The Conformational Change and Active Site Structure of Tetrahydrodipicolinate *N*-Succinyltransferase[†,‡]

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ABSTRACT: Tetrahydrodipicolinate (THDP) *N*-succinyltransferase catalyzes the conversion of tetrahydrodipicolinate and succinyl-CoA to L-2-(succinylamino)-6-oxopimelate and CoA. This reaction represents the committed step of the succinylase branch of the diaminopimelate/L-lysine biosynthetic pathway by which many bacteria synthesize *meso*-diaminopimelate, a component of peptidoglycan, and L-lysine from L-aspartate. The crystal structures of THDP succinyltransferase in complex with the substrate/cofactor pairs L-2-aminopimelate/coenzyme A and L-2-amino-6-oxopimelate/coenzyme A have been determined and refined to 2.0 Å resolution. The active site of the enzyme is a long narrow groove located at the interface between two left-handed parallel β-helix (LβH) structural domains of the trimeric enzyme. On binding the amino acid acceptor and cofactor, this groove is covered by residues from the C-terminus of one subunit and a flexible loop excluded from the LβH domain of an adjacent subunit to form a tunnel. This conformational change is directly related to interactions between the enzyme and the bound amino acid substrate and cofactor and serves to shield the ligands from bulk solvent and to orient the nucleophilic amino group of the amino acid acceptor toward the mercaptoethylamine group of the cofactor.

The conversion of tetrahydrodipicolinate (THDP)[1] and succinyl-CoA to L-2-(succinylamino)-6-oxopimelate and CoA is the committed step of the succinylase branch of the DAP/lysine biosynthetic pathway used by Gram-negative bacteria, Gram-positive cocci, blue-green algae, and higher plants to synthesize L-lysine from L-aspartate (*1*). The importance of the essential amino acid L-lysine and the intermediate *meso*-DAP, a component of peptidoglycan in bacteria, as well as the absence of this pathway in mammals, has drawn attention to the bacterial DAP/lysine pathway and THDP succinyltransferase (EC 2.3.1.117) in particular as targets for inhibitor design. Peptides containing L2AP exhibit antibacterial activity against a wide range of Gram-negative organisms by inhibiting DAP biosynthesis (*2*). A chemical mechanism for the reaction catalyzed by THDP succinyltransferase has been proposed on the basis of a study of a series of cyclic and acyclic analogues of THDP (*3*). Features of the proposed mechanism include enzymatic hydration of the cyclic imine form of THDP followed by succinylation and ring opening to yield the acyclic succinylated product (Scheme 1).

The crystal structure of THDP succinyltransferase in the absence of bound ligands has been previously determined to 2.2 Å resolution and has confirmed this enzyme's membership in the "hexapeptide acyltransferase" superfamily of enzymes (*4*). Enzymes of this family share amino acid sequence similarity in tandem repeated copies of an imperfect hexapeptide sequence, characterized as [LIV]-[GAED]-X_2-[STAV]-X (*5*). Most enzymes of this family are acyltransferases that utilize a phosphopantetheine-based substrate donor, most often as an acyl-CoA. The "hexapeptide repeat" sequence has been shown to encode a left-handed parallel β helix (LβH) in the three-dimensional structure of *Escherichia coli* UDP-*N*-acetylglucosamine acyltransferase (*6*). Residues from this domain were found to bind two inhibitors of *E. coli* THDP succinyltransferase, *p*-(chloromercuri)benzenesulfonic acid and cobalt ion, suggesting that this domain serves a catalytic function (*4*).

To date, the precise active site location and conformation of substrates bound to THDP succinyltransferase have not been reported. We describe here the crystal structures of two ternary complexes of the enzyme formed with L2AP/CoA and L2A6OP/CoA. These structures define the active site location and conformation of the bound amino acid and cofactor and also reveal a substantial conformational change that accompanies binding.

EXPERIMENTAL PROCEDURES

Purification and Crystallization. THDP succinyltransferase was purified as previously described and crystallized by the hanging drop vapor diffusion method from solutions of 10−13% (w/v) poly(ethylene glycol) 4000, 94 mM MES, pH 6.4, 94 mM ammonium sulfate, and 4.7% (v/v) 2-propanol in the presence of 16 mM (D,L)-2-aminopimelate and

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[‡] The coordinates corresponding to the tetrahydrodipicolinate *N*-succinyltransferase ternary complexes have been deposited in the Brookhaven Protein Data Bank (identification codes 2TDT and 3TDT).

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[1] Abbreviations: DAP, diaminopimelate; THDP, 2,3,4,5-tetrahydrodipicolinate; CoA, coenzyme A; LβH, left-handed parallel β helix; MES, 2-(*N*-morpholino)ethanesulfonic acid; Tris, tris(hydroxymethyl)-aminomethane; L2AP, L-2-aminopimelate; L2A6OP, L-2-amino-6-oxopimelate (IUPAC nomenclature, 2-oxo-L-6-aminopimelate); PCMPS, *p*-(chloromercuri)benzenesulfonic acid; RMS, root mean square; σ, root-mean-square units.

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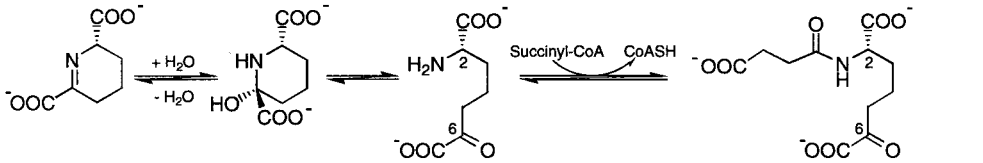
Scheme 1



Tetrahydrodipicolinate (THDP) L-2-amino-6-oxopimelate (L2A6OP) L-2-(succinylamino)-6-oxopimelate

Table 1: Data Measurement and Structure Refinement Statistics[a]

	L2AP/CoA	L2A6OP/CoA
resolution (Å)	2.0	2.0
completeness (%)	98.1 (92.6)	97.1 (87.2)
redundancy	2.7 (1.3)	3.0 (1.5)
R_{merge} (%)	4.7 (21.5)	4.5 (10.3)
protein atoms	2075	2078
substrate atoms	60	61
solvent atoms	85	91
RMS deviation from ideality		
bond lengths (Å)	0.023	0.019
bond angles (deg)	1.94	1.71
average thermal factors (Å^2)		
protein atoms	28.4	23.5
substrate atoms	28.9	36.8
solvent atoms	39.7	34.7
R_{free} (%)	24.6 (31)	22.5 (27)
R-factor (%)	17.1 (26)	15.4 (21)

[a] Data measurement and refinement statistics for THDP succinyltransferase/substrate ternary complexes. R_{merge} (%) $= \Sigma|I_i - \langle I\rangle|/\Sigma|I_i| \times 100$. R_{free} (%) $= \Sigma|F_o - F_c|/\Sigma|F_o| \times 100$ for a 5% subset of X-ray diffraction data omitted from refinement calculations. *R*-factor (%) $= \Sigma|F_o - F_c|/\Sigma|F_o| \times 100$ for all available data (99−2.0 Å resolution). Values in parentheses refer to data in the highest resolution bin.

2.5 mM CoA (*7*). Crystals produced from these experiments appeared as large rhombohedra measuring approximately 0.6 × 0.5 × 0.5 mm and yield X-ray reflections to 2.0 Å resolution. These crystals belong to the rhombohedral space group *R*3 with unit cell parameters $a = 95.7$ Å and $c = 72.8$ Å (hexagonal setting) and contain one subunit of the trimeric enzyme in the asymmetric unit. In an attempt to determine the binding site location and conformation of the natural substrate, THDP was prepared synthetically according to a previously described procedure (*8*), and intact crystals were soaked for 18 h in a solution containing 16% (w/v) poly(ethylene glycol) 4000, 100 mM Tris, pH 8.0, 200 mM ammonium sulfate, 5% (w/v) 2-propanol, 0.1 mM dithiothreitol, 5 mM THDP, and 20 mM CoA. These crystals were nearly isomorphous with those produced from the original crystallization and diffracted X-rays to a similar resolution.

Data Measurement and Molecular Replacement. X-ray diffraction data to 2.0 Å resolution was measured from one crystal for each of the two ternary complexes using a Siemens X1000 area detector mounted on a Rigaku RU2000 rotating anode generator operating in fine focus at 50 kV and 80 mA. The data collection strategy was designed by the program SWEEPS (S. L. Roderick, unpublished program) and reduced with XDS (*9*) (Table 1).

The structure of the L2AP/CoA ternary complex crystal was solved by molecular replacement with the X-PLOR program package (*10*) and the known apoenzyme structure of THDP succinyltransferase (*4*) (PDB identification code 1TDT). The search model consisted of all atoms corresponding to a single subunit of the trimeric apoenzyme (residues 1−256) and was used to calculate a cross-rotation function with 15−4 Å resolution data. The top-ranked solution (6.6σ) was used to orient the search model for input to a translation function calculation using 10−4 Å data. The best translation solution from this calculation (10.4σ) was used to define the location of the oriented subunit in the *xy* plane. The crystalline packing arrangement corresponding to this oriented and translated model was inspected on molecular graphics and revealed an association of subunits surrounding the crystallographic 3-fold axis that produced the familiar trimeric structure of the apoenzyme and an acceptable array of intermolecular contacts.

Model Building and Atomic Parameter Refinement. To properly assess atomic parameter refinements, approximately 5% of the X-ray diffraction data were assigned to a test set and omitted from refinement procedures in order to calculate the ordinary working set residual R_{work} and the cross-validation statistic R_{free} (*11*, *12*). Rigid body refinement was initially carried out using 10−4 Å data with the model divided into four rigid body fragments ($R_{work} = 38.6\%$, $R_{free} = 40.1\%$) and then using 10−3.0 Å data and 18 fragments ($R_{work} = 33.6\%$, $R_{free} = 38.0\%$). This model was subjected to a round of model building using the graphics program O (*13*) and to simulated annealing positional refinement of coordinates from 3000 K using data to 2.8 Å resolution ($R_{work} = 24.6\%$, $R_{free} = 36.6\%$). Further model building was followed by conjugate direction refinement of positional and thermal factors using the TNT program suite (*14*) and employing a disordered solvent model ($R_{work} = 22.1\%$, $R_{free} = 32.0\%$). Difference Fourier maps calculated at this stage indicated the location of CoA and clearly identified the bound amino acid substrate as the L-enantiomer despite the use of racemic 2-aminopimelate in crystallization solutions. In addition, density corresponding to the C-terminal 18 residues (257−274), absent from the apoenzyme model, was apparent and could be modeled at this stage. The last several cycles of model building and refinement were used to extend resolution to 2.0 Å and add 85 water molecules ($R_{work} = 16.9\%$, $R_{free} = 24.6\%$). The final crystallographic *R*-factor for the L2AP/CoA complex model using all available data to 2.0 Å resolution (0.98 complete) was 17.1% (Table 1).

The structure of crystals transferred to a solution containing THDP and CoA was determined by difference Fourier maps phased with protein atomic coordinates from which residual bias had been removed with a 4000 K simulated annealing refinement. The difference electron density clearly indicated that the cyclic THDP molecule present in the crystal soak solution was not bound, but rather the acyclic compound formed by hydration of THDP and ring opening to yield L2A6OP (Scheme 1). Proof that this compound had indeed

FIGURE 1: Superposition of the apoenzyme (gray) and L2A6OP/CoA complex (orange) structures of THDP succinyltransferase. The location of the C-terminal 18 residues (257A−274A) and the residues corresponding to the flexible loop (166B−175B) of the ternary complex are depicted in red. (Left, A) View parallel to the crystallographic 3-fold axis relating subunits of the trimeric enzyme. The three triangular forms correspond to traces of the left-handed parallel β-helix structural domain (see text). (Right, B) Stereoview nearly perpendicular to the 3-fold axis depicting one active site. The A subunit is depicted on the left and the B subunit on the right. Produced by SETOR (*17*).

substituted for the structurally similar L2AP present in the original crystallization solution was provided by the appearance of a very strong electron density feature (7.7σ) on an $F_o - F_o$ map corresponding to the 6-oxo group of L2A6OP. Least squares refinement of positional and thermal factors at 2.0 Å resolution was carried out using TNT with the addition of 91 water molecules (R_{work} = 15.2%, R_{free} = 22.5%). The final crystallographic *R*-factor for the L2A6OP/CoA complex using all available data to 2.0 Å resolution (0.97 complete) was 15.4% (Table 1).

RESULTS AND DISCUSSION

Atomic Models of THDP Succinyltransferase Complexes. The structures of the two THDP succinyltransferase complexes consist of atoms corresponding to all 274 residues of the polypeptide and all atoms corresponding to bound substrates. These models include the C-terminal 18 residues that could not be modeled in any of the three crystallographically independent subunits of the previously reported apoenzyme structure due to disorder (*4*). The L2AP/CoA structure, determined at pH 6.4, contains 85 water molecules and 7 residues truncated to alanine due to weak electron density. The L2A6OP/CoA structure was determined from crystals that had been transferred to a synthetic mother liquor solution containing THDP and CoA at pH 8.0 (activity pH optimum 8.2). This model contains 91 water molecules and 6 residues truncated to alanine. The three-dimensional structures of the two complexes are nearly identical and display an RMS discrepancy of 0.15 Å for protein α carbon atoms, 0.18 Å for coenzyme atoms, and 0.11 Å for common atoms of the amino acid acceptor substrates. Because the two complexes differ in structure by only the 6-oxo group of L2A6OP, the ensuing discussion will focus on just the structure of THDP succinyltransferase in complex with L2A6OP and CoA.

Structure of THDP Succinyltransferase. The overall structure of THDP succinyltransferase is composed of an NH_2-terminal domain containing four α helices (residues 1−101), a structural domain composed primarily of coiled parallel β structure termed a left-handed parallel β-helix (LβH) (contained in the range 102−233), and a C-terminal domain (234−274) (Figure 1). The LβH domain is formed by tandem repeated copies of a hexapeptide repeat sequence motif described as [LIV]-[GAED]-X_2-[STAV]-X and adopts a coiled conformation. The overall shape of this domain is similar to an equilateral prism with its three sides formed by three parallel β-sheets composed of short β-strands. The regular coiled fold of this domain is interrupted at two locations by loops that project from vertices of this prismatic domain and whose sequence disobeys the hexapeptide repeat sequence motif rule (residues 166−175 and 210−224). The trimeric structure of the enzyme in the crystals studied here consists of three subunits that are related by a crystallographic 3-fold rotation axis. This rotation axis is nearly parallel to the axis of each individual prismatic LβH domain. Structural elements that form the apoenzyme subunit−subunit interface are conserved in the ternary complex structures reported here, although some additional intersubunit interactions occur in the ternary complexes (see below).

Active Site Location and Conformational Change. The substrate binding site of THDP succinyltransferase is located at the junction of two LβH domains belonging to separate subunits (Figure 1). The shape of the active site is that of a long and narrow tunnel oriented parallel to the 3-fold axis of the enzyme as well as the axis of each LβH domain. A distance of 34 Å measured parallel to this axis separates the most distal substrate groups: the 7-carboxylate group of L2A6OP and the ribose 3′-phosphate of CoA. This region of the molecule is generally that which had been proposed to form the active site on the basis of the proximity of binding sites for two inhibitors: PCMBS and cobalt ion (*4*). The use of two subunits to form a single active site suggests that each trimeric enzyme molecule possesses three equivalent active sites, each formed at the interface of three different subunit pairs. Further description of the active site requires reference to a pair of subunits. For a single active site, these subunits will be referred to as either A or B and residues belonging to each subunit will be denoted using the subunit name as a suffix. In Figures 1B, 2, 3, and 4, the A subunit is depicted on the left side and the B subunit on the right side.

An intersecting initial velocity pattern has been reported for the *E. coli* THDP succinyltransferase using either THDP or L2AP as succinyl acceptors (*3*, *15*), indicative of a

sequential kinetic mechanism requiring both succinyl-CoA and the amino acid acceptor to bind prior to catalysis. These kinetic studies are confirmed by the observation of a remarkable conformational change in the three-dimensional structure of the enzyme upon the binding of L2A6OP and CoA. After superposition of the LβH domain α carbon coordinates of the apoenzyme and the L2A6OP/CoA ternary complex structures (RMS deviation 0.33 Å), significant conformational changes are apparent in three distinct polypeptide segments. These conformationally flexible segments are (1) a portion of the NH_2-terminal domain containing the first three α-helices (residues 1B−70B; RMS α carbon deviation relative to the apoenzyme of 1.7 Å), (2) a polypeptide loop excluded from the LβH domain (residues 166B−175B; RMS deviation 5.6 Å), and (3) the C-terminal 18 residues (257A−274A; not observed in the apoenzyme structure due to disorder). These conformational changes are not likely to be due to the pH differences of the crystal soak solutions since the two complex structures described here were determined from crystals soaked in solutions of pH 6.4 and 8.0 and the apoenzyme structure was determined at pH 7.5.

The NH_2-terminal domain and the flexible loop excluded from the LβH domain (166B−175B) are in contact and move in the same direction to cover the active site (Figure 1). In the apoenzyme structure, these segments are in contact predominantly via the side chain ammonium group of Lys 60B, a residue of the third α helix (α3; 57B−70B) of the NH_2-terminal domain. The side chain ammonium group of Lys 60B projects toward the lumen of the flexible loop and interacts with four main chain carbonyl groups of this loop in the structure of the apoenzyme (Leu 168B, Pro 170B, Leu 171B, and Ala 173B; N−O distances 2.5−3.2 Å) (Table 2). On binding substrates, the loop undergoes a large conformational change, promoting more intimate interaction with residues on the side of α3 that faces the loop (Thr 55B, Gln 57B, Lys 60B, Leu 64B) as well as three residues of the A subunit and the amino acid acceptor substrate (see below). The interactions between the loop residues and α3 promoted by substrate binding appear to pull the NH_2-terminal domain toward the active site as a rigid body but do not significantly change the main chain conformation of this domain.

The C-terminal 18 residues were not visible in the apoenzyme structure in any of the three crystallographically independent subunits, despite the presence of full-length protein as determined by electrospray mass spectrometry of dissolved crystals (data not shown). The C-terminal 18 residues form a short α-helix (257A−263A) and an irregular, somewhat extended structure (264A−274A) directed nearly parallel to the 3-fold rotation axis (Figure 1B). The C-terminal segment is ordered by its interaction with the 3′-phosphate ADP moiety of the cofactor as well as its interactions with the adjacent B subunit. The subunit−subunit interactions formed as a result of the conformational change include hydrogen bonds from Lys 263A (NZ) to Ser 211B (OG) and from Asn 267A (ND2) to Leu 168B (O), a salt bridge between Arg 271A and the flexible loop residue Glu 169B, and a hydrophobic interaction between the side chains of Leu 270A and Phe 67B of the NH_2-terminal domain α3 helix.

The C-terminal 18 residues (from the A subunit) and the residues that form the flexible loop excluded from the LβH structural domain (from the B subunit) form one wall of the extended active site tunnel as a result of the substrate-induced conformational change. Space-filling diagrams illustrating the degree of active site closure prepared from the observed coordinates of the L2A6OP/CoA complex and resulting from an experiment in which the apoenzyme enzyme structure was docked with substrate atoms in order to achieve a similar association are depicted in Figure 2 and visually indicate the degree of active site closure that occurs on substrate binding. The solvent-accessible surface areas of L2A6OP and CoA are reduced by 98% and 86%, respectively, on association with the enzyme.

Table 2: Hydrophilic Interactions from the Flexible Loop (Residues 166−175)[a]

loop residue	apoenzyme	L2A6OP/CoA complex
Gly 166B (N)		WAT 383 (2.9)
Gly 166B (O)	WAT 189 (3.0)	WAT 330 (3.0)
		WAT 331 (3.0)
		WAT 382 (3.1)
Val 167B (N)	Ser 148B (O) (3.1)	Ser 148B (OG) (2.9)
	WAT 47 (3.2)	
Val 167B (O)	Lys 60B (NZ) (3.3)	Gln 172B (N) (3.1)
Leu 168B (N)		L2A6OP (O12) (2.9)
Leu 168B (O)	Lys 60B (NZ) (3.0)	Asn 267A (ND2) (2.9)
Glu 169B (N)		L2A6OP (O12) (3.1)
Glu 169B (OE1)		Asn 267A (N) (2.8)
		Arg 271A (NH1) (2.6)
Glu 169B (OE2)		His 157A (ND1) (2.8)
		Arg 271A (NH2) (3.0)
Glu 169B (O)		L2A6OP (N2) (2.8)
Pro 170B (O)	Lys 60B (NZ) (3.1)	WAT 382 (2.6)
	Ala 173B (N) (3.1)	
Leu 171B (N)		WAT 358 (2.8)
Leu 171B (O)	Lys 60B (NZ) (3.1)	WAT 340 (2.9)
		WAT 370 (2.9)
Gln 172B (N)		Val 167B (O) (3.1)
Gln 172B (OE1)		WAT 339 (3.2)
Gln 172B (NE2)		WAT 314 (2.8)
Gln 172B (O)		Lys 60B (NZ) (2.8)
Ala 173B (N)	Pro 170B (O) (3.1)	WAT 339 (3.0)
Ala 173B (O)	Lys 60B (NZ) (2.6)	Gln 57B (NE2) (3.0)
		Lys 60B (NZ) (2.9)
Asn 174B (N)		WAT 341 (2.9)
Asn 174B (OD1)		Thr 55B (OG1) (2.9)
Asn 174B (ND2)	Pro 175B (O) (2.8)	WAT 342 (2.6)
	Gly 193B (O) (3.0)	
Asn 174B (O)		WAT 330 (2.9)
		WAT 341 (3.0)
Pro 175B (O)	Asn 174B (ND2) (2.8)	

[a] Distances between hydrophilic groups of the flexible loop (residues 166B−175B) and other hydrophilic groups for the apoenzyme (1TDT) and L2A6OP/CoA ternary complex structures. Distances (in Å) are given in parentheses.

Coenzyme A Binding Conformation and Interactions. The conformation of CoA bound to THDP succinyltransferase is shaped like a fishhook and bent at the pyrophosphate group. The pantetheine arm adopts an extended conformation and interacts directly with the adenine group via the pantetheine hydroxyl (Figures 1, 3, and 4). Several features of the cofactor conformation and its interaction with the protein have been frequently observed in the structures of other CoA binding proteins (*16*). These include the location of the cofactor binding site at a subunit−subunit interface, an overall bent conformation of the cofactor and extended pantetheine arm, an apparent 2′-endo conformation of the ribose, an anti glycosidic torsion angle, a solvent-exposed ribose 3′-phosphate group, and direction of the adenine toward the protein and away from solvent.

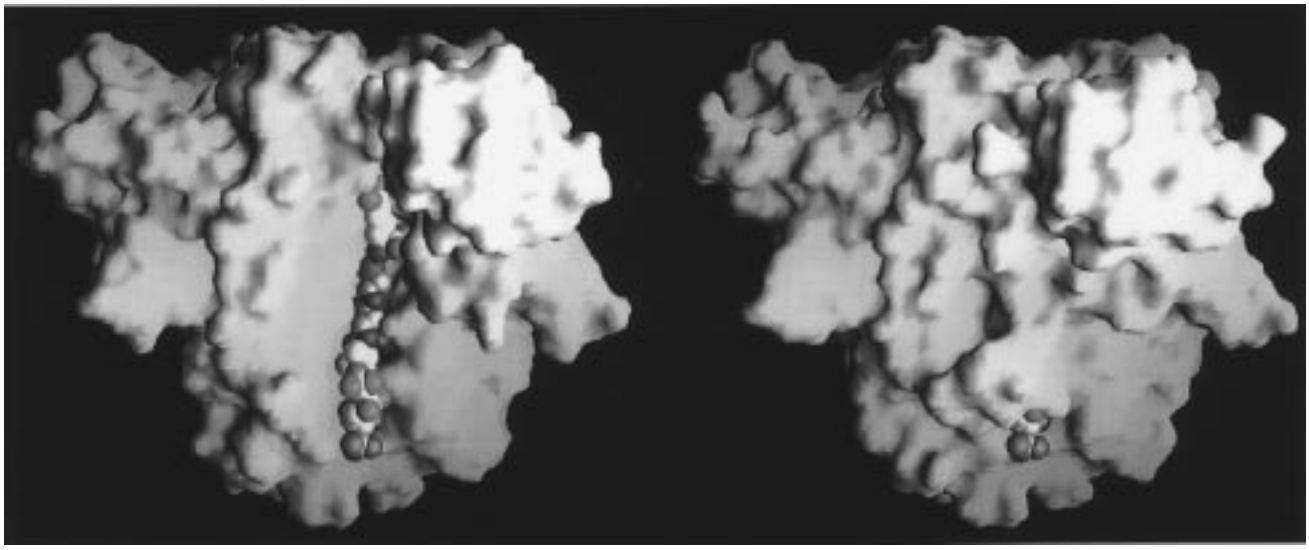


FIGURE 2: Space-filling diagrams of the THDP succinyltransferase. The viewing direction is identical to that of Figure 1B. (Left) Apoenzyme structure (*4*) with docked substrates. (Right) Holoenzyme structure determined here with observed positions of CoA and L2A6OP indicating the reduced degree of solvent accessibility due to the conformational change. Produced by GRASP (*18*).

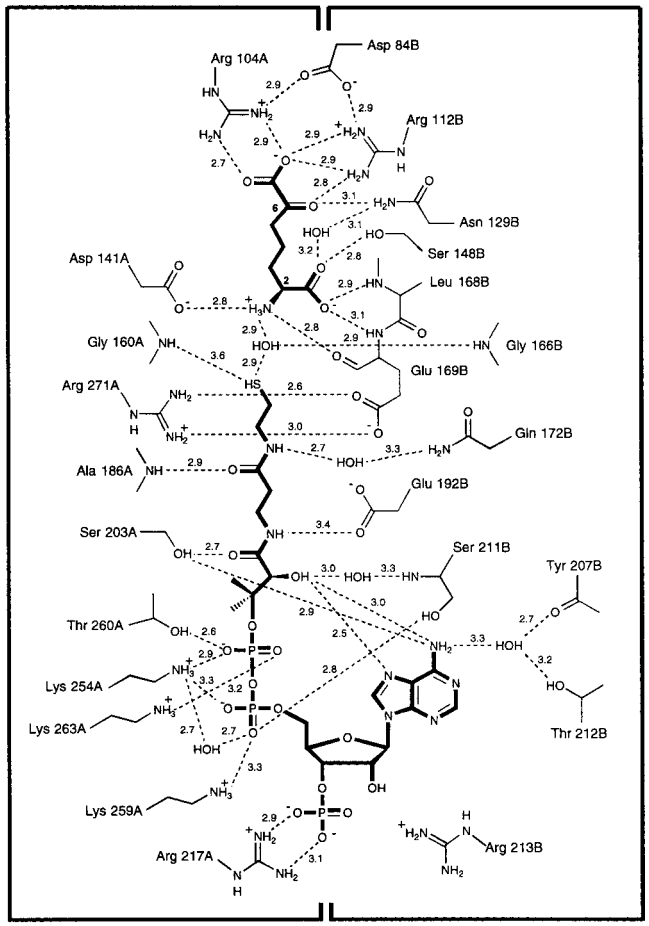


FIGURE 3: Interactions of the substrates CoA and L2A6OP at the active site of THDP succinyltransferase (arbitrary resonance forms). Residues belonging to different subunits are denoted with A and B suffixes. The surrounding brackets suggest the spatial extent of these subunits. Interatomic distances between hydrophilic groups are also given (in Å).

The negatively charged 3′-phosphate and pyrophosphate moieties of the cofactor are bound through electrostatic interactions with Arg 217A, Lys 254A, Lys 259A, Lys 263A, and Arg 213B and hydrogen bonds to Thr 260A (OG1) and Ser 211B (OG) (Figure 3). Three of these interactions are made by positively charged residues from the C-terminus of the A subunit and contribute to the ordering of this segment that is observed only in the presence of substrates. The adenine exocyclic amino group forms hydrogen bonds with Ser 203A (OG) and the pantetheine hydroxyl and indirect, water-mediated interactions with Tyr 207B (O) and Thr 212B (OG1). The amide carbonyl oxygen atoms of the pantetheine β-alanine residues accept hydrogen bonds from Ser 203A (OG) and Ala 186A (N) of the A subunit. These residues are members of adjacent coils of the LβH structural domain and serve to impose an extended conformation on the cofactor pantetheine arm by distance matching between the parallel β strands of this domain (interstrand separation 4.9 Å) and that of the amide carbonyl oxygens of the pantetheine arm in its observed extended conformation (5.0 Å). The nitrogen atoms from the β-alanine units hydrogen bond to Glu 192B (OE2) and indirectly via water to Gln 172B (NE2) of the B subunit. The sulfhydryl group is rather disordered in the ternary complex structures reported here but appears to interact with a water molecule that bridges this group and the amino group of the L2A6OP acceptor substrate and receive a hydrogen bond from the peptide nitrogen of Gly 160A.

Amino Acid Binding Conformation and Interactions. The side chain of the amino acid acceptor, L2A6OP, adopts an extended all-trans conformation. The 1-carboxylate group is staggered with respect to the amino group and side chain α carbon substituents. The side chain of the amino acid and the pantetheine arm of the cofactor are in rough collinear alignment and oriented parallel to the 3-fold axis of the enzyme (Figure 1).

Whereas the cofactor is bound predominantly by residues from the LβH domains and the conformationally mobile C-terminal segment of the A subunit, the amino acid acceptor L2A6OP is bound by residues from the two LβH domains and the conformationally flexible loop of the B subunit. The 7-carboxylate group of L2A6OP is in salt linkage with Arg 104A and Arg 112B, two residues that do not change conformation and appear to be nearly prearranged in the

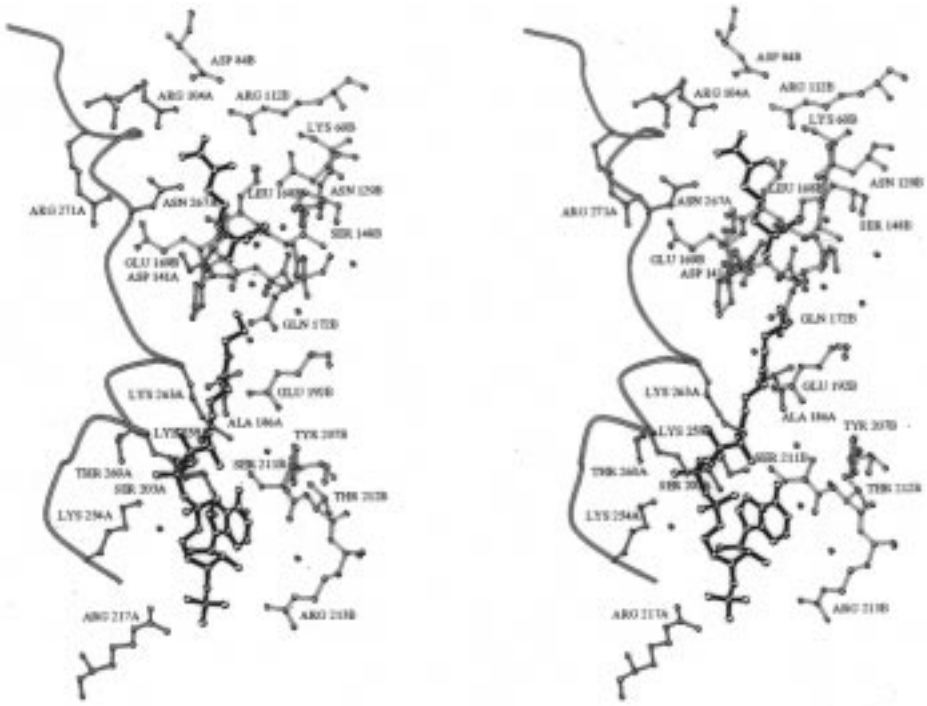


FIGURE 4: Stereoview of the THDP succinyltransferase active site. Residues from the A subunit (blue) and B subunit (yellow) are depicted as well as the substrates (bold). The polypeptide chain path of the C-terminal 18 residues (257A−274A) is depicted as a rope. Produced by MOLSCRIPT (*19*).

apoenzyme structure through salt linkage to Asp 84B. The 6-oxo group of L2A6OP accepts hydrogen bonds from the side chain carboxamide of Asn 129B and the guanidinium of Arg 112B. Ser 148B (OG1) and a water molecule interact with one oxygen atom of the acceptor 1-carboxylate group; the remaining oxygen accepts hydrogen bonds from the main chain amide groups of Leu 168B and Glu 169B of the flexible loop.

Although there are a large number of contacts that characterize the conformational change of THDP succinyltransferase and its interaction with substrates, the role of Glu 169B is particularly noteworthy (Figure 3). The α carbon of this residue moves 7.7 Å on binding substrates, and the hydrophilic groups of this residue make six interactions in the ternary complex structure, none of which occur in the absence of substrates. The side chain carboxylate of Glu 169B interacts with the A subunit by forming a salt bridge with Arg 271A and by accepting hydrogen bonds from the peptide nitrogen of Asn 267A and the imidazole ND1 of His 157A (not shown in Figure 3). The main chain oxygen of Glu 169B accepts a hydrogen bond from the 2-amino group of the acceptor, and the main chain nitrogen of this residue donates a hydrogen bond to the substrate 1-carboxylate. In this way, Glu 169B plays a role in ordering the C-terminal segment of the A subunit and promoting the conformational change that creates the active site tunnel. In addition, it participates in two interactions that orient the amino group of the acceptor toward the cofactor binding pocket and its mercaptoethylamine group.

It may be significant that the amino group of the amino acid substrate L2A6OP accepts a nearly tetrahedral arrangement of substituents, considering its covalent bond to the α carbon atom of the amino acid and hydrogen bonds formed with Glu 169B (O), Asp 141A (OD2), and water. The water molecule bridges the amino acid acceptor amino group and the cofactor sulfhydryl group (N−S distance 4.0 Å) and with one additional water molecule occupies a volume located between these substrates. Modeling experiments indicate that if these water molecules are removed, this volume is large enough to accommodate the succinyl group of succinyl-CoA, although the orientation of this unobserved acyl group cannot be unambiguously assigned in the absence of further observations.

Mechanistic Implications. On the basis of the study of a series of cyclic and acyclic analogues of THDP, a model for the succinylation of THDP has been proposed (*3*). Features of this mechanism include enzymatic hydration of the cyclic imine, THDP, followed by succinylation and then ring opening to form the acyclic succinylated product, L2A6OP. The observed characteristic of several acyclic compounds such as L2AP to serve as good substrates [$K_{m(app)}$ = 1.0 mM, V_{max} = 0.67V_{max} (THDP)] was explained by proposing that acyclic substrates such as this bind in a pseudocyclic conformation to the enzyme.

The crystal structures determined here define the conformation of two enzyme-bound acyclic substrates and demonstrate that an obligatorily acyclic substrate such as L2AP and the hydrated and acyclic product of THDP, L2A6OP, are both bound to the enzyme in nearly identical extended conformations. Although it is not clear whether the hydration and ring opening of THDP occurs in solution or is catalyzed, the orientation of the primary amino group of the amino acid in close proximity to the mercaptoethylamine group of the cofactor suggests that these structures involving acyclic intermediates may indeed characterize the structure of a precatalytic complex. The amino group of both L2AP and L2A6OP interacts with the side chain carboxylate of Asp 141A, and it is tempting to speculate that this residue could function as a base to deprotonate the primary amine prior to nucleophilic attack on the thioester or to aid in the decom-

position of a tetrahedral intermediate. Support for such ideas may be gained from determination of the three-dimensional structures of enzyme complexes containing the natural succinylated cofactor and from preparation and analysis of site-directed mutants of THDP succinyltransferase.

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