the experiments in this report were conducted with the intact fusion protein. The activity of the fusion enzyme is comparable with the wild type TPH1 (30) and TPH2 (28) enzymes (both fusion and native versions) as characterized previously.

**Enzyme Activity Assay**—TPH2 activity was assayed by using a radioenzymatic 3H2O release assay as previously described by Reinhard et al. (31) and modified by Vrana et al. (32). Activity values derived from each assay were normalized to total protein present in the homogenate, as determined by the BCA protein assay (Pierce), and were expressed as nmol/h/mg. All materials for activity assays were obtained from Sigma with the exception of activated charcoal (Darco G-60 from Fisher Scientific).

Michaelis-Menten kinetic analyses were conducted by varying substrate (tryptophan or BH4) concentrations at a fixed concentration of the other substrate (50 μM BH4 and 50 μM tryptophan, respectively) and a fixed and ambient concentration of oxygen. Resulting data were converted to specific enzyme activities (correcting for varying radioactive amino acid concentrations, as nec-
was created by inserting a stop codon (TGA) into the N

was subjected to the same procedures as described for the wild
type construct throughout the study.

Enzyme Mutations by Deletion Analysis—As described in Fig. 3, two different deletion mutations were constructed. For the NA150 mutation, a Ndel restriction sequence was inserted into the wild
type hTPH2 between amino acids 150 and 151 using the following
primers: 5′-GAAGAGAGCTAGGATCATATGGTGCCC-
TGGTTCCCTCG-3′ (forward) and 5′-GAAGGGGACCAGGG-
CACCATAGTCCTCTGCCTCTCCCT-3′ (reverse). Then
this mutated plasmid was digested with Ndel and religated (elim-
ation of the first 150 codons). The NA150/CΔ24 deletion construct was created by inserting a stop codon (TGA) into the NA150
mutation from the COOH terminus between amino acids 24 and
25 (residues 151–476). This was accomplished with the following
primers: 5′-AGTATGAAATAATGTTGATAGGTGCAGGAC-
GCGAAGGTCCTGCACCTA (forward) and 5′-CGGAAAGGTCTTGCACCAT-
TCACATTTCAATCT-3′ (reverse). Deletion mutagenesis
for each construct was carried out, in a termocycler, under the
following conditions: denaturation at 95 °C, annealing at 55 °C,
and 20 cycles using QuikChange™ site-di-

Enzyme Solubility—Aliquots of E. coli (5 ml) expressing wild
type hTPH2 and deletion mutants were incubated at 37 °C, and samples were removed
and placed on ice every 15 min from initiation to 180 min.
Enzyme activity was then determined as described above and
analyzed as a function of activity decay (on a logarithmic plot).

Analysis of hTPH2 Quaternary Structure—Purified wild type
hTPH2, NA150 and NA150/CΔ24 were subjected to size-exclu-
sion chromatography using a Superdex 200 10/300 GL column
(Amersham Biosciences) with buffer containing 25 mM HEPES,

amount of soluble hTPH2 expressed as a percentage of that present
in the homogenate.

Enzyme Stability—Purified wild type hTPH2 and deletion
mutants were incubated at 37 °C, and samples were removed and
placed on ice every 15 min from initiation to 180 min.
Enzyme activity was then determined as described above and
analyzed as a function of activity decay (on a logarithmic plot).

RESULTS

Active TPH2 Expression following Autoinduction—The
expression system in the present study takes advantage of a
recent report by Dr. W. Studier (Bookhavenn National Labora-
tories) that permits the induction of expression from pET-
based expression plasmids in an IPTG-free medium (29). We
compared autoinduction and IPTG expression systems at two
different incubation temperatures: 15 and 25 °C. In both cases,
significantly more soluble protein was obtained at 15 °C com-
pared with 25 °C. For the autoinduction system, this corre-
sponded to a 15-fold increase in active protein, and for IPTG
induction it was a 3.5-fold increase. Comparison of the relative
yields of the two induction systems at 15 °C showed that auto-
induction resulted in 11.8-fold more active hTPH2 protein
compared with IPTG induction of mid-log growth cells (data
not shown). The onset of the autoinduction of the expression
of the wild type hTPH2 construct was determined to be 33 h after
the initiation of the culture (Fig. 2). Activity increased dramat-
Human TPH2 Domain Structure

A

Wild Type

NH₂

R

C

COOH

Hexa-his tag

VPWFP

Tetramerization Domain

NΔ150

NΔ150/CΔ24

B

MW

WT

NΔ150

NΔ150/CΔ24

H

S

H

S

H

S

hTPH2 Structure

hTPH2 NΔ150

hTPH2 NΔ150/CΔ24
Based upon our growth/activity measurements, per unit of optical density of the culture declined. This is consistent with the observations reported by McKinney et al. (27). Based upon our growth/activity measurements, E. coli cultures expressing TPH2 and TPH2 variants were harvested 45 h after the initiation of the culture for the following purification and activity studies. The onset of the autoinduction was observed to coincide with the initiation of the culture for the following purification and activity studies. The onset of the autoinduction was observed to be similar in other E. coli cultures that express hTPH2 deletion constructs (data not shown).

Although the expression of active enzyme was found to be high, this system was limited in its production of large quantities of soluble hTPH2 as determined by the SDS-gel electrophoresis of the lysate (crude sample) and high speed supernatant (soluble proteins) (Fig. 3B). This is similar to what we have seen with other aromatic amino acid hydroxylases such as TH and TPH1. A number of attempts to increase solubility by harvesting cells at earlier time points or growing the cells at lower temperatures did not further improve solubility (data not shown). Based on our previous studies and published reports by others, it is our belief that the limited solubility of full-length hTPH2 is due the NH2-terminal regulatory domain of the enzyme. Therefore, the next series of the studies tested this hypothesis by deleting regions of the enzyme.

Deletion of Both Regulatory and Tetramerization Domains Results in Higher Solubility—In concordance with the notion that the NH2-terminal regulatory domain decreases solubility, deletion studies showed an increase in TPH1 solubility. The highest solubility was achieved when both regulatory and tetramerization domains were truncated. Deletion of the regulatory domain resulted in dramatically increased solubility, while deletion of the amino and carboxyl termini generated nearly completely soluble enzyme (Fig. 3B). Solubilities of the three recombinant proteins were estimated to be 6.9, 62, and 97.5% for full-length hTPH2, NΔ150, and NΔ150/CΔ24, respectively. Interestingly, deletion of just the COOH-terminal tetramerization domain did not increase the solubility or stability (data not shown).

A major advantage of the pET28-tev expression system is the ability to conduct a rapid purification following a single-step Ni2+ affinity column. Both NΔ150 and NΔ150/CΔ24 deletion products were purified with high efficiency as detected by Coomassie staining (Fig. 4, A and B). By contrast, the full-length, wild type hTPH2 purifies as a minor component (Fig. 4, A and B) that can then be enriched by size exclusion chromatography (Fig. 4C). Unfortunately, based on the instability of the wild type hTPH2 (see Fig. 5), even this relatively rapid, two-step approach produces inactive enzyme (data not shown). The enhanced purity of the NH2-terminal truncation mutants stems largely from their greater solubility and consequently they represent a higher fraction of the soluble protein in extracts compared with constructs containing the NH2-terminal domain.

Stability Analysis—Inactivation time courses were conducted for the recombinant enzymes (Fig. 5). Half-lives for inactivation at 37 °C were found to be 30, 203, and 147 min for the resulting products from nickel-column purification of hTPH2, NΔ150, and NΔ150/CΔ24. A total of 0.5 μg of purified protein was used per lane. MagicMark XP (Invitrogen) was used as a molecular weight marker. C, wild type hTPH2 was further purified on a Superdex 200 sizing column following nickel chromatography. BenchMark PreStain (Invitrogen) was used as a molecular weight marker.

**FIGURE 4.** A, SDS-PAGE gels stained with Coomassie depicting the resulting products.

**FIGURE 3.** A, the wild type hTPH2 was truncated at the amino terminus (NΔ150) or both amino and carboxyl termini (NΔ150/CΔ24) to delete the regulatory (R) or the subunit assembly (tetramerization) domains, respectively. C, catalytic domain. B, solubility of wild type hTPH2, its amino (NΔ150) and amino/carboxyl (NΔ150/CΔ24) deletion mutants expressed in ZYP5052-autoinduction medium. Upon lysis of the host bacteria, total homogenate protein (M) and high speed supernatant protein (S) samples were prepared as described under “Experimental Procedures.” The samples were then resolved on denaturing polyacrylamide gels and were Coommassie-stained. BenchMark PreStain (Invitrogen) was used as a molecular weight (MW) marker. C, the predicted structure of hTPH2 and its deletion mutation forms.
Human TPH2 Domain Structure

![Image](image_url)

**FIGURE 5.** The stability of hTPH2 and deletion mutations was measured during a 3-h incubation at 37 °C. The activity for each enzyme was measured every 15 min. Half-lives were found to be 30, 203, and 147 min for hTPH2, NΔ150, and NΔ150/CΔ24, respectively. The wild type hTPH2 activity (solid triangles) rapidly decreased. In contrast, the activity of NΔ150 (open circles) and NΔ150/CΔ24 (solid circles) remained stable.

hTPH2, NΔ150, and NΔ150/CΔ24, respectively (Fig. 5). Although not shown, the CΔ24 truncation mutant was also found to be very unstable with a half-life of less than 30 min.

**Analysis of hTPH2 Quaternary Structure**—Based on size-exclusion chromatography, both wild type hTPH2 and NΔ150 were determined to be tetramers with a size of 236 and 168 kDa, respectively, reflecting their differential subunit size (hTPH2 = 59 kDa; NΔ150 = 42 kDa). On the other hand, NΔ150/CΔ24 was determined to be monomer with a size of 39 kDa (the size of the monomeric protein; Fig. 6).

**Kinetic Analysis**—Given the caveat that the three recombinant enzymes have very different stabilities, we performed enzyme kinetic analysis to determine Michaelis-Menten constants (Table 1). To summarize, removal of the NH2-terminal regulatory domain (NΔ150) produced no significant change in either the $K_m$ for BH$_4$ or tryptophan. Ironically, deletion of both the NH2-terminal regulatory and COOH-terminal tetramerization domains (NΔ150/CΔ24) produced opposite effects in $K_m$ values. That is, the $K_m$,BH$_4$ was increased 2-fold, while the $K_m$,Trp was decreased almost 3-fold. For both deletion constructs, there were apparent increases in $V_{max}$ values that we believe reflect their increased stability and differences in purity. Finally, attempts to extend these findings to the CΔ24 deletion of just the COOH-terminal tetramerization domain produced an insoluble enzyme of such limited stability that it failed to provide reliable kinetic values (data not shown).

**DISCUSSION**

In 2003, the central nervous system tryptophan hydroxylase isoform, TPH2, was discovered (10). Up until this point, we (and others in the field) had been studying the peripheral form of the enzyme traced back to the original cloning of pineal TPH in 1987 by Woo and colleagues (33). Interestingly, during this period, there were hints that the brain TPH behaved differently and that there were difficulties reconciling apparent discrepancies in the regulation (reviewed in Ref. 11). The discovery of TPH2 opens new vistas of central nervous system research and provides tools to better understand human mental health and disease. However, characterization of the structure, activity, and regulation of hTPH2 (human tryptophan hydroxylase 2; central nervous system isoform) is a prerequisite. The present report describes the expression of human TPH2 in bacteria and its characterization.

As has been observed for TPH1 (16–18), there are several problems that arise in working with recombinant hTPH2. First, while it is expressed at very high levels in bacteria (Fig. 2), it is not well tolerated and is “packaged” into insoluble inclusion bodies. This is shown in Fig. 3B as the low level of protein in the soluble fraction for the full-length, wild type hTPH2. While this low level of soluble hTPH2 can then be enriched by nickel column chromatography, it then presents a second problem whereby it co-purifies with other proteins that are expressed in bacteria (Fig. 3B). We have already identified the source of this problem as the NH2-terminal regulatory domain. It is noteworthy that these problems of solubility are identical to those described by Haavik and colleagues (27) for recombinant fusion construct of human TPH2.

In our first efforts to characterize the recombinant hTPH2, we have conducted deletion mutagenesis to confirm our prediction of a regulatory/catalytic domain structure that is common to all of the aromatic amino acid hydroxylases (reviewed in Refs. 34–36). The design of these studies is presented in Fig. 3A. Sequence comparisons suggest that, as with the other hydroxylases, hTPH2 is composed of a COOH-terminal catalytic core and an NH2-terminal domain that presumably functions as a regulatory domain. Interestingly, all four of the hydroxylases (phenylalanine hydroxylase, tyrosine hydroxylase, TPH1, and TPH2) share a conserved pentapeptide (Val-Pro-Trp-Phe-Pro) that marks the beginning of the catalytic domain, which in the case of hTPH2, begins at residue 151. In addition, McKinney et al. (27) report that hTPH2 is phosphorylated at Ser-19 within this NH2-terminal domain. Deletion of the regulatory domain (NΔ150) has a dramatic impact on the enzyme. First, it improves expression of soluble protein (Fig. 3B). This then improves the purification of the protein (Fig. 4). In the latter case, we think this is simply a consequence of the fact that the recombinant truncation mutant represents a high percentage of the total protein and so more effectively competes for binding to the affinity column. This conclusion is reached because a second purification step can produce nearly homogenous enzyme (Fig. 4C), suggesting that there is no strong interaction between wild type hTPH2 and bacterial proteins.

A common problem with TPH1 is a marked instability (reviewed in Ref. 35). In particular, it has been known for some time that the enzyme is difficult to purify, in part, because it loses enzymatic activity during isolation. This is not the result of a proteolytic degradation; rather, the enzyme appears to undergo a loss of function related to denaturation or oxidative damage (the AAAHs all generate reactive oxygen as part of their reaction mechanisms). The same is true of hTPH2 as illustrated in Figs. 4 and 5. First, while we can purify hTPH2 to homogeneity, the enzyme is generally devoid of activity. Previous studies on rabbit TPH1 (21) suggested that the difference between TH (stable) and
TPH1 (unstable) resides in their removal of the NH$_2$-terminal regulatory domains. In the present context, we have established that removal of the regulatory domain stabilizes the protein by a factor of six. However, more detailed fine mapping strategies will be required to identify the specific residues responsible for this destabilization phenomenon.

All of the hydroxylases exist as tetramers (34, 35). Significant work has been conducted to map the interaction domains. The existence of a COOH-terminal coiled-coil (putative leucine zipper) was first postulated in 1991 (37). The existence of this interaction motif has subsequently been confirmed by a number of laboratories for all of the hydroxylases (38–41) including observations in x-ray crystallography studies of a 41 antiparallel coiled coil at the extreme carboxyl terminus of TH (30). The same observations hold true for hTPH2. That is, removal of the carboxyl terminus converts the enzyme from a tetramer to a monomer (Fig. 6). This is in contrast to TPH1 (18) where motifs within the amino-terminal regulatory domain contribute to some subunit interactions. Unfortunately, as noted above, the C/D mutant (that does not contain an amino-terminal deletion) fails to provide reliable, stable, and soluble enzyme for analysis.

Using the modeling programs Accelrys DS Modeling 1.1 and Accelrys DS ViewerPro 5.0 (Accelrys Software Inc.), SwissPdb Viewer (42), and LOOPP (43–45), we have predicted the structure of hTPH2 and its amino and amino/carboxyl-terminal deletions (N$_150$ and N$_150$/C$_24$) (Fig. 3C). The term “presumably” is advisable at this point, because deletion of the NH$_2$-terminal domain produces only modest increases in activity and alterations in kinetic constants (Table 1). The kinetic constants determined for the recombinant hTPH2 enzymes are very comparable for reported values for multiple species of TPH1 (16, 46) and TPH2.

**TABLE 1**

The steady-state kinetic parameters of wild type hTPH2 and its deletion mutations (n = 3–8)

|          | $K_{m}$,L-Trp | $V_{max}$,L-Trp | $K_{m}$,BH$_4$ | $V_{max}$,BH$_4$ |
|----------|---------------|----------------|---------------|-----------------|
| Wild type hTPH2 | 24.26 ± 5.1 | 26.35 ± 4.26 | 17.89 ± 3.0 | 21.7 ± 2.6 |
| N$_{150}$ | 20.1 ± 3.4 | 57.33 ± 6.25 | 16.88 ± 3.9 | 123.6 ± 22.3 |
| N$_{150}$/C$_{24}$ | 41.79 ± 3.12 | 242 ± 21.9 | 6.22 ± 1.4 | 157.6 ± 3.2 |
Human TPH2 Domain Structure

(27, 28). Most importantly, from a practical standpoint, deletion of the NH2-terminal domain dramatically increases the solubility of the enzyme in bacteria (see Fig. 3B) and provides an opportunity to generate sufficient amounts of material for future crystallography studies.

Acknowledgments—We thank George Jiang, Kruti Patel, Dr. Peter C. Appelbaum, Dr. Bulent Bozdogan, and Dan Kriissinger for technical assistance.

REFERENCES

1. Grahame-Smith, D. G. (1964) Biochem. Biophys. Res. Commun. 16, 586–592
2. Hakanson, R., Lombard des Gouttes, M. N., and Owman, C. (1967) Life Sci. 6, 2577–2585
3. Jequier, E., Lovenberg, W., and Sjoerdsma, A. (1967) Biochem. Biophys. Res. Commun. 242, 813–821
4. Reiter, R. J. (1991) Endocr. Rev. 12, 151–180
5. Craig, D. A., and Martin, G. R. (1993) Br. J. Pharmacol. 109, 609–611
6. Walther, D. J., Peter, J. U., Winter, S., Hofte, M., Paulmann, N., Grohmann, M., Vowinckel, J., amo-Benthencourt, V., Wilhelm, C. S., Ahnert-Hilger, G., and Bader, M. (2003) Cell 115, 851–862
7. Matsuda, M., Imaoka, T., Vomachka, A. J., Gudelsky, G. A., Hou, Z., Mistry, M., Bailey, J. P., Nieport, K. M., Walther, D. J., Bader, M., and Horsemann, N. D. (2004) Dev. Cell 6, 193–203
8. Grider, J. R., Kuemmerle, J. F., and Jin, J. G. (1996) Am. J. Physiol. 270, G778–G782
9. Jacobs, B. L., and Azmitia, E. C. (1992) Physiol. Rev. 72, 165–229
10. McKinney, J., Knappskog, P. M., Flatmark, T., Aarden, J. M., Haavik, J., and Martinez, A. (2001) Biochemistry 40, 15591–15601
11. Mockus, S. M., Kumer, S. C., and Vrana, K. E. (1997) J. Mol. Neurosci. 9, 35–48
12. Solubility of the enzyme in bacteria (see Fig. 3B) and provides an opportunity to generate sufficient amounts of material for future crystallography studies.