Crystal Structure of 1-Deoxy-d-xylulose 5-Phosphate Synthase, a Crucial Enzyme for Isoprenoids Biosynthesis*§

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Isoprenoids are an extensive class of extraordinarily diverse natural products and have important functions in all living organisms (1–4). Isopentenyl pyrophosphate (IPP)§ is a common precursor for the synthesis of all isoprenoids, which have important functions in living organisms. IPP is produced by the mevalonate pathway in archaea, fungi, and animals. In contrast, IPP is synthesized by a mevalonate-independent pathway in most bacteria, algae, and plant plastids. 1-Deoxy-d-xylulose 5-phosphate synthase (DXS) catalyzes the first and the rate-limiting step of the mevalonate-independent pathway and is an attractive target for the development of novel antibiotics, antimalarials, and herbicides. We report here the first structural information on DXS, from Escherichia coli and Deinococcus radiodurans, in complex with the coenzyme thiamine pyrophosphate (TPP). The structure contains three domains (I, II, and III), each of which bears homology to the equivalent domains in transketolase and the E1 subunit of pyruvate dehydrogenase. However, DXS has a novel arrangement of these domains as compared with the other enzymes, such that the active site of DXS is located at the interface of domains I and II in the same monomer, whereas that of transketolase is located at the interface of the dimer. The coenzyme TPP is mostly buried in the complex, but the C-2 atom of its thiazolium ring is exposed to a pocket that is the substrate-binding site. The structures identify residues that may have important roles in catalysis, which have been confirmed by our mutagenesis studies.

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2 The abbreviations used are: IPP, isopentenyl pyrophosphate; DXS, 1-deoxy-d-xylulose 5-phosphate synthase; TPP, thiamine pyrophosphate; GAP, glyceraldehyde 3-phosphate; TK, transketolase; PDH, pyruvate dehydrogenase.

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Isoprenoids are an extensive class of extraordinarily diverse natural products and have important functions in all living organisms (1–4). Isopentenyl pyrophosphate (IPP)§ is a common precursor for the synthesis of all isoprenoids. Although it has long been known that IPP can be generated from the mevalonate-independent pathway, recent studies have revealed a mevalonate-independent pathway for IPP biosynthesis in most bacteria, algae, and plant plastids. 1-Deoxy-d-xylulose 5-phosphate synthase (DXS) catalyzes the first and the rate-limiting reaction in the mevalonate-independent pathway, the condensation of glyceraldehyde 3-phosphate (GAP), and pyruvate (Fig. 1A) (5–8, 12–15). The 1-deoxy-d-xylulose 5-phosphate product is also used for the biosynthesis of thiamine (vitamin B₁₂) and pyridoxal (vitamin B₆) (16, 17). The dxs gene is essential for Escherichia coli, and disruption of this gene in Arabidopsis produces an albino phenotype (13, 18) because of the lack of chlorophylls and carotenoids. These studies demonstrate the crucial role of DXS in bacteria and plants. Although absent in humans, dxs and the mevalonate-independent pathway is highly expressed in cohabitating microbiota in the human intestines (19).

DXS is highly conserved in plants and bacteria (Fig. 1B). Weak sequence homology (about 20% identity) has also been identified with transketolase and pyruvate dehydrogenase E1 subunit (5–8, 18, 20). These enzymes catalyze similar biochemical reactions, and they all require the coenzyme thiamine pyrophosphate (TPP). However, DXS is distinct from these other enzymes and represents a novel class of transketolase-like proteins (5, 6). We report here the first crystal structures of DXS, from E. coli and Deinococcus radiodurans, in complex with the coenzyme TPP.

**MATERIALS AND METHODS**

Protein Expression and Purification—E. coli and D. radiodurans dxs were amplified from genomic DNA, inserted into vectors pET26b and pET28a, respectively, and overexpressed in E. coli at 20 °C. The recombinant proteins were purified by nickel-agarose affinity chromatography, anion exchange, and gel filtration chromatography. E. coli DXS, with a C-terminal His tag, was concentrated to 20 mg/ml in a buffer containing 20 mM Tris (pH 7.5), 250 mM NaCl, 10 mM dithiothreitol, and 5% (v/v) glycerol. D. radiodurans DXS, with an N-terminal His tag, was concentrated to 20 mg/ml in a buffer containing 20 mM Tris (pH 7.5), 50 mM NaCl, 10 mM dithiothreitol, and 5% (v/v) glycerol. The protein samples were frozen in liquid nitrogen and stored at −80 °C. Selenomethionine-labeled E. coli DXS was produced in E. coli B834 cells (Novagen) grown in defined LeMaster medium (21) and purified following the same protocol as that for the native enzyme.
Protein Crystallization—Crystals were obtained at room temperature with the sitting drop vapor diffusion method. Prior to crystallization, the DXS proteins were supplemented with 1 mM thiamine pyrophosphate and 5 mM MgCl₂. It was discovered that the *E. coli* enzyme crystallized only after *in situ* proteolysis by a fungal protease, and SDS gels of the crystals showed two bands (at 20 and 40 kDa). The reservoir solution, containing 20% (w/v) PEG3350 and 200 mM Na,K-tartrate, had been infected by a fungus, and this *in situ* proteolysis was also crucial in the crystallization of two other proteins in our laboratory (22, 23). The crystallization condition was optimized using this solution for the drop, and the reservoir solution contained 30% (w/v) PEG3350 and 200 mM Na,K-tartrate. The crystals were cryo-protected by 25% (v/v) ethylene glycol and flash frozen in liquid nitrogen for data collection at 100 K. They belong to space group *P*₂₁ with cell parameters of *a* = 86.8 Å, *b* = 171.2 Å, *c* = 94.8 Å, and *β* = 107.2°. There are four molecules of DXS in the asymmetric unit.

*D. radiodurans* DXS was crystallized against a reservoir solution containing 100 mM Tris (pH 7.5), 200 mM ammonium acetate, 150 mM NaCl, and 20% (w/v) PEG6000 or PEG8000. The crystals were cryo-protected by 25% (v/v) ethylene glycol and flash frozen in liquid nitrogen. They belong to space group *P*₂₁ with cell parameters of *a* = 78.3 Å, *b* = 154.1 Å, *c* = 124.9 Å, and *β* = 98.9°. There are four molecules of DXS in the asymmetric unit.

Data Collection and Processing—A selenomethionyl single-wavelength anomalous diffraction data set to 2.4 Å resolution

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**FIGURE 1.** Primary structure of DXS. A, the reaction catalyzed by DXS. The covalent intermediate between pyruvate and TPP is also shown. B, amino acid sequence alignment of DXS from *D. radiodurans*, *E. coli*, and *A. thaliana*. The secondary structure elements are indicated. The residue numbers are for the *D. radiodurans* enzyme. Conserved residues are highlighted in red, and homologous residues are in yellow. The dots below the sequences indicate residues in the active site of DXS.
Crystal Structures of DXS

TABLE 1

| Structure   | E. coli DXS | D. radiodurans DXS |
|-------------|-------------|---------------------|
| Resolution range (Å) | 30–2.4 | 30–2.9 |
| Number of observations | 608,969 | 221,373 |
| 
| Rmerge (%) | 8.1 (27.3) | 11.4 (42.0) |
| 
| completeness (%) | 93 (84) | 97 (96) |
| 
| Rfree (%) | 19.8 (21.8) | 20.9 (29.0) |
| 
| Root mean square deviation in bond lengths (Å) | 0.008 | 0.011 |
| 
| Root mean square deviation in bond angles (%) | 1.6 | 1.4 |

was collected at the X4A beamline of the National Synchrotron Light Source on a CCD detector. A native data set to 2.9 Å resolution was collected at the X4C beamline of National Synchrotron Light Source on a MarResearch image plate detector. The diffraction data were processed with the HKL package (see Table 1) (24).

**Structure Determination and Refinement**—A total of 84 selenium sites were expected for the four *E. coli* DXS molecules in the asymmetric unit. Two sites were identified by the program Solve in the first cycle (25). By feeding these two sites back to Solve, six sites were identified. Starting with these six sites, Solve was able to locate 76 selenium atoms, and Resolve could automatically trace about 80% of the asymmetric unit after phase improvement. The atomic model was completed manually using O (26), and the structure refinement was carried out with CNS (27).

The structure of *D. radiodurans* DXS was solved by the molecular replacement method with the program Molrep (28). The *E. coli* DXS structure, modified to have the same sequence as *D. radiodurans* DXS with the program Spdbv (29), was used as the search model. The refinement was carried out with Refmac (30), incorporating TLS refinements.

**DXS Assay**—DXS activity was determined using an end point assay. The pyruvate substrate remaining after the reaction was converted to lactate with lactate dehydrogenase, and the concomitant consumption of NADH was determined by fluorescence. The assays were performed in 384-well microtiter plates (Greiner), and each well contained 25 μl of substrate solution (50 μM NADH, 60 μM pyruvate, 60 μM GAP, 10 mM dithiothreitol, 5 mM MgCl₂, 600 μM TPP, 50 mM Tris (pH 7.5)), 5 μl of water, and 20 μl of enzyme in a buffer of 50 mM Tris (pH 7.5), 600 μM TPP, 5 mM MgCl₂, and 10 mM dithiothreitol. The reaction was allowed to proceed for 60 min at room temperature. Then 50 μl of 5 units/ml lactate dehydrogenase was added, and NADH fluorescence was determined 5 min later.

**RESULTS AND DISCUSSION**

**Structure Determination**—The crystal structure of *E. coli* DXS was determined at 2.4 Å resolution by the selenomethionyl single-wavelength anomalous diffraction method (Table 1) (31). We discovered that in situ proteolysis by a fungal protease was essential for the crystallization of this protein (22, 23), and two segments of the enzyme, residues 183–238 and 292–317, had no electron density. The remaining parts of the enzyme, 1–182, 239–291, and 318–620 are consistent with the 20- and 40-kDa species observed in SDS gels of the crystals (the 239–291 segment is too small to be visible in the gels), confirming that the two missing segments were removed by the fungal protease. Unfortunately, the first segment is located near the active site, and the second segment is the linker between two domains of the enzyme. Consequently, there was ambiguity about the active site and the domain organization of DXS based on this structure (see below).

To determine the structure of unmodified DXS, we screened through six DXS proteins from other bacterial sources and found that the enzyme from *D. radiodurans* could be overexpressed in *E. coli* and purified. Moreover, this enzyme could be crystallized without the need for in situ proteolysis, which allowed us to determine the structure of full-length DXS (Table 1). Clear electron density was observed for the segment linking two domains in the structure (Fig. 2).

The refined atomic models have good agreement with the observed diffraction data and the expected bond lengths and bond angles (Table 1). The majority of the residues are located in the most favored region of the Ramachandran plot. The DXS monomers in both crystals have similar conformations, with root mean square distance of 0.4 Å among their equivalent Cα atoms. Moreover, the structures of the *E. coli* and *D. radiodurans* DXS enzymes are also similar, with root mean square distances of 0.7 Å among their equivalent Cα atoms, consistent with their significant sequence conservation (46% amino acid sequence identity; Fig. 1B).

**Structure of DXS**—The structure of DXS monomer can be divided into three domains, I, II, and III (Fig. 3). All three domains have the α/β fold, with a central, mostly parallel β-sheet that is sandwiched by α-helices (supplemental Fig. S1). Domain I (residues 1–319) contains a five-stranded parallel β-sheet, and domain II (residues 320–495) contains a six-stranded parallel β-sheet. Domain III (residues 496–629) contains a five-stranded β-sheet, but the first strand is anti-parallel to the other four strands. The larger size of domain I is due to several extended surface segments (supplemental Fig. S1) at the N terminus (residues 1–49), after the first strand (residues 81–122), and in the connection between the fourth and fifth strands (residues 184–250). In fact, this connection is one of the two segments in *E. coli* DXS that were removed by proteolysis during crystallization (Fig. 3B). In the structure of *D. radio-
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**durans** DXS, residues 199–242 in this connection are also disordered (Fig. 3A). However, the beginning of this connection is ordered in this structure, and some of these residues are located in the active site (especially residues Asn\(^{183}\) and Met\(^{185}\); see below).

A tightly associated DXS dimer is revealed by the crystallographic analyses (Fig. 3), consistent with our solution light scattering studies\(^3\) as well as earlier native gel and gel filtration results (32, 33). Each monomer contributes more than 3,900 Å\(^2\) of surface area to the dimer interface, which is mostly hydrophobic in nature. The two DXS monomers are arranged side-by-side in the dimer, such that each domain of one monomer is in contact with its equivalent in the other monomer (Fig. 3A). The structure of the *E. coli* DXS dimer (Fig. 3B) is similar to that of the *D. radiodurans* DXS dimer (Fig. 3A), with root mean square distances of 1.0 Å among their equivalent C\(\alpha\) atoms.

A Novel Dimer Organization in DXS—The domains of DXS bear significant structural similarity to that of several other proteins, as identified by the program Dali (34). The closest structural homologs include transketolase (TK; Fig. 4A) (35), pyruvate dehydrogenase (PDH) E1 subunit (Fig. 4B) (36), and 2-oxoisovalerate dehydrogenase (supplemental Fig. S2) (37), with root mean square distance of about 2.5 Å for equivalent C\(\alpha\) atoms among their domains. These proteins share roughly 20% amino acid sequence identity and catalyze similar biochemical reactions (5–8).

However, the three domains in the DXS monomer are arranged differently as compared with TK and PDH. As a result, the organization of the dimer is also different. In the DXS dimer, domain I of one monomer is located directly above domains II and III of the same monomer (Fig. 3A). In contrast, domain I in one monomer is located above domains II and III of the other monomer in the dimers of TK (Fig. 4A) and PDH (Fig. 4B). An important consequence of the difference in dimer organization is that the active site in DXS is located within the same monomer, whereas that in TK and PDH is located at the interface between two monomers (see below).

One reason for the difference in domain organization may be the exceptionally long linker (95 residues) between domains I and II in TK (Fig. 4A) and PDH (Fig. 4B). In comparison, DXS contains only 20 residues in this linker (Fig. 3A), which is too short for DXS to assume the same domain organization as in TK and PDH. This linker was removed by proteolysis in the *D. radiodurans* DXS structure (Fig. 3B). However, we observed clear electron density for this linker in the *D. radiodurans* DXS structure (Fig. 2), and there is no ambiguity in the connectivity between the two domains in DXS.

Residues 199–243 in domain I of *D. radiodurans* DXS are not observed in the structure, very likely because of disorder. This missing segment is located close to the dimer interface (Fig. 3A). Therefore, the possibility of a domain-swapped connection in this segment between the two monomers, linking residue 198 in one domain with 244 in the other domain (supplemental Fig. S3), cannot be completely excluded based on the current structural information. With such a connection, the dimer organization of DXS would be similar to that in TK and PDH, although domain I would then have a novel, domain-swapped organization.

**The Active Site of DXS**—The active site of DXS is located at the interface between domains I and II of the same monomer, with no direct contribution from residues in the other monomer of the dimer (Fig. 5A). The central parallel...
β-sheets of the two domains are oriented such that their C-terminal ends are pointed toward each other, and the TPP coenzyme is located at the bottom of a pocket in this interface (Fig. 5B). Residues in the active site are highly conserved among the DXS enzymes (Fig. 1B), and many of them are also conserved in TK and PDH (supplemental Table S1). This conservation is supported by observations that the dxs-null lethal phenotype in E. coli can be rescued by a PDH mutant (E636Q; see below) (38).

The TPP molecule is bound in a V conformation (Fig. 5A) (39), and this has been observed in all other TPP-dependent enzymes (35–37). However, the active site in TK and PDH is located at the dimer interface, between domain I of one monomer and domain II of the other monomer, because of the differences in dimer organization of these enzymes (Fig. 4). The amino-pyrimidine ring of TPP interacts with domain II, whereas the pyrophosphate group interacts with domain I. The TPP molecule is mostly buried in the structure; only the thiazolium ring, especially its C-2 atom, is accessible from the solvent (Fig. 5B). This is consistent with the catalytic mechanism where the C-2 atom participates in the reaction (see below).

The pyrophosphate moiety of TPP has numerous polar interactions with the enzyme (Fig. 5A). A magnesium ion is bound between the two phosphate groups, which is also coordinated by the side chains of Asp154, Asn183, and the main chain carbonyl of Met185. These ligands form a square pyramidal arrangement around the magnesium ion, and a water molecule as the sixth ligand would complete the octahedral coordination of this cation, as observed in the structures of TK and PDH (35, 36). The Gly153-Asp-Gly155-Asn183 sequence in DXS is consistent with the TPP binding motif of GDG\textsubscript{X25–30}N (40). The phosphates are also involved in direct hydrogen bonding interactions with the enzyme, and the Lys289 side chain may help balance the extra negative charge on the terminal phosphate (Fig. 5A).

One face of the amino-pyrimidine ring of TPP has π-stacking interactions with the side chain of Phe398, and the other face has van der Waals' interactions with the side chain of Ile371, which, together with Ile187, also helps to hold the thiazolium ring in place (Fig. 5A). The three nitrogen atoms in this ring are recognized specifically by hydrogen bonds from the enzyme. Especially, the hydrogen bond between the N-1 atom and the side chain of Glu373 may help the deprotonation of the C-2 atom of the thiazolium ring for catalysis (41). The Glu373 side chain is also ion-paired with the side chain of Arg401, at a distance of 4.5 Å (Fig. 5A).

**Substrate Binding Modes**—The pyruvate substrate is expected to form a covalent, semi-stable adduct with the C-2 atom of the TPP coenzyme (supplemental Fig. S4) (41), and such a TPP adduct has been observed for TK (42). We soaked crystals of D. radiodurans DXS with pyruvate and observed electron density near the C-2 atom (data not shown). However, the electron density could not be readily interpreted based on either the enamine or the carbanion intermediate (supplemental Fig. S4). We built a model for the enamine adduct between TPP and pyruvate (Fig. 5B), based on the structure of the enamine adduct in TK (42). The adduct can be incorporated into the active site without causing any steric clashes, and no conformational changes were observed in TK either upon the formation of this adduct (42).
To obtain the binding mode of the GAP substrate, we soaked DXS with this compound at high concentrations (up to 6 mM), but so far have not been able to observe GAP in the active site. We have therefore produced a model for the binding mode of this substrate based on that of erythrose 4-phosphate in TK (43), which is supported by our mutagenesis studies (see below) and provides significant insight into the catalysis by this enzyme. GAP is located in the pocket (Fig. 5B) and could have interactions with the side chains of His51, His304, Tyr395, Arg423, Asp430, and Arg480 (Fig. 5A). The phosphate group of GAP is expected to be located near the Arg423 and Arg480 side chains, but it is also exposed to the solvent in this model (Fig. 5B). Biochemical studies showed that DXS can use glyceraldehyde itself as the substrate (6).

**Mutagenesis Studies**—Our structural analyses have identified the residues in the active site of DXS (Fig. 5A and supplemental Table S1). Some of these residues have already been shown to be important for the catalysis by DXS or the related enzymes TK and PDH. For example, His49 in E. coli DXS, equivalent to His51 in D. radiodurans DXS (Fig. 5A), is essential for catalysis (20). This residue is involved in GAP binding. It is equivalent to His30 in TK (supplemental Table S1), which has been proposed to play a role in proton transfer during that reaction (35).

The mutation in PDH that can rescue the dxs-null phenotype (38), E636Q, is also located in the active site. This residue is equivalent to Asp430 in DXS, which is located in the GAP-binding site (Fig. 5B). Therefore, it may be possible that the E636Q mutation in PDH can alter the substrate specificity of the enzyme to allow it to compensate for the dxs-null mutation, although it remains to be determined whether this mutant can really produce 1-deoxy-D-xylulose 5-phosphate.

To assess the functional roles of the other residues in the active site, we have mutated several of them and characterized their effects on the catalysis of the E. coli enzyme (Fig. 5C). The mutation sites included Glu370 (equivalent to Glu373 in D. radiodurans DXS), Tyr392 (Tyr395), Arg398 (Arg401), His431 (His434), and Arg478 (Arg480). The data show that Glu370, Arg398, and Arg478 are crucial for catalysis. In comparison, mutations of Tyr392 and His431 have minimal impact on the catalytic activity. These observations are fully consistent with our structural information. The Glu370 and Arg398 side chains interact with each other and with TPP (Fig. 5B) and could have interactions with the side chains of His51, His304, Tyr395, Arg423, Asp430, and Arg480 (Fig. 5A). The phosphate group of GAP is expected to be located near the Arg423 and Arg480 side chains, but it is also exposed to the solvent in this model (Fig. 5B). Biochemical studies showed that DXS can use glyceraldehyde itself as the substrate (6).

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