A Thiamin-bound, Pre-decarboxylation Reaction Intermediate Analogue in the Pyruvate Dehydrogenase E1 Subunit Induces Large Scale Disorder-to-Order Transformations in the Enzyme and Reveals Novel Structural Features in the Covalently Bound Adduct*

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The crystal structure of the E1 component from the Escherichia coli pyruvate dehydrogenase multienzyme complex (PDHc) has been determined with phosphonolactylthiamin diphosphate (PLThDP) in its active site. PLThDP serves as a structural and electrostatic analogue of the natural intermediate α-lactylthiamin diphosphate (LThDP), in which the carboxylate from the natural substrate pyruvate is replaced by a phosphonate group. This represents the first example of an experimentally determined, three-dimensional structure of a thiamin diphosphate (ThDP)-dependent enzyme containing a covalently bound, pre-decarboxylation reaction intermediate analogue and should serve as a model for the corresponding intermediates in other ThDP-dependent decarboxylases. Regarding the PDHc-specific reaction, the presence of PLThDP induces large scale conformational changes in the enzyme. In conjunction with the E1-PLThDP and E1-ThDP structures, analysis of a H407A E1-PLThDP variant structure shows that an interaction between His-407 and PLThDP is essential for stabilization of the tetrahedral intermediate analogue and should serve as a model for the corresponding intermediates in other ThDP-dependent decarboxylases. Regarding the PDHc-specific reaction, the presence of PLThDP induces large scale conformational changes in the enzyme. In conjunction with the E1-PLThDP and E1-ThDP structures, analysis of a H407A E1-PLThDP variant structure shows that an interaction between His-407 and PLThDP is essential for stabilization of the tetrahedral intermediate analogue and should serve as a model for the corresponding intermediates in other ThDP-dependent decarboxylases.

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Thiamin-dependent enzymes play key roles in sugar metabolism, typically catalyzing the decarboxylation of α-keto acids and the transfer of an aldehyde or an acyl group (1–5). Examples include the E1 components in pyruvate dehydrogenase complexes (PDHcs),2 pyruvate decarboxylase, transketolase, etc. Crystallographic studies (6–12) have elucidated many of the structural, stereochemical, and biochemical details in the mechanism of action of these enzymes and in the catalytic role of the cofactor ThDP (thiamin diphosphate, vitamin B1 diphosphate, Fig. 1, top left). Despite the enormous contributions made by these and other studies to our understanding of how such enzymes function, important details still remain obscure. There are, for example, no detailed structural data on the first ThDP-bound intermediate in the presence of any enzyme (for example, α-lactylthiamin diphosphate (α-LThDP) in PDHc E1 and pyruvate decarboxylase), which is postulated to form in the currently accepted mechanism of thiamin catalysis (Fig. 1, top, third object from the right). In an effort to obtain structural information pertaining to this key intermediate, we have determined the crystal structure of PDHc E1 from Escherichia coli in complex with α-phosphonolactylthiamin diphosphate (PLThDP).

PLThDP is the product of the reaction between ThDP and methyl phosphonolactylthiamine, with the latter being an analogue of the true substrate pyruvate and a potent inhibitor of PDHc. The complex formed with PLThDP instead of ThDP therefore mimics the structure of the enzyme-bound, reactive tetrahedral intermediate α-LThDP (13) in the decarboxylation step of the PDHc E1 reaction. It differs from the complex formed with the true substrate only in the replacement of the carbonate group by a methyl phosphonate (PO3Me) group. However, unlike the C2α-CO2 bond normally cleaved in the reaction with pyruvate, the C2α-PO3Me bond remains intact. The reaction is therefore trapped in a pre-CO2 release-like state, and the structure represents a covalently bound, pre-decarboxylation reaction intermediate analogue.

There have been three covalently bound reaction intermediate structures reported for ThDP-dependent enzymes (11, 12, 14), but they all represented the planar enamine intermediate (Fig. 1, top right object) that exists only after decarboxylation. The E1-PLThDP structure is thus the first structural example of a covalently bound, pre-decarboxylation reaction intermediate analogue in any ThDP-dependent enzyme.

2 The abbreviations used are: PDHc, pyruvate dehydrogenase multienzyme complex; ThDP, thiamin diphosphate (vitamin B1 diphosphate); LThDP, lactylthiamine diphosphate (reaction intermediate); PLThDP, phosphonolactylthiamine diphosphate (reaction intermediate analogue); E1, first enzymatic component of multienzyme complexes related to and including PDHc; parental E1, protein only component of E1; E1-ThDP, parental E1 with ThDP cofactor; E1-PLThDP, parental E1 with PLThDP; H407A E1-PLThDP, H407A parental E1 variant with PLThDP.

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The atomic coordinates and structure factors (codes 2G2S and 2G28) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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The E1 component of the PDHc complex catalyzes the rate-limiting step of the overall PDHc reaction and therefore also provides an ideal target for mechanistic structural investigation. Initial crystallographic results for the E1-PLThDP complex have been reported previously (15), and complete, detailed results at 2.1 Å resolution are now presented with the analysis revealing several unique structural features that are absent in the native enzyme. 1) The enzyme-bound conformation of PLThDP is the V form, rather than the S form determined many years ago for the same compound in the absence of enzyme, showing how the enzyme can enforce this highly strained V conformation even in the intermediate analogue. 2) The structure identifies a role for residue His-407, explaining previous biochemical data, and shows the direct interaction (and implied function) of several additional active center residues with the covalently bound adduct. 3) Major reorganization of the active center takes place and includes the ordering of two key loops not seen in any of our other PDHc E1 structures before; thus the PLThDP dramatically diminishes their mobility. 4) A marked deviation from co-planarity of the C2–C2 bond with the thiazolium ring is clearly revealed and suggests likely mechanistic consequences. 5) The structure shows strong interaction of the C2α–OH with the N4’ atom of the 4’-aminopyrimidinium of ThDP, as evidenced by a short contact distance. Elsewhere, we used PLThDP in solution to show that PLThDP exists with the 1,4’-iminoTHDP tautomeric form (16, 17) of the 4’-aminopyrimidinium ring, the first instance in which this putative intermediate could be stabilized. The observed short O–N4’ distance is fully consistent with this tautomerization. The structural reorganization and ordering resulting from the presence of PLThDP deep in the active site extends to the enzyme exterior, providing a new surface likely involved in interactions with other enzymatic components within the PDHc multienzyme complex.

EXPERIMENTAL PROCEDURES

Crystallization and Data Collection—The PDHc E1 from E. coli was purified and assayed according to published procedures (18). ThDP, when present, was removed by gel filtration using an Amersham BioSciences G-25 column. For crystallization, the protein was dialyzed when present, was removed by gel filtration using an Amersham BioG-25 column. For crystallization, the protein was dialyzed

Crystallization and Data Collection—The PDHc E1 from E. coli was purified and assayed according to published procedures (18). ThDP, when present, was removed by gel filtration using an Amersham BioSciences G-25 column. For crystallization, the protein was dialyzed against 20 mM HEPES buffer at pH 7.0, containing 5 mM dithiothreitol, when present, was removed by gel filtration using an Amersham BioG-25 column. For crystallization, the protein was dialyzed...
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TABLE 1
Crystallographic data and refinement statistics

|                      | E1-PLThDP | H407A E1-PLThDP |
|----------------------|-----------|-----------------|
| Space group          | P2₁       | P2₁             |
| Unit cell            |           |                 |
| a (Å)                | 81.6      | 82.0            |
| b (Å)                | 142.5     | 143.2           |
| c (Å)                | 82.1      | 82.5            |
| β (°)                | 102.4     | 102.6           |
| Resolution           | 2.1 Å     | 1.55 Å          |
| Completeness % (last shell) | 94, (66) | 95, (33)       |
| Total reflections    | 466,210   | 685,515         |
| Unique reflections   | 99,699    | 213,020         |
| \( R_{merge} \)      | 0.065     | 0.076           |
| Refinement statistics|           |                 |
| Resolution range (Å) | 39.2–2.10 | 42.0–1.85       |
| Number of reflections| 93,771    | 150,327         |
| \( R \) factor (last shell) | 0.203 (0.213) | 0.216 (0.267) |
| \( R_{free} \) (last shell) | 0.254 (0.291) | 0.240 (0.300) |
| Number of residues   | 1662      | 1602            |
| Number of waters     | 564       | 550             |
| Average B factor (Å²)|           |                 |
| Main chain atoms     | 9.50      | 21.04           |
| Side chain atoms     | 11.15     | 21.17           |
| Solvent atoms        | 16.0      | 23.68           |
| Root mean square deviations|          |                 |
| Bond lengths (Å)     | 0.006     | 0.009           |
| Bond angles (°)      | 1.3°      | 1.5°            |

factor is 0.203 (0.213 in the last shell), and the \( R_{merge} \) (based on 5.3% of data) is 0.254 (0.291 in the last shell). The final structure has good stereochemistry with root mean square deviations from ideality of 0.006 Å and 1.3° for bond lengths and angles, respectively. The final model contains two independent subunits, each with 831 amino acids out of a possible 886, as well as two PLThDP-Mg\(^{2+}\) cofactor pairs. This model also includes 564 water molecules, five phosphate ions, and two cisprolines (Pro-463) per asymmetric unit. The model was analyzed with PROCHECK (25), and 89% of the residues are in the most favored region in the Ramachandran plot (26). The Ramachandran plot shows no residues in disallowed regions in either subunit.

The structure of the H407A E1-PLThDP complex was determined and refined in a similar manner. However, in this structure, although the PLThDP was clearly present in strong density, the regions 1–55, 401–413, and 541–557 were completely disordered. The new structure was refined using the program CNS (27). The slightly higher \( R \) factor for the H407A E1-PLThDP variant may be attributed to the fact that this structure contains 60 disordered residues absent from the model (the regions 401–413 and 541–557 in each molecule) that were ordered in the E1-PLThDP structure. Ramachandran statistics are similar to those for the E1-PLThDP structure. All data collection and refinement statistics are given in Table 1. Graphical representations of protein models were generated by using the program RIBBONS (28). Coordinates and structure factors for the E1-PLThDP and H407A E1-PLThDP structures have been deposited in the Protein Data Bank with the access codes 2G25 and 2G28, respectively.

RESULTS

The main chain folds in PDHc E1-ThDP (8) and in its E1-PLThDP counterpart are very similar, with both structures containing two subunits in an asymmetric unit. The two independent subunits are almost identical stereochemically and are related by a 2-fold non-crystallographic symmetry axis. The complete dimer is shown in Fig. 2a. Each E1-PLThDP subunit consists of a single polypeptide chain folded into three domains: the N terminus (1–470), the middle (471–700), and the C terminus (701–886). Least-squares fitting of 801 common \( \alpha \)-carbon atoms in the E1-ThDP and E1-PLThDP structures results in a root mean square deviation of 0.40 Å. However, significant main-chain deviations (up to 2.7 Å) occur in the middle domain helix containing residues 525–535, which are involved in close contacts with residues present in the active site channel. Except for a few side chains at the surface of the protein, the electron density over the entire molecule is generally very well defined, but as in the E1-ThDP structure, there is no interpretable electron density for the N-terminal residues 1–55.

A surprising observation in the E1-PLThDP map is the presence of well defined electron density for loop residues 401–413 and 541–557 that was absent in the map of the E1-ThDP structure due to complete disorder. These two loops form part of the active site channel situated at the dimer interface. The 401–413 loop forms part of the active site, and residue His-407 in this loop forms a critical hydrogen bond to an oxygen atom in the substrate analogue phosphonyl group. This loop stabilizes the other newly ordered loop 541–557, assisted by interactions created from the large movement in the helical region 525–535. The ordering creates new interface interactions between residues Lys-403–Asn-404 from one subunit and residues Gln-548–Asp-549 from the other subunit. The two newly ordered regions thus interact with each other and propagate outward from the active site to the enzyme surface. The newly ordered regions are shown in terms of the overall structure in Fig. 2a and in greater clarity regarding the active site and enzyme surface in Fig. 2b. Electron density for the 401–413 loop and its interactions with PLThDP are shown in Fig. 3.

The average \( B \) value for main and side chain atoms is 9.5 and 11.2 Å\(^2\), respectively. These values are significantly lower than those observed in the E1-ThDP structure (16.0 and 18.6 Å\(^2\), respectively) (8), implying a more ordered assembly. Accordingly, the presence of the reaction intermediate analogue in the active site supports the formation of a tighter E1 dimer. The now ordered loop regions provide a new exterior surface that can conceivably interact with other subunits of the PDHc complex and also partially seal the active site entrance, leading to a more hydrophobic catalytic region.
It is important to note that the E1-ThDP and E1-PLThDP complex crystals are isomorphous; thus the ordering cannot be attributed to new packing contacts in a different crystal form and arises only from the presence of the adduct now covalently bound to the cofactor.

The H407A E1-PLThDP structure (not shown) reveals the active site to be intact and the structure generally unchanged when compared with the E1-ThDP (8) and E1-PLThDP structures. As in E1-ThDP and unlike in the E1-PLThDP structure, however, a final difference electron density map did not contain interpretable electron density for loop regions 401–413 and 541–557; the N-terminal region 1–55 is unobserved in all of the structures. When combined with the other structures, analysis of the H407A E1-PLThDP structure therefore confirmed the need for the His-407-PLThDP interaction in inducing the disorder-to-order transformation in two loops as the loops are ordered only when both the intermediate and His-407 are present. Clearly, the ordering is induced by the presence of PLThDP via direct hydrogen-bonding to residue His-407 and results in providing previously missing structural elements to complete both the exterior surface and the interior surface leading to the active sites.

For E1-PLThDP, a difference electron density map calculated with...
the phosphonyl oxygen atoms. Other residues (Tyr-177, Gln-408, and hydroxyl oxygen atom. Tyr-599 also forms a hydrogen bond with one of the C2–C2 bonds distant from N4. 

The enzyme-bound PLThDP, however, adopts the V conformation of the molecule assumes the S conformation (\(\Phi_p = -99.5^\circ\), \(\Phi_p = -173.6^\circ\)), which is characteristic of other C2-substituted thiamins and places C2 and its bound adduct distant from N4. The thiazolium ring, in the vicinity of the covalent adduct on C2, is a cluster of four histidine residues. Of these, His-142 is involved in binding with the diphosphate group of the cofactor, whereas His-407 and His-640 interact with oxygen atoms in the adduct. The fourth, His-106, is bound to a terminal methyl carbon making a 3.52 Å contact distance to the one of the phosphonyl oxygen atoms of the adduct.

In addition to inducing disorder-to-order transformations, the presence of PLThDP in the active site also induces conformational changes in nearby protein residues that were previously ordered. Relative to the E1-ThDP structure, a conformational rearrangement of protein residues Leu-264, Glu-522, and Tyr-599 occurs in the E1-PLThDP structure. Residue Glu-522 shows a large conformational change as its side chain moved more than 4 Å. The movement of residues Leu-264 and Tyr-599 and the presence of the phosphonyl oxygen atom close to the thiazolium ring play an important role in the movement of the side chain of Glu-522, which is no longer pointing toward the active site. Although it may not play a direct role in the mechanism of catalysis at this stage, Glu-522 still interacts with the cofactor through water molecules. The key interactions with PLThDP are all likely to be present in the corresponding LThDP intermediate formed from the natural substrate pyruvate, as indicated in Fig. 6, since the majority of good hydrogen bonds can still be formed.

Perhaps most surprisingly, analysis of the E1-PLThDP structure revealed a significant distortion in planarity for the C2–C2 bonds of the substrate analogue to the planar thiazolium ring, as seen in Fig. 4. This out-of-plane distortion implies considerable strain and persists despite incorporation of a planarity restraint during refinement, even after significantly increasing the restraint weight well beyond that normally used. To determine whether this effect is real or simply induced by other possibly incorrect refinement restraints, refinements and pure energy minimizations were carried out with a variety of stand-
ard force fields. In all cases, the out-of-plane distortion persists (to varying but significant degrees), provided that any reasonable van der Waals radii are used for the aminopyrimidine N\textsuperscript{4} and adduct C2\textalpha-hydroxyl O atoms, and in any event, the distortion is required to fit the electron density. The resulting distance between these atoms (2.5 Å) is reasonable, but it would be unacceptably short (2.2 Å), producing a strong repulsive force in the absence of the distortion, thus explaining the strained conformation that is observed. Furthermore, since only three essentially rigid groups (coordinates obtained from the highly accurate small molecule PLThDP crystal structure (29)) are involved, the short contact is dictated by only the thiazolium and aminopyrimidine relative ring orientations and by the fact that the C2\textalpha-PO\textsubscript{3}Me bond is nearly perpendicular to the thiazolium ring plane. The \(\Phi_{i}\) and \(\Phi_{\pi}\) torsion angles defining the ring orientations, as well as the \(S-\text{C2}--\text{C2}\alpha--\text{P}\) torsion angle, are clearly required to fit the strong and unambiguous electron density, and the former are also consistent with those observed in all reported ThDP-containing enzyme structures. Therefore the short contacts cannot be explained away by coordinate errors due to data resolution arguments. In addition, the distortion and short contacts are also present in the higher resolution H407A E1-PLThDP variant structure.

It is important to realize that the strain is removed immediately upon decarboxylation if the enamine (Fig. 1, top right object) is formed as the formerly tetrahedral C2\textalpha carbon then adopts sp\(^2\) hybridization, increasing the distance between N\textsuperscript{4} and the oxygen. It thus appears that removal of this strain may be a contributing force behind decarboxylation. If this is the case, the cofactor V conformation imposed by the enzyme environment is important not only in creating the initial reactive ylide (6) (Fig. 1, third object from the left) but also in inducing stress to facilitate decarboxylation. This is a very important finding as the same forces should be operating in all ThDP-dependent decarboxylases. This view is strongly supported by the fact that when the PLThDP molecule is free of the enzyme (and therefore in the S conformation), it shows no evidence of the strain that occurs when bound to the enzyme in the V conformation (29). For example, in the S form, the C2\textalpha-C2 exocyclic bond is coplanar with the thiazolium ring, the valence angles subtended at C2\textalpha are all near the ideal tetrahedral value, and O2\alpha and N\textsuperscript{4} are not in contact. Therefore the strain associated with the V conformation imposed by the enzyme is avoided entirely in the isolated PLThDP molecule. Structural differences between a planar and severely strained pre-decarboxylation intermediate, the observed partially strained, non-planar pre-decarboxylation intermediate, and totally relaxed post-decarboxylation intermediate are shown in Fig. 7.

**DISCUSSION**

The C2\textalpha-C (carboxyl) bond in \(\alpha\)-LThDP is expected to be oriented relative to the thiazolium ring nearly the same (roughly orthogonal) as the C2\textalpha-P bond seen in \(\alpha\)-PLThDP, and this orientation had been predicted for both compounds (29). Given the structural and electronic similarities, active site residues adjacent to C2\textalpha, OH(2\alpha), CH3(2\alpha), and some of the phosphoryl and carboxylate oxygens are then also expected to have very nearly identical locations in both the catalytically active and the analogue intermediates, and the short N\textsuperscript{4}-O2\alpha distance and out-of-plane deformation of the C2--C2\alpha bond observed in the E1-PLThDP complex should occur in the E1-\(\alpha\)-LThDP complex as well. For example, from Fig. 6, it is clear that the carboxyl group could form the same favorable hydrogen bonds to His-407 and Tyr-599 without any changes in the residue positions, and only very subtle changes in these positions would be required to make the interactions even stronger. Accordingly, the E1-PLThDP structure should be an excellent model for the natural intermediate involving \(\alpha\)-LThDP, and conclusions drawn from the E1-PLThDP structure should be applicable to the transient intermediate.

The disorder-to-order transformation induced by the presence of the
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intermediate analogue PLThDP (and presumably α-LThDP) is particularly important as it not only completes the interior of the active site but also results in structural changes leading from it out to the protein exterior. This was shown in Fig. 2b. With this ordering, a tunnel that can accommodate the lipoyl-lysine side chain bound to an E2 component in PDHc is completely formed, and a new surface is created at the mouth of the funnel, which, based on the distance from the active site and the known length of the lipoyl-lysine side chain, is likely to bind to the corresponding E2 lipoyl domain. These results are consistent with a previous report indicating that E1-E2 lipoyl domain binding stability is increased in the presence of the substrate pyruvate (31). Given the strong structural similarity between substrate and analogue, we suggest that the ordering observed in the E1-PLThDP complex would also be present when the intermediate from the natural substrate pyruvate is formed, and the increased E1-E2 stability may be explained by E2-lipoyl domain interactions with the resulting newly ordered E1 surface residues.

It appears that nearly all of the induced structural changes begin with the critical His-407-PLThDP interaction within the active site and then propagate outward, both to another previously disordered loop and to the helical region that was always ordered but now has its location shifted. This can be understood as follows. The first newly ordered loop (401–413) is initially stabilized by the direct and water-mediated hydrogen bonds formed between a phosphoryl (or carboxyl in the case of LThDP) oxygen atom and His-407 and Gln-408 as seen in Fig. 3. The second newly ordered loop (541–557, located at the mouth of the funnel), although not forming any direct interactions with PLThDP/LThDP, is then initially stabilized through the formation of hydrogen bonds between Asn-404 in the (now ordered) first loop and the side chains of Gln-548 and Asp-549. Further stabilization of this segment occurs via interactions near the ends of the (always ordered) helical segment Thr-525–Ile-535, which shifts by 2.7 Å relative to the native structure. These interactions involve formation of hydrogen bonds between Ile-537 and Lys-557 and between Arg-524 and Ala-554, with the latter being water-mediated. Although only the directed and specific hydrogen bonds were described, numerous favorable van der Waals contacts are also present and contribute to the stabilization. The end result is that the reaction intermediate, both newly ordered loops, and the shifted helical segment are all interlinked through hydrogen-bonding and van der Waals contacts to form a more tightly packed dimer with a completed active site channel and a new surface near its entrance. These results are consistent with the fact that the disorder-to-order transformation occurs only when both His-407 and the intermediate analogue PLThDP (or presumably LThDP) are present in the enzyme.

Our previous biochemical studies on the H407A E1 variant clearly indicated its importance in post-decarboxylation steps for the PDHc reaction (19). The mutation was shown to dramatically decrease the rate of reductive acetylation, which could be caused either by direct participation in the catalytic reaction at the active site or by interfering with proper E1-E2 association within the PDHc multienzyme complex. The evidence was that the specific activity of this variant in terms of the overall PDHc assay is less than 0.15%, whereas there are only minor activity effects on the initial decarboxylation reaction step. The current results and interpretation are consistent with this study and imply that His-407 does indeed play a significant role in binding E1 to the E2-lipoyl domains, although direct participation, at least in the later stages of catalysis, cannot be ruled out. The observed change in the active site and its entrance pathway in the presence of substrate/analogue likely facilitates binding with the E2-lipoyl domain and insertion of the lipoyllysine side chain, so that the required (post decarboxylation) reductive acetylation can occur.

We also previously suggested that His-407 functions as a proton donor to a lipoamide sulfur prior to the addition of the enamine to the disulfide (19). It is now tempting to suggest that this protonation occurs after carbon dioxide release because of the availability of His-407 at that instant. When pyruvate adds to form the pre-decarboxylation complex, His-407 would form a hydrogen bond with the pyruvate adduct and trigger the disorder-order transformation in preparation to receive the lipoamide of the lipoyl domain. As the interaction with E2 progresses and the Cα-C (carboxyl) bond is severed, releasing CO₂, the hydrogen bonds from the carboxyl group to His-407 and Tyr-599 obviously will no longer exist, and the lipoyl domain lipoamide can move into the space vacated by the CO₂ molecule in preparation to seize the acyl group. An intriguing question arises about events that occur during the interm period between decarboxylation and reductive-acetylation. Does a reversal of the conformational transformation (order-to-disorder) take place upon severance of the hydrogen bond between His-407 and the carboxylate group, or does the ordered structure remain intact, at least temporarily, until reductive acetylation is completed? In the first instance, the implication is that the ordered structure is unnecessary for the remaining reductive acetylation function to be completed in E1. In the second case, His-407 would be required to form a hydrogen bond to another group (on the lipoyl domain or on E1) until reductive acetylation is completed. An answer to these questions hopefully will be obtained when structural information on a complete E1-E2 complex becomes available.

It is noteworthy that the order-disorder transformation in this α2 E1 dimer differs considerably from those that have been observed in certain α2β2 E1 heterotetramers (14, 32). In the latter cases, the addition of the cofactor alone to the disordered apoenzyme produced an ordered holoenzyme. In the α2 homodimeric E1 protein, disorder occurs in both the apoenzymes and holoenzymes, and it is only the formation of the pre-decarboxylation reaction intermediate that triggers ordering. In addition, the loops in α2 E1 that exhibit transformation are not the same as those reported to undergo transformation in the α2β2 cases (14, 32, 33).

The purpose of the disorder-to-order transformation in homodimeric α2 E1s induced by substrate binding apparently is to activate the enzyme for reductive acetylation by preparing the channel in the active site to receive the E2 lipoyl domain lipoamide group. Interestingly, the phosphorylation regulatory mechanism responsible for deactivating an α2β2 heterotetrameric E1b component operating on branched chains also involves an order/disorder transformation in the corresponding sequence segment (34) but functions in reverse! In that case, substrate binding is not required as the E1b holoenzyme structure is already fully ordered, and phosphorylation deactivates the enzyme by inducing disorder within the segment. It is noteworthy that the phosphorylation site, Ser-292, corresponds to Gln-408 in the α2 E1 structure, which is not only in the newly ordered segment but is adjacent to the critical His-407 residue! Thus both dimeric and tetrameric E1s appear to effectively utilize disorder/order transformations in the same regions with similar trigger points but in different ways and in response to different stimuli.

Finally, with regard to general, enzyme-mediated thiamin catalysis, we now suggest that removal of strain created by the expected, corresponding, out-of-plane distortion in the α-LThDP complex may be a contributing force behind the accelerated decarboxylation that is seen when the thiamin-catalyzed reaction occurs in enzymes and that the strained structure may represent a transition state analogue. We have
shown that the out-of-plane distortion is caused primarily by a short and repulsive N4′−O2α distance, which is the result of the V conformation imposed on the ThDP cofactor by the enzyme. This is apparent since in the absence of enzyme, the PLThDP structure has a C2−Cα bond precisely in the plane of the thiazolium ring but has no short contacts because it is not in the V conformation (29). Once forced in the V conformation by the enzyme, however, if left in the planar state, an extremely short and excessively repulsive contact of 2.2 Å would result (Fig. 7c). To reduce the highly undesirable effects of this short contact, the non-planar distortion occurs, increasing the “bad” contact distance to a more tolerable 2.5 Å as is found in the E1-PLThDP structure and shown in Fig. 7b, although this still represents a strained molecule. Complete removal of the strain would occur only upon decarboxylation as then, the totally planar enamine form (Fig. 1, top right object) is created in which the formerly bad contact distance is increased to a favorable 2.8 Å as seen in Fig. 7c. The distorted, observed structure reported here and shown in Fig. 7b is clearly shifted toward the product enamine structure, both in terms of the N4′−O2α distance and in terms of the methyl carbon and O2α locations, which are now close to being in the plane of the thiazolium ring. It could thus be described as a transition state, in which the energy trapped in the C2α out-of-plane distortion is utilized to help break the C=O bond to form the enamine, in a manner requiring minimal atomic motion for the remaining atoms to attain their positions in the product state. Note that if the alternative initial decarboxylated form, the tetrahedral C2α carbanion (Fig. 1, top second object from the right), were created, the same strained out-of-plane distortion observed in the original pre-decarboxylation complex would persist, suggesting that the planar enamine form is indeed preferred. This is consistent with the fact that in the published post-decarboxylation crystal structures (11, 12, 14), the intermediates that were reported to best fit the electron density corresponded to planar enamines rather than tetrahedral C2α carbanions.

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