X-ray Structures of the Three Lactococcus lactis Dihydroxyacetone Kinase Subunits and of a Transient Intersubunit Complex*  

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The atomic coordinates and structure factors (codes 3CT6, 3CR3, and 3CT4) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org). The on-line version of this article (available at http://www.jbc.org) contains supplemental text, Tables S1–S3, Figs. S1–S3, and references.

Bacterial dihydroxyacetone (Dha) kinases do not exchange the ADP for ATP but utilize a subunit of the phosphoenolpyruvate carbohydrate phosphotransferase system for in situ rephosphorylation of a permanently bound ADP-cofactor. Here we report the 2.1-Å crystal structure of the transient complex between the phosphotransferase subunit DhaM of the phosphotransferase system and the nucleotide binding subunit DhaL of the Dha kinase of Lactococcus lactis, the 1.1-Å structure of the free DhaM dimer, and the 2.5-Å structure of the Dha-binding DhaK subunit. Conserved salt bridges and an edge-to-plane stacking contact between two tyrosines serve to orient DhaL relative to the DhaM dimer. The distance between the imidazole Ne2 of the DhaM His-10 and the β-phosphate oxygen of ADP, between which the γ-phosphate is transferred, is 4.9 Å. An invariant arginine, which is essential for activity, is appropriately positioned to stabilize the γ-phosphate in the transition state. The (Bβα)4 fold of DhaM occurs a second time as a subfold in the DhaK subunit. By docking DhaL-ADP to this subfold, the nucleotide bound to DhaL and the C1-hydroxyl of Dha bound to DhaK are positioned for in-line transfer of phosphate.

Dihydroxyacetone (Dha)4 is the structurally simplest among all metabolized carbohydrates. It is formed as an intermediate of methanol assimilation by methylotrophic yeast (1) and as the end product of dissimilative glycerol oxidation in the respiritory chain of Gluconobacter (2). Dha is taken up and metabolized by bacteria and eukaryotes. The first step of Dha metabolism is either a reduction to glycerol followed by glycerol phosphorylation or, alternatively, direct phosphorylation to the glycolytic intermediate Dha-P. There are two kinases that phosphorylate Dha, glycerol kinase and Dha kinase. Glycerol kinase does not discriminate between glycerol and Dha. In contrast, Dha kinases are specific for Dha, α-glyceraldehyde, and possibly other short-chain aldehydes and ketones. The chemical basis for this specificity is the formation of a covalent hemiaminal bond between the carbonyl carbon of Dha and the Ne2 of the active-site histidine (for a review see Ref. 3).

Dha kinases can be grouped in two structurally homologous but functionally different families. The first are the two-domain Dha kinases (DAK) occurring in eukaryotes and some bacteria. They consist of a Dha binding (K) and an ATP binding (L) domain (4). Characteristically for kinases, ADP is exchanged by ATP after each catalytic cycle.

DAK

\[ \text{ATP} \rightarrow \text{Dha} \]

**REACTION 1**

The second family comprises Dha kinases that occur only in eu-bacteria and a few archaebacteria. These kinases consist of three subunits (5). Two of them, DhaK and DhaL, are homologous to the K and L domains. Intriguingly, the ADP moiety is not exchanged for ATP (6) but remains permanently bound to the DhaL subunit where it is rephosphorylated in situ by the third subunit, DhaM. These Dha kinases function according to Reaction 2.

\[ \text{PEP} \rightarrow \text{EII} \rightarrow \text{HPr} \rightarrow \text{DhaM} \rightarrow \text{DhaL-ADP} \rightarrow \text{Dha} \]

**REACTION 2**

Phosphate is transferred sequentially from PEP via Enzyme I to HPr, hence to DhaM, the ADP cofactor of DhaL, and finally to Dha bound to the DhaK subunit. All subunits except for DhaK are transiently phosphorylated at a histidine and DhaL on the ADP cofactor. In some bacteria, DhaM is a multidomain protein consisting of a domain predicted to belong to the mannose family (EIIMan) of the PTS (5) plus additional domains homologous to HPr and Enzyme I of the

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4 The abbreviations used are: Dha, dihydroxyacetone; PEP, phosphoenolpyruvate; PTS, PEP-dependent carbohydrate phosphotransferase system; DhaK, Dha binding subunit of the Dha kinase; DhaQ, Dha binding activator of the L. lactis transcription activator DhaS; DhaL, nucleotide binding subunit; DhaM, phosphotransferase subunit (Dha kinase-specific phosphotransfer protein of the PTS); EDD, EIIA domain, dihydroxyacetone kinase, DegV; r.m.s.d., root mean square deviation.
Dihydroxyacetone Kinase

PTS. In summary, this second family of Dha kinases that are exclusively bacterial have switched from ATP to PEP as a source of high energy phosphate.

Besides covalent substrate binding by DhaK (7) and containing ADP as cofactor, bacterial Dha kinases display another noteworthy property. The DhaK and DhaL subunits or paralogues thereof function as coactivators of dha operon transcription. For instance, in Escherichia coli, DhaL-ADP (but not DhaL-ATP), and DhaK without bound Dha act antagonistically as coactivators and corepressor, respectively, of the transcription factor DhaR (8), an enhancer-binding protein (9) consisting of a receiver, an AAA + (ATPases associated with various cellular activities), and a DNA binding domain. In Lactococcus lactis, DhaQ, a paralogue of DhaK, acts as coactivator of the transcription factor DhaS, and Dha acts as inducer by binding to DhaQ. DhaS belongs to the tetracycline repressor family but exceptionally functions as activator of the dha operon (10).

Here we present three x-ray structures of the DhaM subunit alone (1.1-Å resolution), of the transient complex between the DhaM and DhaL subunits (2.1-Å resolution) and of the DhaK subunit (2.5-Å resolution).3 We notice that the characteristic fold of DhaM, the prototype of the so-called EDD fold (for EIIA domain, dihydroxyacetone kinase, and DegV (11, 12)) also appears again as a core fold of the much larger DhaK subunit of the Dha kinase.

Using the EDD fold for modeling showed that the contact interfaces between DhaM and DhaL on one hand and between DhaL and DhaK on the other are by and large identical. The same scaffolds control the phosphate transfer from DhaM phosphohistidine to DhaL-ADP, and from DhaL-ATP to the hydroxyl group of Dha.

EXPERIMENTAL PROCEDURES

Cloning, Mutagenesis, Protein Expression, and Purification—The open reading frames encoding the L. lactis Dha phosphotransferase proteins were amplified by PCR and cloned into multicopy vectors encoding N- and C-terminal hexahistidine (H6) tags. Proteins were expressed in E. coli K12 strains and purified by metal chelate and gel-filtration chromatography. N-terminal tags were removed with thrombin. Selenomethionine-labeled proteins were produced using the pathway inhibition method (13). Site-directed mutagenesis was done using the QuickChange protocol (Stratagene). The details are given in the supplemental materials.

Crystallization, Structure Determination, and Refinement—Crystrals of DhaMH6, DhaKH6 and the DhaM-DhaL complex were obtained by sitting drop vapor diffusion at 16 °C. 2 μl of DhaMH6 (15 mg/ml) was mixed with 2 μl of reservoir solution containing 200 mM NaNO3, 20% polyethylene glycol 3350 acidified with HCl to pH 2.8. 2 μl of DhaKHex (7 mg/ml) was mixed with 2 μl of buffer containing 50 mM Tris/HCl, 8.5, 200 mM NH4Cl, 10 mM CaCl2, 30% polyethylene glycol 4000 in a 1:1 ratio. For cryoprotection, the crystals were soaked in 10 μl of crystallization condition plus 18% glycerol. 2 μl of DhaM and DhaL (15 mg/ml of each) was mixed with 2.5 μl of buffer containing 0.1 M Tris/HCl, 8.5, 0.2 M LiSO4, 36% polyethylene glycol 4000. For cryoprotection, the crystals were soaked for 5 min at 16 °C in 10 μl of crystallization condition (0.1 M Tris/HCl, 8.5, 0.2 M LiSO4, 36% polyethylene glycol 4000) supplemented with 5% 2-methyl-2,4-pentanediol and for 3 min at 16 °C in 10 μl of crystallization condition plus 10% 2-methyl-2,4-pentanediol. Crystals were frozen in liquid nitrogen, and the datasets were collected at the X12 beamline (Deutsche Elektronen-Synchrotron, European Molecular Biology Laboratory, Hamburg) employing a MAR Mosaic225 detector (Mar Research, Hamburg, Germany). The details of data processing and structure solution are given in the supplemental materials.

Dha Kinase Activity Assays—The Dha phosphotransferase assay was performed with the ion exchange procedure as described previously (14). [14C]Dha binding was assayed by aceton precipitation of the complex as described previously (15). The details are given in the supplemental materials.

RESULTS

Overall Structure of DhaM—DhaM is a dimer of identical subunits (termed A and B). The DhaM subunit assumes a (βα)nα fold consisting of a four-stranded parallel β sheet (strand order 2, 1, 3, 4) flanked by three and two helices (1, 4, 5 and 2, 3) on either side (Fig. 1A).

The intersubunit contact surface buries 1981 Å2 (15% of the monomer surface). The major contacts are between the two C-terminal helical turns of α1A and α1B and between the C-terminal ends of helices α4A and α4B (Fig. 1A). The interface is composed of 14 apolar residues, 4 hydrogen bonds, and 2 salt bridges. The carboxylate of Gru-13B forms a hydrogen bond to Ser-16B and a salt bridge with Lys-20B. The Glu-94A carboxylate forms a hydrogen bond to the backbone amide of Leu-92B and a salt bridge with Lys-123B (Fig. 1D). The His tag lies in the groove between the two subunits. It expands the interface by 17% and covers the active site. Therefore, the tagged DhaM was catalytically inactive (results not shown) and refractory to cocrystallization with DhaL (see below). The His tag is fixed by two hydrogen bonds between the backbone amide of the His-127B and the OH of Ser-69B and between the backbone carbonyl of His-126B to Tyr-97A. The latter also plays a prominent role at the DhaM-DhaL interface as it will be shown below. The active site conformations of the His-tagged DhaM and DhaM in complex with DhaL (see below) are indistinguishable indicating that inactivation of DhaM is caused by blocking of access to and not distortion of the active site. Furthermore, the His tag is responsible for 36% of the crystal contact interface in the b/c-plane of the protein crystal (supplemental Fig. S3).

The transphosphorylation reaction catalyzed by DhaM proceeds through a phosphohistidine intermediate. The active site His-10 is located in the solvent-exposed β1α1 loop in a groove between the two monomers (Fig. 1D). Adjacent to His-10B is a hydrophobic patch formed by residues Ile-42B, Gly-43B, Leu-67B, Ala-70B, and Leu-21A. The N81 amide of the imidazole ring facing the protein interior is in hydrogen-bonding distance to the completely buried and not charge-compensated Asp-66 in the loop between β3 and α3 (Fig. 1D). The His-Asp pair is

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5 The amino acid sequence and X-ray structures of the proteins can be accessed through the Swiss Protein Database and the RCSB Protein DataBank codes: DhaM, Q9CIV6 and 3CT6; DhaL, Q9CIV7 (DhaM-DhaL complex 3CR3); and DhaK, Q9CIV8 and 3CT4.
invariant in all homologues of DhaM. By acting as a general base the carboxylate group of Asp-66 increases the nucleophilicity of the His-10 imidazole ring. N/H9280 of His-10 is located close to the N-terminal end of helix /H9251, and the charge of the phosphoryl group at N/H9280 would thus be partially compensated by the helix macro dipole. Charge-dipole stabilization (16) is often found in phosphate binding sites.

Comparison of the x-ray structure of DhaM with known folds using the DALI-server (17) gave significant scores for EIIA\textsuperscript{Man} domain of the E. coli mannose transporter (PDB code 1pdo, The C termini are highlighted as transparent surfaces. C, DhaK monomer with Dha bound. The EDD fold is rainbow colored with the N terminus in blue and the C terminus in red, the N-terminal /alpha/ beta extension and the C-terminal subdomain are colored in magenta and cyan, respectively. Dha in the hemiaminal linkage to the active-site His-220 (black stick) is highlighted as gray transparent spheres. Note, that numbers in A–C refer to /beta/-strands. D, details of the DhaM-dimer interface with the active site and the His tag. The subunits are colored differently; subunit A in blue and subunit B in orange. Two clusters of ionic interactions are shown as sticks, and the hydrophobic patch around the active site His-10 is a gray surface. The active-site His-10\alpha-Asp-66\beta dyad is covered by the His tag. The hydrogen bonding interaction between Tyr-97\alpha and Ser-69\beta (yellow sticks), and the His tag is shown as a red dotted line. E, DhaK dimer with Dha bound. The EDD fold is rainbow colored, and the N- and C-terminal subdomains are colored in magenta and cyan, respectively. The second chain is colored in green. The colored subunit is generated by a 45° and a 90° rotation of C. Images were prepared with PyMOL (W. L. DeLano (2002) PyMOL, DeLano Scientific, San Carlos, CA).

FIGURE 1. Structures of the L. lactis Dha kinase subunits DhaM and DhaK. A, DhaM: the secondary structure elements ( /beta/-strands. D, details of the DhaM-dimer interface with the active site and the His tag. The subunits are colored differently; subunit A in blue and subunit B in orange. Two clusters of ionic interactions are shown as sticks, and the hydrophobic patch around the active site His-10 is a gray surface. The active-site His-10\alpha-Asp-66\beta dyad is covered by the His tag. The hydrogen bonding interaction between Tyr-97\alpha and Ser-69\beta (yellow sticks), and the His tag is shown as a red dotted line. E, DhaK dimer with Dha bound. The EDD fold is rainbow colored, and the N- and C-terminal subdomains are colored in magenta and cyan, respectively. The second chain is colored in green. The colored subunit is generated by a 45° and a 90° rotation of C. Images were prepared with PyMOL (W. L. DeLano (2002) PyMOL, DeLano Scientific, San Carlos, CA).
Overall Structure of DhaK-Dha Complex—DhaK is a homodimer and crystallized in space group P3121 with three DhaK monomers (A, B, and C) in the asymmetric unit. By applying the symmetry operations to the asymmetric unit the physiological dimers AC and BB of each monomer (A, B, and C) are generated. Refinement of the native protein structure against 2.5-Å data resulted in an R-factor of 20.4% and an R<sub>free</sub> of 26.7%, with reasonable stereochemistry. Only residues 200–209 are disordered. This segment is without electron densities in all known DhaK structures (7, 12) with the exception of the L. lactis DhaQ where the loop becomes ordered in the course of a conformational change triggered by Dha binding (10). As observed before with DhaK of E. coli (7), DhaK of L. lactis contains a molecule of dihydroxyacetone (Dha) covalently bound in hemiaminal linkage to the Ne2 nitrogen of His-220.

At the core of the DhaK structure is an EDD fold (residues Val-48 to Asn-185) similar to DhaM (Fig. 1, C and E, rainbow color). A comparison of the two folds calculated by DaliLite (19) resulted in a Z-score of 11.1 and a r.m.s.d. of 2.2 Å for 108 Cα atoms. The core, however, accounts for only 40% of the total mass of DhaK. Attached to the N terminus is an aβαβ motif (residues 1–47), which enlarges the β sheet of the EDD fold by two strands and provides one of the two dimerization helices. Attached to the C-terminal α-hairpin is a two-layered αβ fold (residues 186–331). The inner layer, a six-stranded mixed β-sheet, covers the EDD fold. An α helix of the outer layer provides the second dimerization helix.

The overall structure of L. lactis DhaK is very similar to the orthologous DhaK of E. coli (PDB entry 1OI2 (7)) and the paralogous transcription coactivator DhaQ of L. lactis (PDB entry 2IU4 (10)). The sequence identity for both proteins is 45%, and the r.m.s.d. values are 1.1 Å for 305 Cα atoms with a Z-score of 47.1, and 1.6 Å for 310 Cα atoms with a Z-score of 46.2, respectively, as determined with Dali (17). The main difference is the two-stranded β-hairpin of E. coli DhaK. Inserted in the two-layered αβ fold it covers the hydrophobic edge strand of the EDD fold and possibly prevents edge-to-edge aggregation (7). In DhaK of L. lactis aggregation may be prevented by a different mechanism, namely electrostatic repulsion (21), between charged residues protruding from the edge, for instance Glu-130 in DhaK of L. lactis or a lysine, arginine, and aspartate in other homologues.

Structure of the Transient DhaM-DhaL Complex—Two molecules of DhaL are complexed to one DhaM dimer (Fig. 2A). The structure of the DhaM subunit has been described above, that of the homologous E. coli DhaL subunit before (12, 22). In summary, DhaL is an eight-helix barrel of regular up-down topology and slightly conical shape. The ADP cofactor is coordinated by two Mg<sup>2+</sup> ions, which in turn are anchored to the protein scaffold by three aspartates in the loop connecting helices 1 and 2 (Fig. 2B).

The orientation of DhaM and DhaL in the complex is conducive to phosphoryl transfer between His-10 of DhaM and ADP of DhaL. The distance between the imidazole Ne2 and the β-phosphate oxygen is 4.9 Å (Fig. 2B), allowing enough space for a γ-phosphate that is missing in the model (the complex is formed of non-phosphorylated DhaM and DhaL complexed with ADP). This distance is only 30% more than the experimentally determined distance of the pentacoordinate phosphate observed in phosphoglucomutase near the transition state (23). Additional evidence for the physiological state of the complex is Arg-161 of DhaL. Reaching from the lid (loop between helices 7 and 8) into the empty space, Arg-161 is appropriately positioned to stabilize the putative trigonal bipyramidal transition state of the γ-phosphate. This Arg is invariant in all DhaL sequences and is part of the strongly conserved “signature sequence” GRAsYXGERSiG.

The total buried surface area is 1953 Å<sup>2</sup> per DhaM-DhaL binding site of which 566 Å<sup>2</sup> and 383 Å<sup>2</sup> are from DhaM subunit A (contributing the active site His-10) and B, and 1003 Å<sup>2</sup> from DhaL. About 50% of the total interaction surface is contributed by hydrophobic residues located at the center of the interface around the phosphotransfer site, His-10 of DhaMA and the ADP cofactor of DhaL. Salt bridges are formed between Asp-75<sup>B</sup> and Glu-79<sup>A</sup> of DhaM and Lys-113, Arg-114, and Lys-116 of DhaL (Fig. 2B). A prominent hydrogen bond is formed between the hydroxyl group of Tyr-97<sup>B</sup> of DhaM and Arg-161 of DhaL, the Arg that is being implicated in transition state stabilization of the γ-phosphate. Tyr-97<sup>B</sup> of DhaM in addition forms a T-shaped aromatic-aromatic interaction with Tyr-164 of DhaL (24) (Fig. 2B). A comparison with homologous DhaM and DhaL sequences reveals strong conservation of the acidic residues 75 and 79 of DhaM and the basic residues 113, 114, and 116 of DhaL, suggesting that these interactions may play an important role in orienting the subunits. The tyrosines also are strongly conserved in DhaM and invariant in the signature sequence GRAsYXGERSiG of DhaL.

DhaL of L. lactis and E. coli (PDB code 2BTD) display 35% sequence identity, a Z-score of 30.1, and an r.m.s.d. of 1.5 Å for 209 Cα atoms (17). The only obvious difference between the two DhaLs is the glycosidic bond rotational isomer of the adenine, anti in L. lactis and syn in E. coli (Fig. 2D). The reason for this difference is not clear. There is one hydrogen bond between the purine N6 and the Gly-119 carbonyl, which is invariant in both conformations and therefore unlikely to determine it. However, a water molecule bridging the purine ring and the protein backbone could tip the balance toward the anti conformation (Fig. 2D). It has been reported that about one-third of the purine nucleoside analogues in the Cambridge data bank are syn isomers, indicating that the free energy difference between the two conformations is quite small (26).
proportional to the product of the DhaM and DhaL concentrations suggesting that the rate of phosphoryl transfer is diffusion-controlled. The $K_d$ of the complex is unknown, but was found to be 30 μM in a comparable “complex” between HPr and EIIAMan (27).

To evaluate the importance of the ionic and aromatic-aromatic interactions for Dha kinase function, the activities of the wild-type and mutant DhaM/DhaL 1:1 complexes were assayed in the presence of E. coli DhaK (supplemental Fig. S1A). Wild-type DhaM and DhaL were used as the positive control, a com-

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**TABLE 1**

Dha kinase activity of DhaM:DhaL with mutations at the subunit contact interface

$k_{cat}$ values are given in min$^{-1}$, in parenthesis % of wild-type activity is shown. Given are the standard errors of the linear regression through the values in the linear concentration/activity range of the curves of supplemental Fig. S1.

| DhaM     | DhaL | R114A     | R114E     | Y164A    | R161A     |
|----------|------|-----------|-----------|----------|-----------|
| DhaM     |      |           |           |          |           |
| wt       |      | 21.7 ± 1 | 6.1 ± 1.7 (28) | 0.2 ± 0.03 (1) | 0.8 ± 0.09 (4) | 0.0 ± 0.005 (0) |
| E79A      |      | 6.6 ± 2  | 6.4 ± 1.2 (29) | 0.2 ± 0.03 (1) | 0.1 ± 0.05 (0)  |
| E79R      |      | 5.5 ± 1  |           |          |           |
| Y97A      |      | 0.0 ± 0.03 (0) | |          |           |
The combination of wild-type DhaM with DhaL R161A as the negative one. The results are summarized in Table 1. The missing activity of the DhaL R161A mutant confirms that this invariant residue indeed is functionally important and might stabilize the transition state of the ATP γ-phosphate. The disruption of the ionic interaction between DhaM Glu-79 and DhaL Arg-114 by exchange of one or both charged residues with an alanine reduced the activity 3-fold. Inverting putative Coulomb attraction into repulsion has a stronger effect. DhaM E79R paired with wild-type DhaL has <25% of control activity, and wild-type DhaM paired with DhaL R114E is inactive. The T-shaped aromatic-aromatic interaction between DhaM Tyr-97 and DhaL Tyr-164 appears to be important, too. The DhaM Y97A mutant is inactive and the DhaL Y164A mutant retains only 4% activity. A possible explanation for this difference is that Tyr-97 of DhaM strikes an intersubunit hydrogen bond to the invariant active site Arg-161 of DhaL, whereas Tyr-164 is partly surface-exposed and probably less constrained.

DISCUSSION

The structure of the DhaM subunit of the PTS-dependent dihydroxyacetone kinase is the first experimental model of a prototypical EDD fold. Here we have characterized three embodiments of this fold: the free DhaM homodimer, DhaM in a transient complex with the DhaL subunit of the Dha kinase, and the EDD fold as the core of the larger DhaK subunit of the Dha kinase.

Only DhaM with a C-terminal His tag afforded well diffracting crystals, whereas only the untagged form of DhaM was active and could be co-crystallized with DhaL. The conformation of the His tag in the crystal suggests that it not only forms intrasubunit hydrogen bonds, the latter form the catalytic dyad. As the point of reference ADP is shown while the polypeptide chain of DhaL is omitted.

Equivalent salt bridges could be formed between helix 3 of DhaM (blue) and helix 5 of DhaL (orange) and between helix 3 from the EDD fold of DhaK (orange) and helix 5 of DhaL. The distances are 4.5 Å between Arg-114 (DhaL) and Glu-120 (DhaK), and 3.7 Å between Lys-116 (DhaL) and Glu-116 (DhaK).
other hand this conformation would interfere with the alignment of the phosphoryl donor and acceptor active sites in the DhaM-DhaL complex. This complex is transient and cannot be isolated by gel filtration. Nevertheless, the conformation of the subunits in the crystalline complex represents a physiological relevant interaction as evidenced by the close proximity of the phosphoryl donor and acceptor sites (Fig. 2, A and B). The structures of free and DhaL-complexed DhaM are almost identical with an r.m.s.d. = 0.65 Å. Only two small changes occur upon complex formation (Fig. 2C): (i) Tyr-164 of DhaL pulls Tyr-97 of DhaM from 4.6 Å to 3.8 Å edge to plane stacking distance. This brings the Tyr-97 η oxygen into hydrogen bonding distance (3.05 Å) of the Arg-161 η nitrogen, the residue that might assist the transfer of the γ-phosphate between DhaM and DhaL. (ii) The α3 helix of DhaM undergoes a 3.10 to α transition that might be induced by the three salt bridges between this helix and helix α5 of DhaL.

The DhaM-DhaL complex provides a model of how DhaL interacts with DhaM and by extrapolation also with the EDD module of DhaK. It is important to notice that one DhaL subunit makes contacts to both subunits of the DhaM dimer, of which the more extensive one is to the subunit bearing the active site histidine. A model of the putative DhaL-DhaK complex was produced as follows: The two EDD folds, namely DhaM residues 3–123 of the DhaM-DhaL complex and residues 48–185 of DhaK were structurally aligned as rigid bodies using the DALI alignment tool. The 48–185 of DhaK were structurally aligned as rigid bodies using the DALI alignment tool. The DhaM residues 3–123 of the DhaM module of DhaK. It is important to notice that one DhaL subunit makes contacts to both subunits of the DhaM dimer, of which the more extensive one is to the subunit bearing the active site histidine. A model of the putative DhaL-DhaK complex was produced as follows: The two EDD folds, namely DhaM residues 3–123 of the DhaM-DhaL complex and residues 48–185 of DhaK were structurally aligned as rigid bodies using the DALI alignment tool. The 48–185 of DhaK were structurally aligned as rigid bodies using the DALI alignment tool. The Z-score of the alignment is 11.1 and the r.m.s.d. 2.2 Å for 108 Cα atoms (Fig. 3, A–E). This alignment brings into perfect register Nε2 of the His-10 of DhaM and the primary hydroxyl group of Dha in the active site of DhaK (Fig. 3C), the two sites, which donate and accept, respectively, the phosphoryl group of DhaL. The electrostatic interactions that steer the contact between DhaM and DhaL in the experimental structure are also present in the predicted model of the DhaL-DhaK complex, with Glu-116 and Glu-120 of DhaK corresponding to Asp-75 and Glu-79 of DhaM (Fig. 3D). Only one region of DhaL, which in the experimental model binds to the DhaM subunit with the smaller interaction surface, in the docking model clashes with a loop of DhaK. This loop of DhaK is involved in non-equivalent crystal contacts in the asymmetric unit, thereby assuming different conformations (Fig. 3E). It appears to be intrinsically flexible and becomes fixed only in contact with a binding partner, either another DhaK subunit in the crystal or a DhaL subunit in solution. The DhaL-DhaK docking model is further corroborated by a comparison with the experimental structure of the C. freundii Dha kinase (Fig. 3A). This ATP-dependent Dha kinase is a homodimer of two subunits consisting of two domains (K and L), which are connected by a twenty-residue linker. The domains are swapped such that the N-terminal K-domain of one subunit is complexed with the C-terminal L-domain of the other (12). The K-L contact interface in both structures is formed by the same helices of the DhaL barrel. Only the surface potentials are different, of opposite charge in the DhaL-DhaK complex, and apolar in the Dha kinase of C. freundii (22), as expected of soluble and only transiently associating subunits on one hand and of domains that remain permanently associated, on the other.

Several complexes between subunits of the PTS, which have proved refractory to crystallization, could be characterized by multidimensional NMR in the laboratory of M. Clore: Enzyme I (N-terminal domain)–HPr complex (28), HPr-EIIAGlc (29), EIIAGlc–EIIBGlc (30), HPr–EIIAMan (31), and HPr–EIIAMan complex (27) (for a review see Ref. 32). Of particular interest in the context of the DhaM-DhaL complex is the NMR structure of the complex between the EIIAMan domain of the mannose transporter and HPr (27). EIIAMan not only features the same EDD fold as DhaM (Figs. 1A, 1B, 3A, and 3B), but it also assumes the same position in the phosphotransfer cascade of the PTS. Both proteins share HPr as phosphoryl donor and then relay the phosphate to the EIIBMan domain (20, 25) and DhaL-ADP, respectively.

\[
\begin{align*}
\text{HPr} & \leftrightarrow \text{DhaM} \leftrightarrow \text{DhaL-ADP} \\
\text{HPr} & \leftrightarrow \text{EIIAMan} \leftrightarrow \text{EIIBMan}
\end{align*}
\]

DhaL-ADP and HPr have different folds. A comparison of the DhaL-DhaM (dimer) with the HPr–EIIAMan (dimer) interface suggests that the same goal, namely bringing the phosphoryl donor and acceptor residues in close proximity is achieved by different topological interactions; e.g., the buried surface of the DhaL and HPr bodies and their orientation relative to the EDD folds are completely different. Whereas HPr makes a minor contact to the EIIAMan subunit bearing the active site His-10, it is opposite for DhaL-ADP that makes its major contact to the DhaM subunit presenting the active site (Fig. 3B). On the other hand, shapes and potentials of the interactive surfaces are similar, concave, and negatively charged for DhaM and EIIAMan, convex and positively charged for HPr and DhaL. The gap between Nδ1 of HPr His-15 and Nε2 of EIIAMan His10 is 5.3 Å, the one between Nε2 of DhaM His-10, and the β-phosphate oxygen of ADP is 4.9 Å.

In conclusion, using the EDD fold (11, 12) as common structural element, the experimental x-ray structure of the transient DhaM-DhaL complex could be used to model the complex between DhaL and the Dha-DhaK holoenzyme, as evidenced by the near coincidence of the phosphate binding residues Nε2 of His-10 and C1-OH of Dha (Fig. 3D). On the basis of these structures, it appears that phosphoryl transfer from DhaM to DhaL-ADP, and from DhaL-ADP to Dha-DhaK, can proceed with minimal conformational changes of the donor and acceptor proteins.

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