Structural Determinants of Enzyme Binding Affinity: The E1 Component of Pyruvate Dehydrogenase from *Escherichia coli* in Complex with the Inhibitor Thiamin Thiazolone Diphosphate[†,‡]

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ABSTRACT: Thiamin thiazolone diphosphate (ThTDP), a potent inhibitor of the E1 component from the *Escherichia coli* pyruvate dehydrogenase multienzyme complex (PDHc), binds to the enzyme with greater affinity than does the cofactor thiamin diphosphate (ThDP). To identify what determines this difference, the crystal structure of the apo PDHc E1 component complex with ThTDP and Mg^{2+} has been determined at 2.1 Å and compared to the known structure of the native holoenzyme, PDHc E1−ThDP−Mg^{2+} complex. When ThTDP replaces ThDP, reorganization occurs in the protein structure in the vicinity of the active site involving positional and conformational changes in some amino acid residues, a change in the V coenzyme conformation, addition of new hydration sites, and elimination of others. These changes culminate in an increase in the number of hydrogen bonds to the protein, explaining the greater affinity of the apoenzyme for ThTDP. The observed hydrogen bonding pattern is not an invariant feature of ThDP-dependent enzymes but rather specific to this enzyme since the extra hydrogen bonds are made with nonconserved residues. Accordingly, these sequence-related hydrogen bonding differences likewise explain the wide variation in the affinities of different thiamin-dependent enzymes for ThTDP and ThDP. The sequence of each enzyme determines its ability to form hydrogen bonds to the inhibitor or cofactor. Mechanistic roles are suggested for the aforementioned reorganization and its reversal in PDHc E1 catalysis: to promote substrate binding and product release. This study also provides additional insight into the role of water in enzyme inhibition and catalysis.

PDHc[1] E1 is the initial member of the pyruvate dehydrogenase multienzyme complex, an assemblage that plays a pivotal role in cellular metabolism catalyzing the oxidative decarboxylation of pyruvate and the subsequent acetylation of coenzyme A to acetyl-CoA. E1 catalyzes the first step of the multistep process, using ThDP and Mg^{2+} as cofactors. In all thiamin-dependent enzymes, the reaction is initiated by the formation of a covalent adduct between the substrate and cofactor through the C2 atom of the thiazolium ring, as seen in the reaction path for E1 (Figure 1). As would be expected, blocking this site, for example, by replacing the proton on C2 with an oxygen atom as in thiamine thiazolone diphosphate (ThTDP, Figure 1) or with a sulfur atom as in thiamine thiothiazolone diphosphate (ThTTDP), inactivates the enzyme (*1*, *2*).

The binding affinities of ThTDP or ThTTDP relative to that of the cofactor ThDP are vastly different for the various thiamin-dependent enzymes. Gutowski and Lienhard (*1*) and more recently Nemeria *et al*. (*2*) have shown that ThTDP exhibits a significantly stronger binding affinity (>1000-fold) for *Escherichia coli* PDHc E1 than does ThDP, based on the lower limit of K_i (0.003 μM) for ThTDP with isolated *E. coli* PDHc E1, compared to the K_d (1.58 μM) for ThDP. In contrast, ThTDP is not a particularly potent inhibitor of several other ThDP-dependent enzymes. ThTDP binds to *E. coli* pyruvate oxidase (POX) only ~30 times more strongly ($K_{d.ThTDP}$ = 0.20 μM) than does the cofactor ThDP (*3*). On the basis of the rate of release of ThTDP from the enzyme, Kluger *et al*. (*4*) observed that ThTDP binds only slightly

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[‡] Coordinates have been deposited in the Protein Data Bank as entry 1RP7.

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[1] Abbreviations: PDHc, pyruvate dehydrogenase multienzyme complex; ThDP, thiamin diphosphate; ThTDP, thiamin thiazolone diphosphate; ThDP complex, PDHc E1−ThDP−Mg^{2+} complex; ThTDP complex, PDHc E1−ThTDP−Mg^{2+} complex; LThDP, C2α-lactylthiamin diphosphate; HEThDP, C2α-hydroxyethylthiamin diphosphate; 2-AcThDP, C2α-acetylthiamin diphosphate; E1, first enzymatic component of multienzyme complexes related to and including PDHc; POX, pyruvate oxidase; PDC, pyruvate decarboxylase; TRK, transketolase; PEG, polyethylene glycol; NCS, noncrystallographic symmetry; RMS, root-mean-square; BNLSLS, Brookhaven National Laboratory Synchrotron Light Source.

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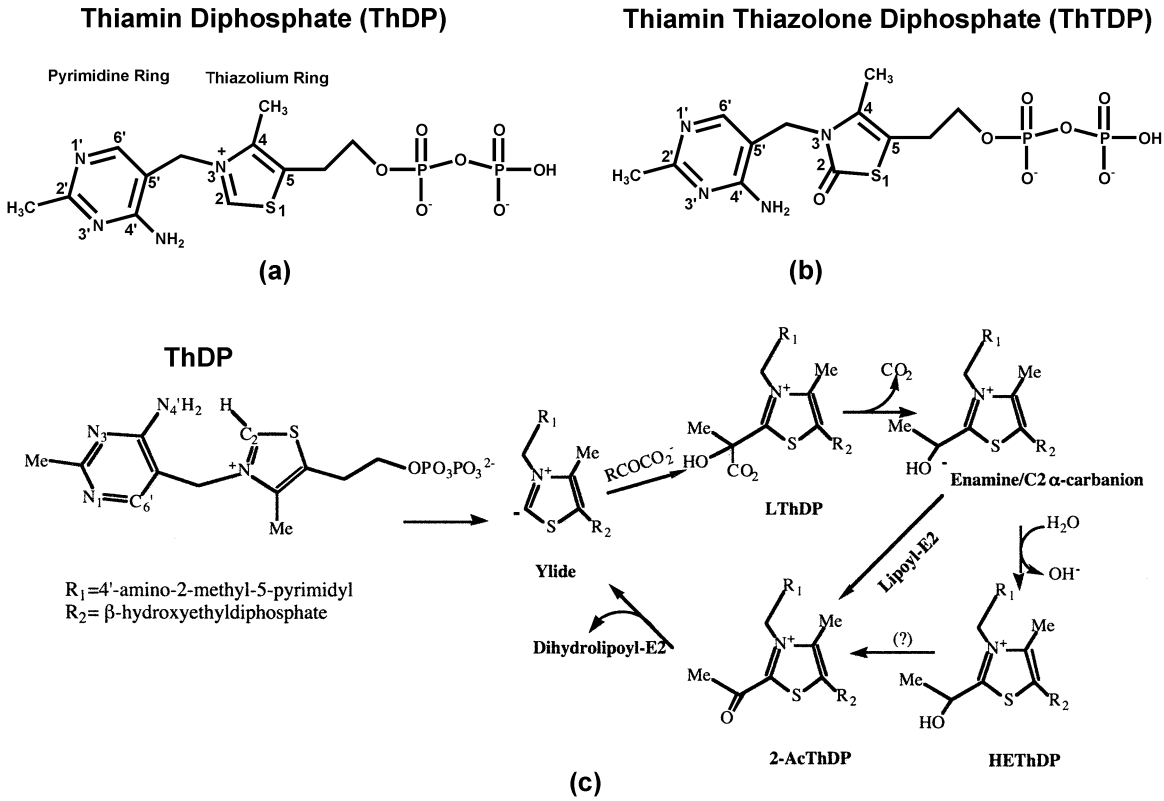

FIGURE 1: (a) Schematic representation of thiamin diphosphate (ThDP). (b) Schematic representation of thiamin thiazolone diphosphate (ThTDP). (c) Path for the E1 reaction. The initial steps involve formation of the ylide (deprotonation at C2) and the pyruvate adduct, lactyl-ThDP (LThDP). Decarboxylation of the latter results in the enamine−C2α carbanion intermediate, which proceeds to form C2-acetyl-ThDP (2-AcThDP). Formation of HEThDP, a hydroxyethyl intermediate, may or may not be involved.

more tightly ($K_{i.ThTDP}$ = 2 μM), perhaps ~10 times, to pyruvate decarboxylase (PDC) than does ThDP. Also, ThTDP is a competitive inhibitor of baker's yeast transketolase (TRK), but binds to it with roughly the same affinity ($K_{i.ThTDP}$ = 0.028 μM) as ThDP (*5*). Structurally, ThTDP was found to bind *Saccharomyces cerevisiae* transketolase (*6*) in a manner essentially identical to that of ThDP, but no solvent molecules were reported by the authors in their description of this structure.

The crystal structure of the *E. coli* PDHc E1 holoenzyme complex has recently been reported (*7*) at 1.85 Å resolution, and interactions involving the cofactors have been discussed in detail. The crystal structure of the analogous PDHc E1−ThTDP−Mg^{2+} complex is presented here, and the structures of the two complexes are compared in an attempt to elucidate structural features that contribute to the much greater affinity of this enzyme for the inhibitor ThTDP than for the cofactor ThDP. The comparison reveals structural features that are very likely the source of most of the difference in the stabilities of the complexes. The results of this analysis also explain the wide variation exhibited by thiamin-dependent enzymes in their affinities for ThTDP relative to ThDP. Unexpectedly, it provides additional insight into the role of water in enzyme catalysis.

EXPERIMENTAL PROCEDURES

The apoenzyme from *E. coli* was purified according to the procedure of Nemeria *et al.* (*8*). The enzyme was cocrystallized with ThTDP by the sitting drop vapor diffusion method. Extensive screening of protein and inhibitor concentrations, divalent cations, additives, pH, and temperature allowed the crystallization conditions to be optimized. The best crystals were obtained using a reservoir solution of 15−20% PEG2000 monomethyl ether, 5−10% 2-propanol, 0.2% NaN_3, and 100 mM HEPES buffer (pH 7.05) at 22 °C. Crystals grew in ~4 weeks. Data collected on the X12C beamline at the Brookhaven National Laboratory Synchrotron Light Source (BNLSLS, Brookhaven National Laboratory, Upton, NY) from a single crystal (0.40 mm × 0.20 mm × 0.15 mm) were processed with the DENZO software package (*9*). The crystal diffracted to 2.09 Å. The Matthews coefficient V_m was calculated to be 2.33 Å^3/Da, based on a dimer per asymmetric unit (*10*).

The PDHc E1−ThTDP−Mg^{2+} complex is isomorphous with the native holoenzyme (PDHc E1−ThDP−Mg^{2+}) structure and crystallizes in the same space group ($P2_1$) with cell parameters that differ by less than 0.5%. The structure of the complex was therefore determined by starting with the final refined coordinates of the native enzyme omitting water molecules, ThDP, and neighboring protein residues and re-evaluating the conformation of the cofactor analogue ThTDP as well as the details of its binding site and solvent structure. A difference Fourier map revealed the locations of these residues, water molecules, and ThTDP as well.

While the two monomers in the asymmetric unit are related by an approximate 2-fold noncrystallographic symmetry (NCS) axis, they were not constrained to be identical during refinement. The refinement procedure included periodic examinations of residue omit maps and subsequent refitting using the graphics program O (*11*). As in the case with the native structure, 85 residues in three (disordered) regions could not be traced; 499 solvent molecules were identified

Table 1: Crystallographic Data and Refinement Statistics

data	
space group	$P2_1$
cell constants	$a = 81.55$ Å, $b = 141.84$ Å, $c = 82.17$ Å, $\beta = 102.61°$
diffraction limit (Å)	2.09
completeness (%) (last shell)	93.2 (55.1)
total no. of reflections	351214
no. of unique reflections	100232
R_{merge} (on I) (last shell)	0.075 (0.203)
X-ray source	X12C at BNLSLS
wavelength (Å)	1.072
refinement	
resolution range (Å)	8.00−2.09
no. of reflections ($I > 2\sigma$)	85348
no. of reflections for test set	5521
R factor (last shell)	0.192 (0.238)
R_{free} (last shell)	0.252 (0.273)
no. of residues	1602
no. of protein atoms	12686
no. of solvent atoms	499
average B factor (Å^2)	
all atoms	18.16
protein atoms	18.25
solvent atoms	16.69
ThTDP atoms	11.57
estimated coordinate error (Å)	0.08 [from a Luzzati plot (*24*)]
rms deviation	
bond lengths (Å)	0.007
bond angles (deg)	1.267

and included in the refinement. Simulated annealing and positional and temperature factor refinement were performed with XPLOR, version 3.8 (*12*). Crystallographic data and final refinement statistics are described in detail in Table 1. The refined coordinates have been deposited in the Protein Data Bank as entry 1RP7.

RESULTS

The crystal structures of the PDHc E1−ThTDP−Mg^{2+} and PDHc E1−ThDP−Mg^{2+} complexes are very similar. Both complexes are dimers much alike in their respective tertiary and quaternary structures. In each of them, the monomers within the crystallographic asymmetric unit are tightly packed into a dimer, with ThTDP or ThDP situated at the monomer−monomer interface within the dimer. Accordingly, there are two cofactor or inhibitor binding sites per complex. The root-mean-square deviation between the respective α-carbon atoms in the two complexes is 0.18 Å. The positions of many of the water molecules are virtually the same in both complexes, and many hydrogen bonds in one occur in the other as well. Some hydrogen bonds common to both complexes directly link the cofactor or inhibitor to the protein. They function as linkages between conserved structural elements, such as hydrogen bonds that link either side chains of conserved amino acid residues or main chain atoms to the diphosphate group or to the aminopyrimidine ring (Table 2). Other water-mediated interactions in the binding region that are found in both complexes are listed in Table 3. The B factors for water molecules in the active site range from 10 to 24 Å^2 for ThTDP and from 9 to 29 Å^2 for ThDP. The mean B values for solvent molecules are comparable to those for the protein atoms, as seen from Table 1.

On the other hand, the complexes also exhibit pronounced differences in the vicinity of the cofactor or inhibitor binding site. Variations occur in amino acid side chain conformations, in the V conformation (*13*) of the ThTDP and ThDP adducts, in the number and positions of water molecules, and in their hydrogen bonding systems. Figures 2 and 3 show details of the environments of the binding sites in the two complexes. Table 4 shows the hydrogen bond interactions in and around the binding sites that are unique to either of the two complexes. Conspicuous differences are evident in the environment of the thiazolium ring. For example, water molecule W4 occurs only in the ThDP complex where it forms hydrogen bonds to N4′ and NE2 of H640, whereas W6−W8 are present only in the ThTDP complex. In the latter complex, the site occupied by W4 in the former is no longer available for a solvent molecule because space is needed to accommodate the C2 substituent carbonyl group. This group forms an intramolecular hydrogen bond to N4′, corresponding somewhat to the intermolecular hydrogen bond between W4 and N4′ in the ThDP complex. The carbonyl group also forms a hydrogen bond to W6 which

Table 2: Direct Interactions between the Cofactor or Inhibitor and Enzyme that Are Common to both ThTDP and ThDP Complexes[a]

protein atom	ThTDP or ThDP atom	distance (Å) ThTDP complex	distance (Å) ThDP complex
Ser109 OG	O21	2.98(6)	2.99(1)
Ser109 OG	O22	2.80(8)	3.15(3)
Gln140 NE2	O11	3.26(6)	3.41(1)
Gln140 NE2	O21	3.23(4)	3.20(2)
His142 NE2	O22	2.60(1)	2.64(3)
Val192 O	N3′	3.16(4)	3.26(1)
Val192 O	N4′	2.68(4)	2.77(3)
Met194 N	N3′	3.23(1)	3.21(1)
Asp230 OD1	O12	2.93(3)	2.95(3)
Gly231 N	O12	2.79(9)	2.75(2)
Glu232 N	O13	3.00(5)	2.98(3)
Asn260 ND2	O21	3.36(7)	3.40(1)
Asn260 ND2	O23	3.22(6)	3.07(8)
Gln262 O	O12	3.14(2)	3.13(4)
Gln262 O	O23	3.09(1)	3.13(2)
Glu571 OE2[b]	N1′	2.85(1)	2.77(2)

[a] Distances are averaged for the two molecules in an asymmetric unit, with the deviation in the last significant digit for either molecule from the mean given in parentheses. [b] Bonded atoms are from the same molecule.

Table 3: Water-Mediated Interactions Linking the Cofactor or Inhibitor and Enzyme that Are Common to both ThTDP and ThDP Complexes[a]

ThTDP or ThDP atom	solvent atom	protein atom	distance (Å) ThTDP complex	distance (Å) ThDP complex
O21	W1		2.62(13)	2.77(16)
O23	W1		2.97(17)	3.11(1)
	W1	Asp230 N	3.24(5)	3.15(17)
	W1	Asp230 OD1	2.89(7)	3.19(29)
	W1	Asn258 O	2.63(3)	2.55(5)
O21	W2		3.15(13)	3.34(14)
	W2	Ser109 OG	2.68(2)	2.62(6)
	W2	Ser113 OG	2.98(7)	2.98(7)
	W2	Gln140 OE1	2.57(3)	2.60(4)
	W2	Asn258 ND2	2.90(9)	2.78(8)
O13	W3		2.68(6)	2.74(4)
	W3	Gln140 NE2	2.82(8)	2.82(3)

[a] Distances are averaged for the two molecules in an asymmetric unit, with the deviation in the last significant digit(s) for either molecule from the mean given in parentheses.

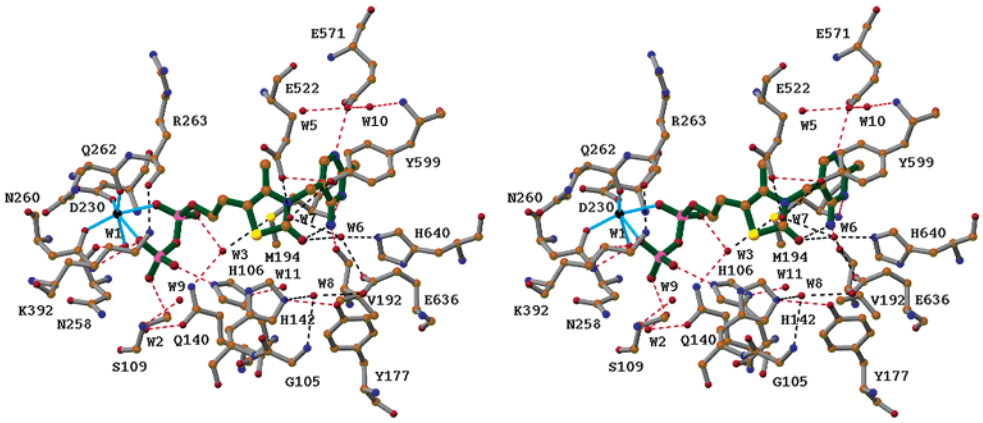

FIGURE 2: Stereodiagram of the ThTDP complex showing hydrogen bonds in the active site. Bonds common to the ThDP and ThTDP complexes are shown in red, and those unique to ThTDP are shown in black. This figure was prepared with the program RIBBONS (*14*).

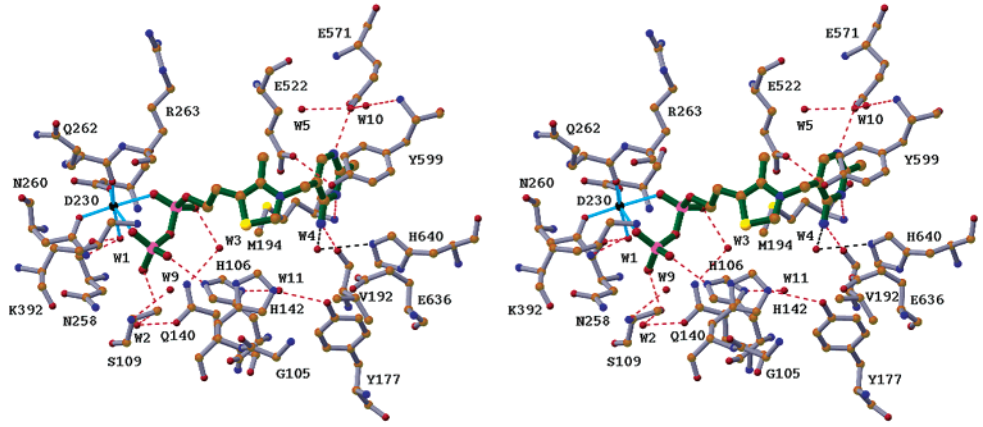

FIGURE 3: Stereodiagram of the ThDP complex showing hydrogen bonds in the active site. Bonds common to the ThDP and ThTDP complexes are shown in red, and those unique to ThDP are shown in black. This figure was prepared with the program RIBBONS (*14*).

itself bonds to NE2 of H640 (analogous to the W4 interaction in the ThDP complex), to OE1 of E636, and to W7. The latter water molecule also makes hydrogen bonds with OE2 of E522 and OH of Y599. The hydrogen bonding architecture associated with W7 is similar to that discussed by Brown and Levy (*15*) in the neutron diffraction study of sucrose. W8 forms hydrogen bonds to N of G105, NE2 of H106, and OE2 of E636. Indeed, it is also apparent in Figures 2 and 3 that there are hydrogen bonds linking the adduct to the protein via solvent atoms which are specific to only one of the complexes. Conformational differences associated with the change in substituent on the thiazolium C2 atom can be seen in Figure 4.

The torsion angles defining the V conformation of the E1-bound ThTDP molecule, viz., Φ_T (C5′−C35−N3−C2) and Φ_P (N3−C35−C5′−C4′), are 97.5° and −66.5°, respectively. While these values are similar to those found in TRK, POX, and PDC, Φ_T is reduced by 10.5° from that observed in the ThDP molecule in the native E1 structure (108.0°). Along with this variation in the V conformation of the adduct, a change in the conformation of the side chain of M194, from a fully extended form in the ThDP complex to a bent form in the ThTDP complex, occurs. As seen from Figures 2 and 3, M194 is adjacent to the thiazolium ring and the conformational variation it undergoes gives rise to two effects. First, it moves the SD atom of M194 ~0.3 Å away from the N3 atom of ThTDP relative to ThDP; second, in the ThTDP complex, this SD atom is now positioned closer to solvent molecule W3 (Table 4). The SD···O distances of 3.36 and 3.47 Å in the two monomers are now conducive to hydrogen bond interactions as discussed in some detail by Gregoret *et al.* (*16*) and Pal and Chakrabarti (*17*). The corresponding distances in the native structure, 3.83 and 3.82 Å, are outside the range of hydrogen bonds and merely indicate van der Waals contacts.

Another conformational change that occurs in the binding site region results in the formation of a new hydrogen bond but does not involve a water molecule; namely, the side chain conformation of K392 exhibits differences in the ThTDP and ThDP complex structures. Residue K392 is in a more extended form in the former structure, which allows a hydrogen bond (~2.7 Å) to be formed between the NZ atom of K392 and the main chain carbonyl oxygen of R263 of the same monomer (Table 4). The interaction between these residues is manifest only as a van der Waals contact in the ThDP complex (3.50 and 4.02 Å in the two monomers).

DISCUSSION

The results presented in Table 4 demonstrate that an important structural source of the greater affinity of PDHc E1 for ThTDP than for ThDP is the larger number of hydrogen bonds linking the ThTDP molecule to protein atoms. This observation leads to the question of exactly what gives rise to the extra hydrogen bonding in the inhibitor. The evidence indicates that the need to accommodate C2═O, W6, W7, and the C2═O···N4′ hydrogen bond triggers a structural

Table 4: Hydrogen Bond Interactions Specific to either One or the Other of the Two Complexes[a]

atom 1	atom 2	distance (Å)	
		ThTDP complex	ThDP complex
ThTDP N4′[b]	ThTDP OC2	2.91(2)	
W6	ThTDP OC2	3.18(9)	
W6	Glu636 OE1	2.73(4)	
W6	His640 NE2	2.92(4)	
W6	W7	3.19(2)	
W7	Glu522 OE2	2.89(2)	
W7	Tyr599 OH	3.30(4)	
W8	Gly105 N	3.22(3)	
W8	His106 NE2	3.31(1)	
W8	Glu636 OE2	2.95(2)	
Glu522 OE2[b]	Tyr599 OH	2.74(13)	2.78(3)
Lys392 NZ[b]	Arg263 O	2.72(3)	[3.76(26)]
W3	Met194 SD	3.42(6)	[3.83(1)]
W4	ThDP N4′		3.03(3)
W4	His640 NE2		3.18(1)

[a] Distances are averaged for the two molecules in an asymmetric unit, with the deviation in the last significant digit(s) for either molecule from the mean given in parentheses. Values in brackets indicate distances outside the range of hydrogen bond interactions in one of the complexes. Solvent molecule W4 in molecule A of the native structure is replaced with solvent molecules W6 and W7 in the inhibitor complex. Solvent molecule W8 in the inhibitor complex does not have an equivalent in the native structure. Solvent molecule W3 is in essentially the same location in both complexes. [b] Bonded atoms are from the same molecule.

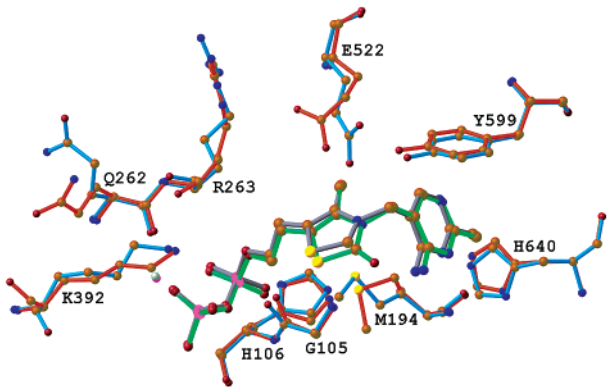

FIGURE 4: Superposition of the native (ThDP complex) and inhibitor complex (ThTDP complex) structures of PDHc E1 based on least-squares alignment of all known α-carbon atoms. Residues in the ThDP complex are shown with blue bonds and those in the ThTDP complex with red bonds. The ThDP molecule is shown in gray and the ThTDP molecule in green. This figure was prepared with the program RIBBONS (*14*).

reorganization in the vicinity of the active site conducive to the formation of a more extensive hydrogen bonding system. A similar but more extensive reorganization would be required to accommodate the bulkier substrate pyruvate during catalysis. The study also shows that with C2(H), W4, and E522 carboxylate oxygen occupying the corresponding active site region in the ThDP complex, a similar structural reorganization does not occur. Accordingly the nature of the substituent on C2 appears to be critical for initiating reorganization of the active site. However, a second condition for forming the more extensive hydrogen bonding system is that the reorganized active site amino acid residues must be chemically and positionally compatible for participation in hydrogen bond formation with water molecules or other members of a given potential hydrogen bond-forming group. Therefore, the amino acid sequence of the protein must be suitable for hydrogen bonding to a particular inhibitor or substrate complex. For example, the amino acid residues whose side chains engage in the extra hydrogen bonds unique to the PDHc E1−ThTDP complex are generally all nonconserved residues in ThDP-dependent enzymes, whereas those residues hydrogen bonded to both the ThTDP and ThDP molecules in their respective complexes are conserved (Table 5). Since PDHc E1 has the greatest affinity for ThTDP relative to ThDP and since the residues engaged in the extra hydrogen bond network in PDHc E1 are known, it is evident that other thiamin-dependent enzymes whose approximate relative affinities for ThTDP and ThDP are much less different do not have suitable side chains in corresponding positions to support such an extensive enhancement in the hydrogen bonding system. It should be noted that the basic affinity of an enzyme for ThDP might be higher than is the case for PDHc E1. For example, ThDP does bind more tightly to PDC than to PDHc E1, and the hydrogen bonding system is indeed seen to be much more extensive in the PDC−ThDP complex [31 hydrogen bonds (*18*)] than in the PDHc E1−ThDP complex (16 hydrogen bonds). POX is stabilized by 15 hydrogen bonds (*19*) linking the enzyme and ThDP, and TRK (*20*) is stabilized by 13 such hydrogen bonds. However, from an analysis of Table 5, it appears that several of the residues forming hydrogen bonds with PDHc E1 would be unable to do so in the PDC−ThTDP, POX−ThTDP, or TRK−ThTDP complex. As a consequence, the ratio of the relative affinities of ThTDP and ThDP for each of these three enzymes is predicted to be much smaller than in the case of PDHc E1. Thus, the extensiveness of the hydrogen bonding system in the vicinity of the cofactor binding site is a good qualitative measure of the tightness of the binding in the complexes.

It should be noted that ThTDP has been cited as an example of a transition state analogue for ThDP-dependent enzymes. The analogy is derived from the notion that the charge distributions of the two contributing resonance structures of ThTDP resemble the charge distribution in the central enamine−C2α carbanion intermediate which is believed to form transiently in all ThDP-dependent enzymes (Figure 5). As in the PDHc E1−ThTDP complex, a similar reorganization of the active site is predicted to stabilize the enamine−C2α carbanion intermediate as well. Albeit, the reorganization of the active site containing the enamine−C2α carbanion intermediate is expected to involve other and more extensive shifts because of the differences in the molecular structures of the adducts.

In the PDHc E1−ThTDP complex, the carbonyl oxygen on C2 occupies a position that is anticipated to be close to the predicted α-carbon location in the enamine−C2α carbanion complex. W7 in the PDHc E1−ThTDP complex corresponds in location to an expected pyruvate carboxyl oxygen site when pyruvate covalently bonds to C2. Thus, the crystal structure of the PDHc E1−ThTDP complex should share structural features that bear a resemblance to the enzymic reaction during or immediately after carbon dioxide and the enamine−C2α carbanion intermediate are formed. Indeed, the structural information from the complexes analyzed here suggests a cyclic mechanistic role for the reorganization in PDHc E1 catalysis. For example, when the bond attaching the enamine−C2α carbanion intermediate

Table 5: Structurally Equivalent Residues in Some Thiamin-Dependent Enzymes[a]

residue in	equivalent residue			
Escherichia coli PDHc E1	*Saccharomyces cerevisiae* TRK	*Lactobacillus plantarum* POX	*Saccharomyces cerevisiae* PDC	comments on atom involved in bonding of PDHc E1 to ThDP or ThTDP (bold)
S109	A33	−	A545	involves OG of Ser
Q140	N67	D393	D387	involves NE2 of Glu
H142	H69	G395	G389	involves NE2 of His
V192	G116	A420	G413	involves main chain O atom
D230	D157	D447	D444	conserved residue
G231	G158	G448	G445	conserved residue
E232	C159	G449	S446	involves main chain N atom
N260	N187	N474	N471	conserved residue
Q262	I189	Q476	G473	involves main chain O atom
E571	E418	E59	E51	conserved residue
G105	G29		L549	**involves main chain N atom**
H106	H30	−	N548	**involves NE2 of His**
H640	H481	Q122	H115	**involves NE2 of His**
E636	D477	−	−	**involves OE1 and OE2 of Glu**
E522	L383	G34	G27	**involves OE2 of Glu**
Y599	F442	S82	T73	**involves OH of Tyr**
M194	L118	M422	I415	**involves SD atom of Met**
R263	T190	Y477	Y474	**involves main chain O atom**
K392	I250	G546	V542	**involves NZ of Lys**

[a] The equivalent residues were determined by a least-squares superposition of the α-carbon atoms of each enzyme with PDHc E1. Coordinates were obtained from crystal structure determinations [PDHc E1 (this work), PDC (*18*), POX (*19*), and TRK (*20*)].

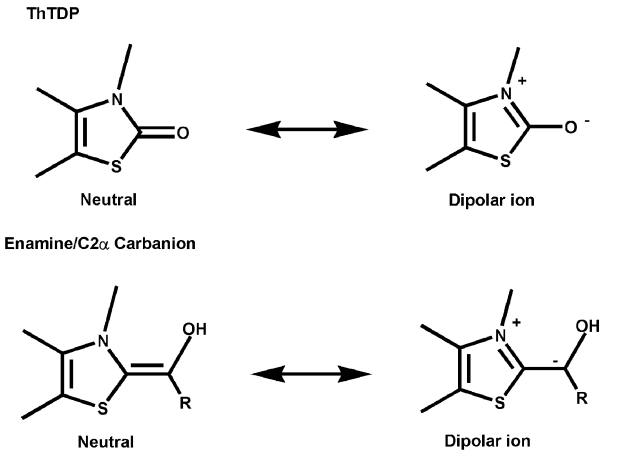

FIGURE 5: Schematic diagram comparing the resonance structure of the thiazolium ring of ThTDP to the enamine−C2α carbanion intermediate.

to C2 is being severed during product release, the structural reorganization of protein residues and hydration sites that occurred during substrate binding would be reversed to lower the energy required to release the product from C2. This cyclic change in the enzyme structure which is localized to the vicinity of the active site can be explained phenomenologically. Interactions between the incoming substrate and enzyme (including the cofactor) trigger a reorganization to stereochemically accommodate the additional substrate atoms. The triggering interactions, which include conformational changes as well as the formation and elimination of hydration sites and hydrogen bonds, lower the energy required to bind the substrate. Once decarboxylation occurs and product release is initiated, some of these stabilizing interactions are lost or disrupted and the enzyme seeks to compensate for the resulting increase in energy by reverting to its original state, which significantly lowers the energy. The release of product is catalytically enhanced by this process; in other words, the process adds to the driving forces breaking the bond between C2 and the α-carbon. Notable in this regard are the roles of E522 and M194 of PDHc E1.

E522 is located directly above the thiazolium ring in the unreacted enzyme with one of its carboxyl oxygens in close contact with N3. During catalysis, E522 must move as in this position it would interfere with the binding of pyruvate since the covalent bond to C2, the internal hydrogen bond to N4′, and the principles of least motion (*21*) and maximum orbital orbital overlap (*22*) require the bond between Cα and the carboxyl group in pyruvate to be perpendicular to the thiazolium ring (*23*). This extension above the plane of the ring would lead to a steric clash with the carboxyl group of E522, and movement of E522 is part of the reorganization. An analogous situation is depicted in Figure 4 where the location of E522 is changed to sterically accommodate W7, which is present in the ThTDP complex but not in the ThDP complex. Likewise, the M194 side chain is displaced but in the opposite direction when the active site is occupied by the bulkier extra groups. This displacement makes room for the hydrogen bond between the carbonyl oxygen of the enamine−C2α carbanion intermediate and N4′ by optimizing the V coenzyme conformation for that purpose. When the group leaves, M194 is predicted to reassume its original conformation with the SD atom moving closer to N3. This restricts the V conformation and disrupts the hydrogen bonding between the existing product and N4′, thus aiding in catalyzing product release.

This interpretation implies that some and possibly all enzymes use structural reorganizations, including the creation and elimination of hydration sites, as a means of controlling reactant binding and release during catalysis by altering the hydrogen bonding network between the enzyme and adduct and between appropriate residues in the active site. An increase in the number of hydrogen bonds promotes substrate binding; the number reaches a maximum in the activated transition state and is reduced during subsequent product release. The capability of an enzyme to time these events favorably during catalysis is sequence-dependent, having

been acquired during its evolution. Accordingly, the catalytic enhancements of substrate binding and product release by the localized cyclically changing enzyme structure are predicted to be sequence-dependent and to vary among thiamin-dependent enzymes, since the protein residues that are involved are not conserved.

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