Three-dimensional Structure of YaaE from Bacillus subtilis, a Glutaminase Implicated in Pyridoxal-5'-phosphate Biosynthesis*

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The structure of YaaE from Bacillus subtilis was determined at 2.5-Å resolution. YaaE is a member of the triad glutamine aminotransferase family and functions in a recently identified alternate pathway for the biosynthesis of vitamin B₆. Proposed active residues include conserved Cys-79, His-170, and Glu-172. YaaE shows similarity to HisH, a glutaminase involved in histidine biosynthesis. YaaD associates with YaaE. A homology model of this protein was constructed. YaaD is predicted to be a (β/α)_8 barrel on the basis of sequence comparisons. The predicted active site includes highly conserved residues 211–216 and 233–235. Finally, a homology model of a putative YaaD-YaaE complex was prepared using the structure of HisH-F as a model. This model predicts that the ammonia molecule generated by YaaE is channeled through the center of the YaaD barrel to the putative YaaD active site.

Pyridoxal-5'-phosphate (PLP),¹ one of the B₆ vitamins, is an essential cofactor in all living systems. Its mechanistic function is the stabilization of carbanions adjacent to amino groups. This cofactor therefore plays a key role in amino acid metabolism; PLP-containing enzymes catalyze racemization, decarboxylation, transamination, and side-chain substitution reactions of amino acids. PLP is biosynthesized in bacteria, fungi, and plants, but it is an essential nutrient in animals (1).

It was clear from studies involving the incorporation of isotopically labeled PLP precursors that the PLP biosynthetic pathway in eukaryotes such as Saccharomyces cerevisiae is different from the well studied Escherichia coli pathway (Fig. 1). In E. coli, PLP is biosynthesized from α-erythrose-4-phosphate, deoxy-α-xylulose-5-phosphate, and glutamate (2), whereas in yeast, it is biosynthesized from an unidentified pentulose or pentose, glyceraldehyde, and glutamine (3–5). All of the genes required for PLP biosynthesis in E. coli have now been identified, the biosynthesis has been fully reconstituted in vitro, and the structures of three of the biosynthetic enzymes have been determined (6). In contrast, PLP biosynthesis in S. cerevisiae has not yet been reconstituted in a cell-free system, and the genes involved in the pyridine ring formation have only recently been identified (7–14). These are PDX1 and PDX2 in S. cerevisiae. Extensive phylogenetic analysis revealed that PLP biosynthesis, by what was considered previously to be the eukaryotic pathway, is widespread, and this pathway has now been found in prokaryotes, eukaryotes, and in the archaebacteria (15).

YaaD and YaaE have been identified as the PLP biosynthetic enzymes in Bacillus subtilis (16). YaaD revealed no homology with any known enzyme class. However, YaaE shows high sequence similarity to glutamine aminotransferase, suggesting that this protein is involved in the hydrolysis of the amide of glutamine, releasing ammonia for incorporation into the pyridine ring in a reaction likely catalyzed by YaaD.

We report here the crystal structure of the B. subtilis YaaE at 2.5-Å resolution. A comparison of this structure with that of other glutaminases reveals a high structural similarity to the histidine biosynthetic protein HisH from Thermotoga maritima (17). We also report a homology model of YaaD and propose a model for the YaaD-YaaE complex.

EXPERIMENTAL PROCEDURES

Molecular Cloning—Standard methods were used for DNA restriction endonuclease digestion, ligation, and transformation of DNA (18). Plasmid DNA was purified with the Wizard™ Plus SV DNA miniprep kit (Promega). DNA fragments were separated by agarose gel electrophoresis, and then excised and purified with the QiaQuick gel extraction kit (Qiagen). E. coli strain DH5α was used as a recipient for plasmid construction and for plasmid propagation and storage. A PerkinElmer Life Sciences GeneAmp PCR System 2400 and Platinum™ Pfx DNA polymerase (Invitrogen) were used for PCR. The plasmid pET-28a was obtained from Novagen. Sequencing was performed at the Cornell BioResources Center. Genomic DNA from B. subtilis CU1065 was used as a template for the PCR amplification using 5'-AGG AGC GCT GCT CAT ATG TTA ACA ATA GGT GTA-3’ (inserts an NdeI site) and 5’-CTG CTA ACG CTT CTC GAG CTT TAT TTG TGC TTA TAA TG-3’ (inserts an XhoI site) for the primer pair. The PCR product was digested with NdeI and XhoI and ligated into similarly digested pET-28a to give pCLIK1501. All PCR-derived DNA was sequenced to ensure that no mutations had been introduced in the cloning process.

Expression and Purification of YaaE—For protein purification, the expression construct was transformed into E. coli B834(DE3) cells (Novagen), which are auxotrophic for methionine. One liter of Luria-Bertani medium containing 25 µg/ml kanamycin was inoculated with 5 ml of saturated starter culture and incubated at 37 °C. When the culture reached an A₆₀₀ of 0.6, the cells were induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 6 h at 25 °C. Cells were harvested by centrifugation and resuspended in 25 ml of cold extraction buffer (10 mM imidazole, 50 mM Tris, 500 mM NaCl, pH 8.0). All subsequent protein purification steps were carried out at 4 °C. Cells were lysed by two passes through a French pressure cell at 15,000 p.s.i., and the insoluble cell debris was removed by high speed centrifugation. The clarified cell extract was mixed with a 3-ml slurry of nickel-nitrilotriacetic acid resin (Qiagen), which was pre-equilibrated in extraction buffer, and gently stirred for 1 h. The resin was centrifuged for 15 min at low speed, and the supernatant was decanted. The resin was resuspended in the ex-

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‡ These abbreviations and trivial names used are: PLP, pyridoxal-5'-phosphate; acivicin, (S)-S-(2-amino-5-chloro-4,5-dihydro-5-isoxazole-acetic acid; APS, Advanced Photon Source; CHESS, Cornell High Energy Synchrotron Source; PDB, Protein Data Bank.
traction buffer and poured onto a nickel column. The column was thoroughly washed with the extraction buffer. The His-tagged protein was then eluted with 130 mM imidazole, 50 mM Tris, 500 mM NaCl, pH 8.0. The eluted enzyme was dialyzed against 10 mM Tris, pH 8.0, and was concentrated to 5 mg/ml.

Production of selenomethionine-labeled YaaE (SeMet-YaaE) follows the same protocol as above with the following modifications. The cells were grown in 1 liter of cultures containing M9 minimal medium supplemented with 40 μg/ml l-amino acids (excluding methionine), 1% BME vitamin solution (Invitrogen), 0.4% (w/v) glucose, 2 mM MgSO4, 25 μg/ml FeSO4·7H2O, 0.1 mM CaCl2, 50 μg/liter kanamycin, and 50 μg/liter L-selenomethionine. This medium was inoculated with a 1:20 dilution of cells from a 50-ml starter culture containing the above medium but with L-methionine in place of L-selenomethionine to encourage growth. Prior to inoculation, the starter cells were pelleted and washed with the induction culture to remove the L-methionine. Purification of the labeled enzyme then proceeded as for the native protein.

Crystallization of YaaE—The optimized conditions for both the native and the selenomethionine-labeled proteins were found to be 10–12% (w/v) polyethylene glycol-8000, 100 mM MOPS, pH 7.0–7.2, 6% (v/v) ethylene glycol, and a protein concentration of 2.5 mg/ml. Crystals of SeMet-YaaE also grew in the presence of 2 mM dithiothreitol. The crystals were grown at 18 °C using the hanging-drop vapor diffusion technique. Drops (8 μl) containing a 1:1 mixture of protein and reservoir solutions were optimal for crystal growth. Crystals of diffraction quality grew over a period of 1–2 weeks. YaaE crystallizes in the orthorhombic space group P212121 with unit cell dimensions of a = 45.50 Å, b = 80.87 Å, and c = 115.39 Å. Each asymmetric unit contains two monomers corresponding to a calculated solvent content of 50%.

Data Collection and Processing—Initially, a single-wavelength anomalous diffraction data set was collected on a single frozen SeMet-YaaE crystal at the Cornell High Energy Synchrotron Source (CHESS) beamline F2. A cryoprotectant solution of 18% ethylene glycol in the mother liquor was used to prevent damage during freezing. An x-ray absorption spectrum in the vicinity of the selenium absorption edge was determined for the SeMet-YaaE crystal by recording x-ray fluorescence as a function of wavelength. Diffraction data were then collected to 2.85 Å at the wavelength corresponding to the peak of this spectrum (0.979 Å).
lowest energy model revealed no additional related structures. The numbers in parentheses are for the highest resolution shell.

\[ \text{Resolution (Å)} = 2.5 \text{ Å} \]

\[ \text{R factor (％)} = 24.01 \]

\[ \text{Rfree (％)} = 27.94 \]

\[ \text{Average B factors (Å}^2\text{)} = 1415 \]

\[ \text{Generously allowed region (％)} = 2.1 \]

\[ \text{Most favored region (％)} = 83.1 \]

\[ \text{Ramachandran plot} \]

\[ \text{Most favored region (％)} = 99.8 \]

\[ \text{Generously allowed region (％)} = 0.0 \]

\[ \text{Disallowed region (％)} = 99.9 \]

\[ \text{Completeness (％)} = 99.8 \]

\[ \text{Average B factors (Å}^2\text{)} = 9.4 \]

\[ \text{Ramachandran plot} \]

\[ \text{Most favored region (％)} = 99.9 \]

\[ \text{Generously allowed region (％)} = 87.0 \]

\[ \text{Disallowed region (％)} = 87.0 \]

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such packing constraint exists for monomer B, in which the loop containing residues 9–14 is permitted to move freely. Residues 9–14 were not visible in the initial MAD phasing map of monomer B and appeared only weakly in the later composite omit maps; residues 45–60 were visible in the initial map but were too disordered to be built until the later stages of refinement. In both monomer A and monomer B, the loop containing residues 87–96 is unconstrained and is somewhat disordered; even at the later stages of refinement, it was possible to build the main chain atoms only approximately. The majority of the residues in the generously allowed region of the Ramachandran plot are from these loops. These poorly ordered regions of

FIG. 2. A stereodiagram showing the electron density surrounding a portion of the YaaE active site. The density is from a composite omit map calculated at 2.5-Å resolution using the final model. The map is contoured at 1.5 times the root mean square value of the map. The strand containing residues Ser-168–Glu-172 lies to the right, and the turn containing Cys-79 and Ala-80 is shown at center, whereas Gly-46 and Gly-47 are at the upper left. Note the broken density for Gly-46 and Gly-47, which may form part of the oxyanion hole in the YaaD-YaaE complex.

FIG. 3. Structure of YaaE. a, stereo diagram showing the differences in conformation between monomer A (blue) and monomer B (green). Note that the ordering of residues 9–14 in monomer A causes the helix containing residues 45–60 to adopt a noticeably different position. The loop containing residues 106–115 in A makes a crystal contact with the C-terminal helix of an adjacent monomer and is not affected by the repositioning of helix 45–60. The loop containing residues 87–96 is somewhat disordered, the different conformations arising from flexibility rather than crystal contacts. b, a topology diagram showing the connections between the strands of the central mixed β-sheet and the flanking α-helices. The beginning and ending sequence numbers are shown for each secondary structural element. The orientation is the same as that for the monomer shown in panel a. c, a view of YaaE looking down the C-terminal end of the β-sheet. YaaE embodies the features of the triad aminotransferase fold (42): a twisted seven-stranded parallel β-sheet flanked by six α-helices. The active site cysteine is located on a tight turn between β4 and α5, which causes its phi, psi values to fall into the generously allowed region of the Ramachandran plot.
the structure contain nearly 15% of the residues and are probably responsible for the slightly high crystallographic R-factor reported in Table II. The remaining residues in the generously allowed region are Cys-79 from both monomers; as detailed below, these residues serve as active site nucleophiles. Finally, the loop containing residues 106–115 is pushed into a slightly different position in monomer A as a result of the C-terminal helix of an adjacent monomer of A packing against this loop; this also had the effect of making the C-terminal residues of monomer A somewhat more ordered than those of monomer B.

**Overall Structure**—YaaE is an α/β three-layer sandwich containing a seven-stranded twisted parallel β-sheet flanked by six α-helices on the N-terminal stretch of the sheet, four on one side and two on the other. The first five strands in the sheet, β1–β4, β12, and the last strand, β10, share the same orientation, whereas β11, the next-to-last strand in the sheet, lies antiparallel to the rest. This type of mixed β-sheet appears to be common to the triad aminotransferases. A pair of antiparallel β-strands is located along the exposed edge of the central β-sheet. A mixed, three-stranded region of short β-strands flanks the other side of the central sheet. The α-helices lie close to the N-terminal edge of the central sheet, whereas the flanking β-strands lie at the C-terminal edge of the central sheet. These connections are illustrated in the topology diagram shown in Fig. 3b.

The overall structure of YaaE is shown in Fig. 3c. The molecule folds as a single domain with approximate dimensions of 27 Å × 22 Å × 50 Å. YaaE adopts the form of a rough cylinder with a cleft between β4 and β12 and below a loop near the center of the upper face. At the bottom of this cleft lies a tight turn between β4 and α5, oriented in such a way that the side chain of Cys-79 protrudes into the center of the cavity. The side chain of His-170 lies 3.4 Å from the sulfur atom of Cys-79 and 2.7 Å from the carboxyl side chain of Glu-172 in an orientation suggesting a Glu-His-Cys catalytic triad. This, along with sequence and structural alignments of YaaE with known triad aminotransferases (38, 39), led us to designate this cleft as the active site.

**Active Site**—The active site crevice lies below a two-strand stretch of antiparallel β-sheet. Cys-79 has (φ, ψ) values of (51.5, −132.2); average values for active site cysteines in triad aminotransferases are (50, −110). In keeping with the active sites of other aminotransferases, His-170 and Glu-172 are separated by Pro-171. Continuing with the analogy with other triad aminotransferases, we expected an oxyanion hole to be formed by the amide NH of Ala-80 and Gly-47; however, no water molecules were found bound in this location. Comparison with the structurally similar HisH from T. maritima leads us to suspect that in the absence of YaaD, the oxyanion hole is not well formed (17). By superimposing the active sites for the two monomers, it may be observed that three bound water molecules in the active site superimpose within 1 Å of their corresponding partner; two of these pairs are located in positions indicative of catalytic significance. One pair is located within hydrogen bonding distance of the histidine ring of His-170, the second is within hydrogen bonding distance of two backbone carbonyls (168 and 134) and one backbone amide (134 N) and appears to be positioned in such a way as to serve the function of nucleophile. When the active site residues of YaaE are superimposed with the active site residues of HisH from T. maritima (17) and S. cerevisiae (40), anthranilate synthase TrpG

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**Table III**

| Protein          | Z     | r.m.s.d.* | LALI ‡ | LSEQ2 § | % IDE ¶ |
|------------------|-------|-----------|--------|--------|---------|
| HisH             | 21.7  | 2.7       | 184    | 200    | 18      |
| HisH-F           | 18.0  | 2.8       | 181    | 537    | 22      |
| CPS              | 15.8  | 3.1       | 172    | 379    | 17      |
| TrpE             | 15.7  | 2.9       | 164    | 195    | 18      |
| GMP synthase     | 15.3  | 2.7       | 162    | 501    | 20      |
| γ-glutamyl hydrolase | 13.6  | 2.8       | 167    | 288    | 19      |

* r.m.s.d., root mean square deviation.
‡ LALI, length of aligned residues.
§ LSEQ2, length of the complete sequence.
¶ % IDE, percentage of identical residues.

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**Fig. 4. The active site residues of YaaE.** a, the structure of HisH (PDB code 1JVN) with inhibitor acivicin has been superimposed to illustrate possible functions of key residues. Active site residues of yeast HisH are shown in cyan; Gln-397 from the HisF subunit is indicated in magenta. By analogy, Glu-172, His-170, and Cys-79 of YaaE form the catalytic triad, whereas Gly-47 participates in the formation of the oxyanion hole. The active site water molecule was found in all other triad aminotransferases examined, within 1 Å of the position illustrated here. b, the structure of acivicin.

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[Acivicin diagram]
from *Serratia marcescens* (41), and the aminotransferase domain of carbamoyl phosphate synthase from *E. coli* (39), analogues for this water molecule superimpose within 1 Å of each other. Furthermore, in carbamoyl phosphate synthetase and anthranilate synthase, this water molecule also lies within hydrogen bonding distance of the imidazole side chain of the catalytic histidine.

**DISCUSSION**

A comparison of the structure of YaaE with those of other known protein structures using the DALI server returned the highest scores (Table III) for free HisH from *T. maritima* (17) and the HisH subunit from the structure of HisH-F complex from *S. cerevisiae*. HisH and the remaining entries in Table III are all triad aminotransferases or triad glutaminase domains of multifunctional proteins. A least squares superimposition of YaaE with each of these other proteins reveals that they all possess the same αβ structure with loops of various lengths and conformations; this motif has been designated as the triad aminotransferase fold (42). Salient characteristics of this fold include a seven-stranded parallel β-sheet flanked by α-helices with the active site located at the C-terminal end of the sheet and below an overhanging loop. The active site cystine residue is generally near the 80th residue in the domain and lies on a nucleophilic elbow: a tight turn between a β-strand and an α-helix at the bottom of the active site. A least squares superimposition of the active sites using the catalytic triad and oxyanion hole residues reveals that the active site of YaaE is largely similar to that of the other aminotransferases; however, there are a few minor, but notable, differences. YaaE lacks a
substrate-interacting glutamine present in the other aminotransferases. Additionally, a conserved histidine, which does not interact directly with the substrate, is absent in YaaE. In its place is a proline that serves to kink a loop into a somewhat different conformation in YaaE from the other glutaminases. Finally, YaaE has three arginine residues located above the active site, which the other glutaminases appear to lack. It is presently not possible to speculate on the functional significance, if any, of these differences. One additional point of similarity exists: a water molecule, located within 3.5 Å of the cysteine sulfur atom, is present in all glutaminases examined in Table III. When the active site residues are superimposed, these water molecules superimpose within 1 Å of each other. In every case, they are located in a position consistent with nucleophilic behavior and are anchored there by at least two hydrogen bonds; in two of the five cases examined, they are close enough to the catalytic histidine to hydrogen-bond to one of the nitrogen atoms of the imidazole ring.

The active site residues of YaaE are shown in Fig. 4 superimposed upon the active site residues of yeast HisH with bound (αS,5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazololacetic acid (acivicin) (40). This inhibitor functions by covalently modifying the nucleophilic sulfur of the active site cysteine. The superposition shows conservation of the catalytic triad (Cys-79-His-170-Glu-172); however, the only other conserved residue is Gly-47, which is believed to contribute to the oxyanion hole. In HisH, the α-amino group of the substrate is positioned to hydrogen-bond to Gln-87, whereas the carboxyl group interacts with the backbone nitrogen atoms of Ser-148 and Phe-149 and also forms a hydrogen bond to Gln-397. The superposition suggests that Arg-106, which is conserved in YaaE sequences, may play a role in substrate binding. If so, the α-amino and carboxyl groups of the substrate glutamine would be interchanged by rotation about the Co-Cβ bond, keeping the terminal amide group near Cys-79. The water molecule shown is found in all triad glutaminase structures and is positioned for nucleophilic attack in the final step of product formation in which the glutamate-enzyme thioester intermediate is hydrated to glutamate and free enzyme.

As seen in Table III, the protein with the closest structural similarity to YaaE is the histidine biosynthesis protein HisH. HisH complexes with HisF to catalyze the reaction shown in Fig. 5. Secondary and tertiary structural prediction of YaaD using the SCOP (structural classification of proteins) database (43) indicated that YaaD most likely folds in a (3/10)/β(8) barrel motif. Since HisF is also a (3/10)/β(8) fold and it complexes with HisH, which had the closest structural match to YaaE, it seemed as though a modeling study would reveal useful information. The lowest energy complex model is shown in Fig. 6. In this model, YaaE binds to YaaD in the same general orientation as HisH binds to HisF: the active site of YaaE faces up into the N-terminal end of the YaaD model. The closest approach of YaaE to YaaD is where strands β7 and β8 pack against the N-terminal end of the β-barrel of YaaD. These strands contain three invariant phenylalanine residues that face in the direction of YaaD. The orientation of β8 appears to be due to an invariant Pro-137, which is also responsible for distorting one of the strands involved in the YaaE active site.
The three invariant arginine residues that line the opening to the active site of YaaE are close enough to YaaD to presumably play some role in the interaction of YaaE with YaaD.

One of the more interesting features of the HisH-F enzyme complex is the hydrophobic tunnel through the center of the β-barrel, finally delivering the ammonia to the HisF active site (17). In modeling YaaD, we looked for a corresponding channel. In the YaaD model, a number of invariant residues were located in the barrel interior and in two adjacent loops, one between residues 212 and 218 and the other between residues 234 and 236 (Fig. 7). The region between these two loops corresponds to one of the phosphate binding sites of HisF and is predicted to be a phosphate binding site in YaaD based on sequence analysis. The remaining loops consist largely of unconserved residues. A number of hydrophobic residues were located in the core of the barrel, but a number of invariant polar or charged residues and two aromatic residues are also present near the surface. Calculating the accessible surface area using SPOCK (44) does not unambiguously indicate either the presence or the absence of a channel large enough for NH₃ to pass through. During the model construction, no attempt was made to place conserved residues at the C-terminal end of the barrel; however, it is known that the interface of the barrel is very highly conserved for HisH-F (17, 40), and it would seem reasonable to expect similar conservation of the interface residues of YaaD-YaaE.

In summary, the model of YaaD reported here allows us to identify possible active site residues, whereas the model of the YaaD-YaaE complex indicates that the two enzymes can bind in an orientation favorable toward channeling of the ammonia molecule. The existence of an ammonia channel through YaaD cannot be definitely inferred from the model because of ambiguities in side chain positions. However, the structural and functional similarities of YaaD-E and HisH-F strongly suggest ammonia channeling.

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REFERENCES
1. Drewke, C., and Leistner, E. (2001) Virol. Immunol. 16, 221–225
2. Tanzya, K., Adachi, Y., Masuda, K., Yamada, K., and Kumaoka, H. (1995) Biochim. Biophys. Acta 1244, 113–116
3. Zeidler, J., Ullah, N., Gupta, R. N., Pauloski, R. B., Sayer, B. G., and Spenser, I. D. (2002) J. Am. Chem. Soc. 124, 4542–4543
4. Zeidler, J., Gupta, R. N., Sayer, B. G., and Spenser, I. D. (2003) J. Org. Chem. 68, 3486–3493
5. Sakai, A., Katayama, K., Katsuragi, T., and Tani, Y. (2001) J. Bacteriol. 183, 4876–4882