Comparative Analysis of the *Escherichia coli* Ketopantoate Hydroxymethyltransferase Crystal Structure Confirms that It Is a Member of the $(\beta\alpha)_8$ Phosphoenolpyruvate/Pyruvate Superfamily

Florian Schmitzberger,¹ Alison G. Smith,² Chris Abell,³ and Tom L. Blundell^{1*}

Department of Biochemistry, University of Cambridge, Cambridge CB2 1GA,¹ Department of Plant Sciences, Cambridge CB2 3EA,² and University Chemical Laboratory, Cambridge CB2 1EW,³ United Kingdom

Received 3 February 2003/Accepted 18 April 2003

***Escherichia coli* ketopantoate hydroxymethyltransferase (KPHMT) catalyzes the first step in the biosynthesis pathway of pantothenate (vitamin B₅), the transfer of a hydroxymethyl group onto α -ketoisovalerate. Here we describe a detailed comparative analysis of the KPHMT crystal structure and the identification of structural homologues, some of which have remarkable similarities in their active sites, modes of binding to substrates, and mechanisms. We show that KPHMT forms a family within the phosphoenolpyruvate/pyruvate superfamily. Based on the analysis, we propose that in this superfamily there should be a subdivision into two groups. This paper completes our structural analysis of the *E. coli* enzymes in the pantothenate pathway.**

Pantothenate (vitamin B₅) is a vital and central metabolic compound in all organisms. It constitutes the invariable precursor of phosphopantetheine, the moiety of coenzyme A and the acyl carrier protein. However, pantothenate is synthesized only in bacteria, fungi, and plants; animals have lost the corresponding biosynthetic pathway. Consequently, enzymes of the pathway are considered attractive antimicrobial, fungicidal, and herbicidal targets. In *Escherichia coli* the pantothenate pathway comprises four enzymes (Fig. 1) (3). Ketopantoate hydroxymethyltransferase (KPHMT; EC 2.1.2.11) catalyzes the first committed reaction, the transfer of the C₁₁ carbon of 5,10-methylene tetrahydrofolate (THF) onto α -ketoisovalerate (α -KIVA) to form ketopantoate. Subsequently, ketopantoate reductase (EC 1.1.1.169; Protein Data Bank [PDB] code 1ks9) reduces ketopantoate to pantoate, using NADPH as a cofactor. Concomitantly, in a separate branch of the pathway L-aspartate- α -decarboxylase (EC 4.1.1.11; PDB code 1aw8) converts aspartate to β -alanine, which is finally coupled to ATP-activated pantoate by pantothenate synthetase (EC 6.3.2.1; PDB code 1iho).

The crystal structure of *E. coli* KPHMT (PDB code 1m3u) was solved to 1.9-Å resolution in complex with Mg²⁺ and the product ketopantoate (30). KPHMT adopts a homodecameric quaternary structure. Based on the analysis of both the hydrophobic and polar interactions and the buried surface area between the subunits, it is best described as a pentamer of dimers. The protomeric tertiary structure of the subunits is a classical $(\beta\alpha)_8$ - or triosephosphate isomerase barrel fold with some minor but important variations (Fig. 2). The helix, usually found between β -strands 7 and 8, is here replaced by a loop, and an additional N-terminal α -helix preceding the usual first β -strand in the sequence is located at the base of the β -barrel. The subunits are arranged in an orientation such that

the vertical axis of the $(\beta\alpha)_8$ -barrel is perpendicular to the fivefold axis of the decamer. In the active site of KPHMT, magnesium is coordinated in an octahedral sphere with ketopantoate, occupying an axial and an equatorial coordination site (Fig. 2a; see also Fig. 5a).

Although KPHMT belongs to the common $(\beta\alpha)_8$ - or triosephosphate isomerase barrel fold, sequence analysis failed to detect any enzymes similar or homologous to KPHMT. However, comparative analysis of X-ray structures is a powerful tool in identifying evolutionary relationships and in gaining insight into the mechanism. Here we have used this approach to identify homologues of KPHMT and to compare their active sites. Based on this analysis, we have been able to classify KPHMT as a member of a $(\beta\alpha)_8$ superfamily, to draw conclusions about the evolutionary relationship of the enzymes within the superfamily, and to gain insight into the conservation of both substrate binding and mechanisms.

MATERIALS AND METHODS

KPHMT amino acid sequence-to-structure searches were carried out by using the homology recognition server FUGUE (27) and the fold recognition server 3D-PSSM (14). The structure of KPHMT was compared to those of all of the proteins in the PDB with DALI software (9), which carries out structure-to-structure comparisons and provides the superposition of C α coordinates. Potential homologues were then aligned with COMPARE (25), a structure-based alignment program, and the structural alignments were formatted in JOY (22). Structural superpositions for the visual inspection of the superposed three-dimensional coordinates were produced with MNYFIT (28). Catalytically important residues were identified from their conservation in multiple sequence alignments and by visual inspection of their X-ray structures. In addition, enzymes which are classified in the same Enzyme Commission group and for which structures were available were compared to KPHMT by using the methodology described above.

The SCOP database (23) superfamily classifications both of proteins considered homologues and of enzymes classified in the same Enzyme Commission group were investigated. Other members of the superfamily in which homologues of KPHMT were identified were subjected to the comparative structural analysis described above. In that way, more-distant homologues could potentially be identified and relationships within the superfamily be revealed. Particular attention was then paid to the conservation of the active-site residues common to homologues and positions in the structures of those having equivalent functions.

* Corresponding author. Mailing address: Department of Biochemistry, University of Cambridge, 80 Tennis Court Rd., Cambridge CB2 1GA, United Kingdom. Phone: 0044-1223-333628. Fax: 0044-1223-766082. E-mail: tom@cryst.bioc.cam.ac.uk.

FIG. 1. Pantothenate pathway in *E. coli*. The names of enzymes are shown in italics; substrates, products, cofactors, and their turnover are shown in roman font. 5,10-meTHF, 5,10-methylene THF.

RESULTS AND DISCUSSION

Structural homologues. A search of protein folds with the program DALI with KPHMT found similarities to numerous proteins with a $(\beta\alpha)_8$ -barrel fold motif, as expected, with the closest structural similarity being observed with phosphoenolpyruvate (PEP) mutase (PEPM; EC 5.4.2.9; PDB code 1pym) and isocitrate lyase (ICL; EC 4.1.3.1; PDB code 1igw). Both of these enzymes could also be detected as homologues by a KPHMT sequence-structure homology search using FUGUE, with PEPM scoring particularly high (Table 1), as it also did in a search with the 3D-PSSM server. In spite of a lack of significant sequence identity between KPHMT and either of the two enzymes (Table 1), structure-based sequence alignment with COMPARE revealed remarkable similarities of both the lo-

cations and the properties of important active-site residues. PEPM from *Mytilus edulis* is a homotetrameric (dimer of dimers) enzyme that catalyzes the conversion of PEP to phosphonopyruvate in the biosynthesis of phosphonates involving cleavage of an O—P bond and formation of a C—P bond (10, 15). The overall globular architecture of the $(\beta\alpha)_8$ -barrel is very similar to that in KPHMT. Both enzymes have an additional N-terminal helix of the same length prior to the $(\beta\alpha)_8$ domain that lies across the N terminus of the barrel. The major difference lies in the helix swapping of the eighth C-terminal helix in PEPM, which, together with two ensuing smaller helices, protrudes from one protomer to pack against a neighboring protomer, a phenomenon that is not observed in KPHMT. Conservation in the overall sequence between

FIG. 2. (a) Top view of the (βα)₈-barrel of KPHMT with α-helices in red, β-strands in blue, and loop and turn regions in grey. Active-site residues D45, S46, D84, K112, H136, E114, and E181 are shown in the ball-and-stick representation (atomic color scheme: carbons, grey; oxygens, red; nitrogens, blue). The octahedrally coordinated Mg²⁺ ion is shown in light blue, and the product ketopantoate is shown in green. (b) Side view of the (βα)₈-barrel of KPHMT. Panels a and b were prepared in MOLSCRIPT (16) and rendered in RASTER3D (21). (c) Schematic secondary structure representation of KPHMT. N-term., N terminus. α-Helices are shown in red, ₃₁₀-helices are in brown, β-strands are in blue, and loops are in grey. Residues bordering the respective secondary structural elements are shown in black.

KPHMT and PEPM is most obvious in α7 and in the loop connecting α7 and β6 (Fig. 3). The reason might be solely structural, since this part of the (βα)₈-barrel has no direct catalytic role and is not involved in dimer formation. Further significant sequence similarity can be observed in β3, which is important for the orientation of Asp 84. The insertion in the loop region between β4 and α5 in PEPM is thought to be involved in conformational change upon substrate binding and blocks the C-terminal region of the active site in the crystal structure. However, we could not identify a similar functional region in KPHMT. The most striking resemblance is in the coordination sphere of the active-site Mg²⁺ ion and the coordination of the ligand in the two enzymes (see Fig. 5). Ser 46, Asp 84 in β3, and Glu 114 at the C terminus of β4, all of which are completely conserved in KPHMT, have their equivalents in the invariant residues Ser 46, Asp 85 in β3, and Glu 114 at the C terminus of β4 in PEPM (Fig. 3). Superposition and inspection

TABLE 1. Comparison of characteristics of enzymes assigned to the PEP/pyruvate superfamily in SCOP^a

Enzyme	PID (%)	DALI Z-score	No. of aligned residues	RMSD (Å)	FUGUE Z-score
PEPM	13.5	17.1	198	3.2	14.47
ICL ^b	16.5	14.6	201	3.8	3.35
DDGA	11.4	12.3	173	3.0	
PK ^b	16.1	11.8	179	3.5	
PPDK ^b	10.6	9.9	178	3.7	
PEPC ^b	12.9	6.2	183	3.8	

^a All comparisons in the table are with respect to KPHMT. Sequence identities were calculated from structure-based COMPARE alignments. PID, percent identity.
^b For the comparison with ICL, the *E. coli* structure was used, and for the comparison with the multidomain proteins PEPC, PPDK, and *E. coli* PK, only their (βα)₈ domains were used.

FIG. 3. Structure-based sequence alignment of KPHMT with all the enzymes currently classified in the PEP/pyruvate superfamily. The full-length sequences of KPHMT, PEPM, and *E. coli* ICL and DDGA are shown. From the three-domain *E. coli* PK, only the ($\beta\alpha$)₈ domain comprising residues 1 to 70 and 171 to 345 was used (residues 70 to 171 have been removed; their position is indicated by a blue box). Likewise, only the ($\beta\alpha$)₈ domain of PPK, including residues 534 to 874, was used. However, in order to simplify and compress the alignment, residues 640 to 696 were removed (the deletion is indicated by a green box). For the initial alignments, the full-length PEPC protein was used. Then the following, mainly α -helical, insertions were removed: residues 1 to 112 at the N terminus, 161 to 232 between $\beta 1$ and $\beta 2$, 289 to 375 between $\beta 3$ and $\beta 4$, 412 to 464 between $\beta 4$ and $\beta 5$, and 671 to 883 at the C terminus. All deletions are indicated by a red box. The yellow and brown boxes around residues highlight the conserved catalytic residues among KPHMT, ICL, and PEPM and among DDGA, PEPC, PPK, and PK.

tion of the active site shows that these residues are in fact in the same absolute position and coordinate the ligand and Mg^{2+} in exactly the same way (Fig. 4). However, a Mg^{2+} ion that uses three water molecules as a bridge to the protein, as in PEPM, is rather unusual.

PEPM shows the closest similarity to ICL and vice versa. *E. coli* ICL and PEPM superimpose with a root mean square deviation (RMSD) of 1.51 Å for 221 structurally aligned residues, and the sequence identity between the two enzymes is 20.5%. The homotetrameric ICL is structurally the second-most similar enzyme to KPHMT. ICL catalyzes the reversible conversion of isocitrate into glyoxylate and succinate, the first step in the glyoxylate bypass pathway. The crystal structures of the *E. coli* enzyme in complex with Mg^{2+} and pyruvate (1), the *Aspergillus nidulans* enzyme in complex with Mn^{2+} and glyoxylate (PDB code 1dqu) (2), and the *Mycobacterium tuberculosis* enzyme in complex with Mg^{2+} and with two inhibitors have been solved (PDB code 1f8i) (26). For the comparison with KPHMT in this paper, we used the *E. coli* ICL structure. Sequence conserved between ICL and KPHMT overlaps largely with the residues that are conserved between KPHMT and PEPM. In *E. coli* ICL there are three α -helices before the first β -strand, of which $\alpha 3$ lies across the N-terminal part of the barrel. As in PEPM, the chain folds in a series of α -helices at the C terminus, and similarly, the $\alpha 12$ and $\alpha 13$ helices are involved in helix swapping. The conserved residues 194 to 199 in the region between $\beta 4$ and $\alpha 5$ in *E. coli* ICL are disordered in three out of four subunits. Comparison with PEPM shows that this region is spatially equivalent to the one expected to undergo conformational change in PEPM between $\beta 4$ and $\beta 5$. The active site of ICL shows almost identical features to those of PEPM, and the key active-site residues in PEPM are also invariant in ICL (Fig. 5). Similarly, the mode of ligand binding is essentially the same. The conserved Asp 157 after $\beta 3$ in *E. coli* ICL is equivalent to Asp 84 in KPHMT and Asp 85 in PEPM, as are the conserved Glu 186 after $\beta 4$, which is equivalent to Glu 114 in KPHMT and PEPM, and the conserved Ser 91, which is equivalent to Ser 46 in KPHMT and PEPM (Fig. 3). The only difference in the coordination of Mg^{2+} between PEPM and *E. coli* ICL seems to be the conservative substitution of Asp 87 to its equivalent Glu 159 in the *E. coli* ICL structure, although in *A. nidulans* and *M. tuberculosis* ICLs, this residue is also an aspartate (Asp 170 and Asp 153, respectively).

Mechanistic comparison with ICL and PEPM. Comparison of the mechanisms of both ICL and PEPM with KPHMT is not straightforward. The reaction mechanism in PEPM is not very well understood but has been proposed to involve a covalent phospho-enzyme intermediate of the phosphoryl group with Asp 58. It is proposed that during formation of this phospho-enzyme intermediate, the Mg^{2+} ion shifts towards the position of the water to the left of the Mg^{2+} ion, as shown in Fig. 5b

(10), interacting directly with the carboxylate groups of Asp 85, Asp 87, and Glu 114, a process for which no equivalent in KPHMT has been found so far. What seems to be much the same in KPHMT and PEPM is the need to stabilize an enolate intermediate. This is derived from pyruvate in PEPM (15) and from α -KIVA in KPHMT. In ICL the initial step, as in KPHMT, is a proton abstraction. As for KPHMT and PEPM, the reaction intermediate for the reverse reaction in ICL involves an enolate, which then undergoes a Claisen condensation. For *E. coli* ICL, it has been suggested that Cys 195, which is located in the loop region between $\beta 4$ and $\beta 5$, is the base for the proton abstraction. In *M. tuberculosis* ICL, a large conformational movement of this cysteine-containing loop together with the ordering of the final helix at the C terminus could be observed. Neither ICL nor PEPM used any cofactor other than Mg^{2+} in the reaction.

Comparison with the other members of the PEP/pyruvate superfamily. The SCOP database currently distinguishes 25 superfamilies of $(\beta\alpha)_8$ -barrel proteins in which the members of the superfamily have a probable common evolutionary origin. Both ICL and PEPM are assigned to the PEP/pyruvate superfamily. Other members of this superfamily include *E. coli* 2-dehydro-3-deoxy-galactarate aldolase (DDGA; EC 4.1.2.20; PDB code 1dxf), *E. coli* PEP carboxylase (PEPC; EC 4.1.1.31; PDB code 1qb4), *E. coli* pyruvate kinase (PK; EC 5.4.2.9; PDB code 1pky), and pyruvate phosphate dikinase (PPDK; EC 2.7.9.1; PDB code 1kbl) from *Clostridium symbiosum*.

Like KPHMT, the hexameric (trimer of dimers) DDGA is a class 2 aldolase with a single-domain $(\beta\alpha)_8$ -fold (11). It catalyzes the reversible aldol cleavage of 2-dehydro-3-deoxy-galactarate into pyruvate and semialdehyde and in the catabolic pathway for the utilization of D-glucarate/galactarate. The enzyme has a low substrate specificity, condensing a wide range of aldehydes with pyruvate. A DALI search with KPHMT listed DDGA (PDB code 1dxe) only as the 31st hit. However, PEPM is structurally the second-most similar enzyme to DDGA within the PEP/pyruvate superfamily. The two enzymes superimpose with an RMSD of 3.1 Å for 174 structurally aligned residues, whereas sequence identity between the two enzymes is not significant (percent identity, 12.8%). DDGA shows some features in common with KPHMT, such as the N-terminal α -helix. Analogous to findings for ICL and PEPM, helix swapping of the eighth α -helix can be observed. The active-site Mg^{2+} ion is directly complexed by Glu 153 in the loop after $\beta 5$ and by Asp 179 in the α -helix after $\beta 6$ (Fig. 5). The cocrystallized pyruvate occupies two Mg^{2+} binding sites and hydrogen bonds to Ser 178. Two water molecules complete the coordination sphere, one of which hydrogen bonds to Glu 49, which aligns with Asp 45 from KPHMT (Fig. 3). Thus, the overall Mg^{2+} and ligand coordination bears some resemblance to that of KPHMT, but with inverted symmetry. The absolute position of the metal ion has moved from between $\beta 3$ and $\beta 4$ to a

respectively. The alignment is numbered according to the KPHMT sequence, with every 10th residue shown. Secondary structural elements are also denoted according to those of KPHMT. The key to the JOY annotation is as follows: lowercase red letter, α -helix; lowercase blue letter, β -strand; lowercase maroon letter, 3_{10} -helix; uppercase letter, solvent-inaccessible residue; lowercase letter, solvent-accessible residue; italic lowercase letter, positive ϕ ; breve, *cis*-peptide; lowercase letter with tilde, hydrogen bond to other side chain; bold lowercase letter, hydrogen bond to main-chain amide; underlined lowercase letter, hydrogen bond to main-chain carbonyl; c with cedilla (ç), disulfide bond. A consensus for the secondary structure elements is shown at the bottom.

FIG. 4. (a) Globular structural superposition of KPHMT (blue), *E. coli* ICL (grey), and PEPM (red). Shown are the secondary structural elements (helices as cylinders) around the first four β -strands comprising residues 1 to 130 of KPHMT, 43 to 224 in ICL (for clarity, the first 42 residues in ICL are omitted), and 1 to 150 in PEPM. Additionally, the catalytically important residues S46, D85, and E114 in KPHMT that correspond to S46, D85, and E114 in PEPM and S91, D157, and E186 in *E. coli* ICL are shown in ball-and-stick representation. (b) Globular structural superposition of DDGA (steel blue) and the $(\beta\alpha)_8$ domain of PEPC (magenta), PPDK (green), and PK (brown). Shown are the secondary structural elements around β -strands 3 to 6 comprising residues 70 to 208 in DDGA, 391 to 571 in PEPC, 613 to 775 in PPDK, and 61 to 271 in PK. For clarity, residues 398 to 412 in PEPC were omitted. Likewise, residues 618 to 700 in PPDK are not shown. The catalytically important residues E153 and D179 in DDGA, E745 and D769 in PPDK, E506 and D543 in PEPC, and E223 and D246 in *E. coli* PK (corresponding to D271 and E295 in rabbit muscle PK; PDB code 1a49) are shown in ball-and-stick representation. Both panels were prepared in MOLSCRIPT (16) and rendered in RASTER3D (21).

FIG. 5. Schematic active-site representations of KPHMT and all the members of the PEP/pyruvate superfamily. All are viewed with the vertical coordination of metal ion in line with the vertical axis of the $(\beta\alpha)_8$ -barrel. (a) KPHMT; (b) PEPM; (c) ICL; (d) DDGA; (e) rabbit muscle PK (PDB code 1a49); (f) PPK; (g) PEPC. *E. coli* PEPC has been solved in complex with Mn^{2+} ; however, no ligand-bound structure of PEPC is available yet.

position between $\beta 5$ and $\beta 6$ (Fig. 4). However, not only the absolute position but also the positions of the Asp and Glu residues relative to each other have changed. The possibility of a circular permutation was investigated; however, no evidence for such an event could be found. A circular permutation in which β -strands are joined and the barrel is opened at another place would not explain why the relative positions of Asp and Glu have been exchanged.

The first step in the reaction catalyzed by DDGA involves the enolization of pyruvate in an aldol reaction chemically very similar to the one in KPHMT. However, no base close enough for the proton abstraction could be identified in the active site of the enzyme. Instead, a phosphate, which was observed in the active site in the ligand-free structure, is thought to accept the proton of the pyruvate's methyl group (11).

PEPC, PK, and PPDK are structurally not very similar to KPHMT. However, investigation of the structural similarity relationships between the first three and DDGA shows that the closest structural similarity is between DDGA and *E. coli* PK (PDB code 1pky). PK is the structurally most similar enzyme in both PEPC (PDB code 1qb4) and PPDK (PDB code 1kc7), and DDGA is the next most similar. The homotetrameric PK, a three-domain protein with a $(\beta\alpha)_8$ core domain, catalyzes the conversion of PEP to pyruvate coupled to the synthesis of ATP (17, 20). The homotetrameric PEPC catalyzes the irreversible carboxylation of PEP to form oxalacetate and inorganic phosphate (12, 19). PPDK catalyzes the interconversion of ATP, phosphate, and pyruvate into AMP, pyrophosphate, and PEP (7, 8), respectively. Structure-based sequence alignment of the $(\beta\alpha)_8$ domain and superpositions of the active sites show that the Mg^{2+} -coordinating residues in PEPC, PK, and PPDK are in identical positions and align with those in DDGA (Fig. 5). The mode of binding of the α -ketoacid moiety of the cocrystallized ligands to Mg^{2+} is essentially the same in DDGA, PK, and PPDK. PEPC, PPDK, and PK, together with PEP, also share the ligand PEP as their substrate or product. Whereas PK uses one ADP molecule and PPDK uses one ATP molecule in the course of the reaction, PPDK does not have any second substrate.

Comparison with other class 2 aldolases and THF binding enzymes. Apart from 2-dehydro-2-deoxy-galactarate aldolase, structures of four class 2 aldolases, all from *E. coli*, are available. These include fructose-1,6-bisphosphate aldolase (6, 24) and tagatose-1,6-bisphosphate aldolase (5), both adopting a $(\beta\alpha)_8$ -fold as well as L-fucose-1-phosphate aldolase (4) and L-ribulose-5-phosphate 4-epimerase (18). As seen in the SCOP database, the latter two enzymes do not adopt the $(\beta\alpha)_8$ -fold and belong to the class 2 family in the class 2 superfamily within the α and β (α/β) fold. The dimeric fructose-1,6-bisphosphate aldolase and the tetrameric tagatose-1,6-bisphosphate aldolase are assigned to the class 2 aldolase family in the aldolase superfamily in SCOP. In each of these structures, the catalytic Zn^{2+} metal ion has an environment different from that of the magnesium coordination site found in KPHMT. However, although all of these class 2 aldolases involve the same chemical mechanism as KPHMT, comparison of their active sites, overall three-dimensional structures, and functions with the respective properties of KPHMT did not show significant analogies. None of the class 2 aldolases use a second substrate that is even remotely similar to 5,10-methylene THF. As the class 2 aldo-

lases appear to be spread out across folds and superfamilies, membership in this functional group is not a particularly helpful criterion in understanding structure and function in KPHMT. The identity of the second substrate or electrophilic donor was also not a basis for classification, as folate binding is not a trait of any existing $(\beta\alpha)_8$ superfamily.

Conclusions. Comparative analysis of $(\beta\alpha)_8$ structures requires careful attention to detail in the structure, as the overall folds can be very similar, even though there is no significant sequence identity among $(\beta\alpha)_8$ enzymes. Comparison of the structure of KPHMT with the structures of PEP and ICL has shown considerable similarity in the overall folds and, more importantly, convincing similarity in their active sites. The two Mg^{2+} -coordinating carboxylate-containing residues, together with a Ser residue that interacts with the ligand, are completely conserved throughout the three enzymes. Additionally, an enolate is an intermediate in each of the reactions catalyzed by these enzymes. On these grounds, we propose that KPHMT should be assigned to the PEP/pyruvate superfamily and should form a family of its own within this superfamily. However, DDGA, PEPC, PPDK, and PK, which are all in the PEP/pyruvate superfamily, show a different active-site architecture, which appears as a distorted mirror image to the one found in KPHMT. A circular permutation cannot explain such a change, and we could not determine the evolutionary step behind such an alteration. Based on the latter observation, we propose that there should be a subdivision within the PEP/pyruvate superfamily into two groups, one comprising KPHMT, PEP, and ICL and the other comprising DDGA, PEPC, PPDK, and PK. However, at this stage we cannot make any inferences from this for the mechanism in KPHMT. That KPHMT is a member of the PEP/pyruvate superfamily is yet another example of the catalytic proficiency of the enzymes of this particular superfamily and of the functional plasticity of the $(\beta\alpha)_8$ -fold in general. Though functionally distinct and mechanistically diverse, members of the PEP/pyruvate superfamily seem to exploit the same structural strategy, namely, the use of a catalytic Mg^{2+} ion in a very specific way, i.e., as an electron sink, and to position and orient the substrate, which always resembles an α -ketoacid moiety. However, apart from KPHMT, only PK and PPDK have a second substrate.

The enzymes in the PEP/pyruvate superfamily appear to have evolved divergently while conserving modes of binding to substrates and producing similar reaction intermediates but not maintaining a similarity in their mechanisms, like proton abstraction. Also, selection for a specific cofactor does not seem to have played any role. The analysis thus stands in contradiction to recent studies (29) that supported the theory that chemistry and cofactor binding are more likely to be conserved during evolution than substrate binding. However, it is not possible to determine whether any enzyme might have evolved from another one within the PEP/pyruvate superfamily. There might have been a common ancestor which no longer exists. Given that pantothenate is likely to have existed in the prebiotic soup (13) and that the pantothenate pathway is part of primary metabolism, it seems reasonable to argue that KPHMT must be one of the oldest members of this superfamily. However, further insight into the reaction of KPHMT will require structural information about the cofactor-enzyme complex, which can then provide a basis for rational drug design.

ACKNOWLEDGMENTS

We gratefully acknowledge funding by BBSRC for structural studies of the pantothenate biosynthesis enzymes. F.S. is the recipient of a Cambridge European Trust scholarship and a research studentship from the University of Vienna.

REFERENCES

- Britton, K. L., S. Langridge, P. J. Baker, K. Weeradechapon, S. E. Sedelnikova, J. R. De Lucas, D. W. Rice, and G. Turner. 2000. The crystal structure and active site location of isocitrate lyase from the fungus *Aspergillus nidulans*. *Structure* **8**:349–362.
- Britton, K. L., I. S. Abeyasinghe, P. J. Baker, V. Barynin, P. Diehl, S. J. Langridge, B. A. McFadden, S. E. Sedelnikova, T. J. Stillman, K. Weeradechapon, and D. W. Rice. 2001. The structure and domain organization of *Escherichia coli* isocitrate lyase. *Acta Crystallogr. Sect. D* **57**:1209–1218.
- Brown, G. M., and J. M. Williamson. 1982. Biosynthesis of riboflavin, folic acid, thiamine, and pantothenic acid. *Adv. Enzymol. Relat. Areas Mol. Biol.* **53**:345–381.
- Dreyer, M. K., and G. E. Schulz. 1996. Catalytic mechanism of the metal-dependent fucose aldolase from *Escherichia coli* as derived from the structure. *J. Mol. Biol.* **259**:458–466.
- Hall, D. R., C. S. Bond, G. A. Leonard, C. I. Watt, A. Berry, and W. N. Hunter. 2002. Structure of tagatose-1,6-bisphosphate aldolase. Insight into chiral discrimination, mechanism, and specificity of class II aldolases. *J. Biol. Chem.* **277**:22018–22024.
- Hall, D. R., G. A. Leonard, C. D. Reed, C. I. Watt, A. Berry, and W. N. Hunter. 1999. The crystal structure of *Escherichia coli* class II fructose-1,6-bisphosphate aldolase in complex with phosphoglycolohydroxamate reveals details of mechanism and specificity. *J. Mol. Biol.* **287**:383–394.
- Herzberg, O., C. C. Chen, G. Kapadia, M. McGuire, L. J. Carroll, S. J. Noh, and D. Dunaway-Mariano. 1996. Swiveling-domain mechanism for enzymatic phosphotransfer between remote reaction sites. *Proc. Natl. Acad. Sci. USA* **93**:2652–2657.
- Herzberg, O., C. C. Chen, S. Liu, A. Tempczyk, A. Howard, M. Wei, D. Ye, and D. Dunaway-Mariano. 2002. Pyruvate site of pyruvate phosphate dikinase: crystal structure of the enzyme-phosphonopyruvate complex, and mutant analysis. *Biochemistry* **41**:780–787.
- Holm, L., and C. Sander. 1993. Protein structure comparison by alignment of distance matrices. *J. Mol. Biol.* **233**:123–138.
- Huang, K., Z. Li, Y. Jia, D. Dunaway-Mariano, and O. Herzberg. 1999. Helix swapping between two α/β barrels: crystal structure of phosphoenolpyruvate mutase with bound Mg^{2+} -oxalate. *Struct. Fold. Des.* **7**:539–548.
- Izard, T., and N. C. Blackwell. 2000. Crystal structures of the metal-dependent 2-dehydro-3-deoxy-galactarate aldolase suggest a novel reaction mechanism. *EMBO J.* **19**:3849–3856.
- Kai, Y., H. Matsumura, T. Inoue, K. Terada, Y. Nagara, T. Yoshinaga, A. Kihara, K. Tsumura, and K. Izui. 1999. Three-dimensional structure of phosphoenolpyruvate carboxylase: a proposed mechanism for allosteric inhibition. *Proc. Natl. Acad. Sci. USA* **96**:823–828.
- Keefe, A. D., G. L. Newton, and S. L. Miller. 1995. A possible prebiotic synthesis of pantotheine, a precursor to coenzyme A. *Nature* **373**:683–685.
- Kelley, L. A., R. M. MacCallum, and M. J. Sternberg. 2000. Enhanced genome annotation using structural profiles in the program 3D-PSSM. *J. Mol. Biol.* **299**:499–520.
- Kim, J., and D. Dunaway-Mariano. 1996. Phosphoenolpyruvate mutase catalysis of phosphoryl transfer in phosphoenolpyruvate: kinetics and mechanism of phosphorus-carbon bond formation. *Biochemistry* **35**:4628–4635.
- Kraulis, P. J. 1991. MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallogr.* **24**:946–950.
- Larsen, T. M., M. M. Benning, I. Rayment, and G. H. Reed. 1998. Structure of the bis(Mg^{2+})-ATP-oxalate complex of the rabbit muscle pyruvate kinase at 2.1 Å resolution: ATP binding over a barrel. *Biochemistry* **37**:6247–6255.
- Luo, Y., J. Samuel, S. C. Mosimann, J. E. Lee, M. E. Tanner, and N. C. Strynadka. 2001. The structure of L-ribulose-5-phosphate 4-epimerase: an aldolase-like platform for epimerization. *Biochemistry* **40**:14763–14771.
- Matsumura, H., M. Terada, S. Shirakata, T. Inoue, T. Yoshinaga, K. Izui, and Y. Kai. 1999. Plausible phosphoenolpyruvate binding site revealed by 2.6 Å structure of Mn^{2+} -bound phosphoenolpyruvate carboxylase from *Escherichia coli*. *FEBS Lett.* **458**:93–96.
- Mattevi, A., G. Valentini, M. Rizzi, M. L. Speranza, M. Bolognesi, and A. Coda. 1995. Crystal structure of *Escherichia coli* pyruvate kinase type I: molecular basis of the allosteric transition. *Structure* **3**:729–741.
- Merritt, E. A., and D. J. Bacon. 1997. Raster3D: photorealistic molecular graphics. *Methods Enzymol.* **277**:505–524.
- Mizuguchi, K., C. M. Deane, T. L. Blundell, M. S. Johnson, and J. P. Overington. 1998. JOY: protein sequence-structure representation and analysis. *Bioinformatics* **14**:617–623.
- Murzin, A. G., S. E. Brenner, T. Hubbard, and C. Chothia. 1995. SCOP: a structural classification of proteins database for the investigation of sequences and structures. *J. Mol. Biol.* **247**:536–540.
- Plater, A. R., S. M. Zgiby, G. J. Thomson, S. Qamar, C. W. Wharton, and A. Berry. 1999. Conserved residues in the mechanism of the *E. coli* class II FBP-aldolase. *J. Mol. Biol.* **285**:843–855.
- Sali, A., J. P. Overington, M. S. Johnson, and T. L. Blundell. 1990. From comparisons of protein sequences and structures to protein modelling and design. *Trends Biochem. Sci.* **15**:235–240.
- Sharma, V., S. Sharma, K. Hoener zu Bentrup, J. D. McKinney, D. G. Russell, W. R. Jacobs, Jr., and J. C. Sacchettini. 2000. Structure of isocitrate lyase, a persistence factor of *Mycobacterium tuberculosis*. *Nat. Struct. Biol.* **7**:663–668.
- Shi, J., T. L. Blundell, and K. Mizuguchi. 2001. FUGUE: sequence-structure homology recognition using environment-specific substitution tables and structure-dependent gap penalties. *J. Mol. Biol.* **310**:243–257.
- Sutcliffe, M. J., I. Haneeff, D. Carney, and T. L. Blundell. 1987. Knowledge based modelling of homologous proteins, part I: three-dimensional frameworks derived from the simultaneous superposition of multiple structures. *Protein Eng.* **1**:377–384.
- Teichmann, S. A., S. C. Rison, J. M. Thornton, M. Riley, J. Gough, and C. Chothia. 2001. The evolution and structural anatomy of the small molecule metabolic pathways in *Escherichia coli*. *J. Mol. Biol.* **311**:693–708.
- von Delft, F., T. Inoue, S. A. Saldanha, H. O. Ottenhof, F. Schmitzberger, L. M. Birch, V. Dhanaraj, M. Witty, A. G. Smith, T. L. Blundell, and C. Abell. *Structure*, in press.