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Theoretical calculation of the binding free energies for pyruvate dehydrogenase E1 binding with ligands

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Abstract—We have tested a computational protocol based on molecular mechanics-Poisson–Boltzmann surface area (MM–PBSA) free-energy calculations to examine the detailed microscopic structures and binding free energies for the pyruvate dehydrogenase multienzyme complex (PDHc) E1 binding with its ligands (cofactor and inhibitors). The calculated binding free energies are all in good agreement with available experimental data, with an average absolute deviation of ~0.7 kcal/mol, suggesting that the computational protocol tested may be valuable in future rational design of new, more potent inhibitors of PDHc E1.

As the initial member of the pyruvate dehydrogenase multienzyme complex (PDHc), PDHc E1 plays a pivotal role in cellular metabolism to convert the product of glycolysis (pyruvate) to acetyl-CoA.[1] The later is one of the two compounds needed for condensation to citrate and required for tricarboxylic acid (Krebs, or citric acid) metabolic cycle:

$$\text{Pyruvate} + \text{coA} + \text{NAD}^+ \rightarrow \text{acetyl-coA} + \text{CO}_2 + \text{NADH} + \text{H}^+ \quad (1)$$

PDHc E1, using thiamin diphosphate (ThDP) and Mg^{2+} as its cofactors, catalyzes the first step of the multistep reaction process. In all thiamin-dependent enzymes, as depicted in Figure 1, the catalytic reaction is initiated by the formation of a covalent adduct between the substrate and cofactor ThDP through the C2 atom of the thiazolium ring. For this reason, blocking this site by replacing the proton on C2 with an oxygen atom as in thiamine thiazolone diphosphate (ThTDP), with a sulfur atom as in thiamine thiothiazolone diphosphate (ThTTDP), or with methylacetylphosphonate (PLThDP), inactivates the enzyme.[2,3] Depicted in Figure 2 are the molecular structures of these three inhibitors and the cofactor.

Nemeria and Jordan et al. reported K_i values for these three inhibitors, that is, 0.003 μM for ThTDP, 0.064 μM for ThTTDP, and 6.69 μM for PLThDP.[3,4] These data show that the binding affinities of ThTDP and ThTTDP are three- or two-orders of magnitude higher than that of cofactor ThDP ($K_d \approx K_M$ = 1.58 μM),[3] whereas the binging affinity of PLThDP is close to that of ThDP. ThTDP and ThTTDP have been considered as 'transition state' analog (TSA)-type inhibitors of the ThDP-dependent decatboxylations. Their structures and bond polarization are similar to those of the 2α-hydroxylethylidene-ThDP (the enamine) intermediate and the transition state involved in the chemical reaction. PLThDP is a stable structural analog of the covalently bound, pre-decarboxylation reaction intermediate and it mimics the structure of the reactive tetrahedral intermediate α-LThDP in the decarboxylation step of the PDHc E1 reaction. The X-ray crystal structures have recently been reported for *Escherichia coli* PDHc E1 binding with ThDP,[5] ThTDP,[6] and PLThDP.[7] The reported X-ray crystal structures demonstrate some important structural features of the enzyme-ligand binding. It has been recognized that the hydrogen bonding in the vicinity of the cofactor-binding site is a crucial factor affecting the relative binding affinities between PDHc E1 and its ligands.

For rational design of more potent inhibitors of PDHc E1, it is essential to establish a reliable computational protocol capable of predicting the relative binding

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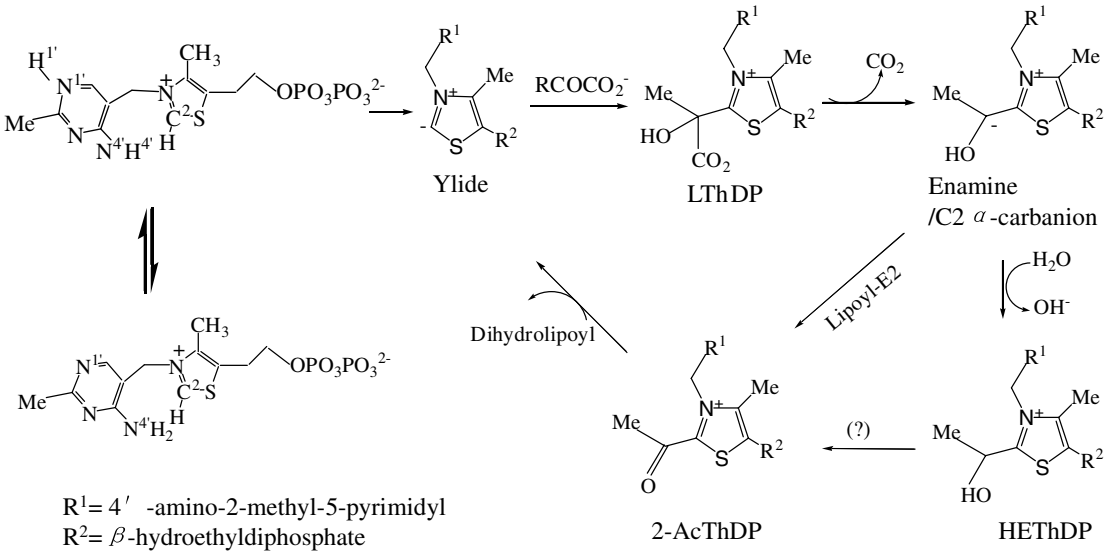

Figure 1. Schematic representation of the reaction pathway relevant to the function of PDHc E1. The initial steps involve formation of the ylide (deprotonation at C2) and the pyruvate adduct, lactyl-ThDP (LThDP). Decarboxylation of LThDP results in the enamine carbanion intermediate, which proceeds to form C2-acetyl-ThDP (2-AcThDP).

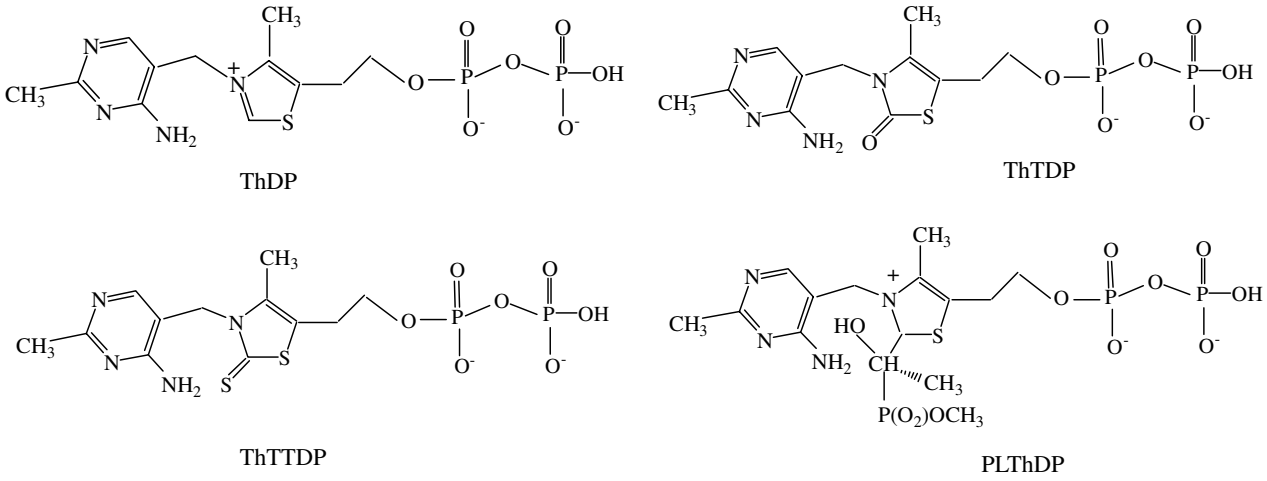

Figure 2. Schematic representation of thiamin diphosphate (ThDP), thiamine thiazolone diphosphate (ThTDP), thiamine thiothiazolone diphosphate (ThTTDP), and methylacetylphosphonate (PLThDP).

affinities of PDHc E1 binding with its ligands. Based on the available X-ray crystal structures, a computational protocol has been examined to model the detailed microscopic structures and binding free energies for the PDHc E1 binding with ThDP, ThTDP, ThTTDP, and PLThDP. The calculated binding free energies are in good agreement with available experimental data, suggesting that the computational protocol used in this study may be useful in future rational design of new inhibitors of PDHc E1.

To model PDHc E1 binding with the ligands, the crystal structures of the *E. coli* PDHc E1-ThDP-Mg^{2+} complex (1L8A),[5] *E. coli* PDHc E1-ThTDP-Mg^{2+} complex (1RP7),[6] and *E. coli* PDHc E1-PLThDP-Mg^{2+} complex (2G25).[7] were used to build the initial structures of the PDHc E1 binding with ThDP, ThTDP, and PLThDP. The initial structure of the PDHc E1-ThTTDP-Mg^{2+} complex was built from the X-ray crystal structure of PDHc E1-ThTDP-Mg^{2+} complex by changing an oxygen atom in the PDHc E1-ThTDP-Mg^{2+} complex to a sulfur atom. The standard protonation states at physiological condition (pH $\sim$ 7.4) were set to all ionizable residues of the protein. The initial structures were energy-minimized by using the Sander module of Amber8 program suite.[8] The non-bonded model was used for the metal ion Mg^{2+} with the default parameters in the program (e.g., the point charge was +2). The partial atomic charges used for the non-standard residues (i.e., the ligands ThDP, ThTDP, ThTTDP, and PLThDP) were calculated by using the restricted electrostatic-potential (RESP) fitting protocol implemented in the Antechamber module of the Amber8 program following electrostatic potential (ESP) calculations at *ab initio* HF/6-31G* level. Each aforementioned initial structure was neutralized by adding counter ions

and was solvated in a rectangular box of TIP3P water molecules with a minimum solute-wall distance of 10 Å. The PDHc E1 including Mg^{2+} ion has a very large negative charge of −44e, and the net charges of ligands ThDP, ThTDP, ThTTDP, and PLThDP are −2e, −3e, −3e, and −3e, respectively. So 46 Na^+ were added to neutralize the solvated system PDHc E1-ThDP-Mg^{2+} complex and 47 Na^+ were added to neutralize the other three solvated systems, that is, the PDHc E1-ThTDP-Mg^{2+}, PDHc E1-ThTTDP-Mg^{2+}, and PDHc E1-PLThDP-Mg^{2+} complexes. The total number of atoms in each solvated protein structure for the energy minimization was larger than 200,000, although the total number of atoms of each enzyme-ligand complex was only about 25,000.

For the energy minimization on each solvated complex, the particle mesh Ewald (PME) method was used to treat long-range electrostatic interactions.[9] Ten angstrom was used as the none-bonded cutoff during the energy minimization. First of all, the protein (including Mg^{2+}) and ligand were frozen and the solvent water molecules with the counter ions were allowed to move during a 2000-step energy minimization process. Then, only the added hydrogen atoms were energy-minimized for 2000 steps. Finally, all the atoms were allowed to relax by a 2000-step full energy minimization.

The binding free energies were calculated by using a molecular mechanics-Poisson–Boltzmann surface area (MM–PBSA) free-energy calculation method.[10] In the MM–PBSA method, the Gibbs free energy of the inhibitor binding, ΔG_{bind}, is obtained from the difference between the free energies of the receptor-ligand complex (G_{cpx}) and the unbound receptor (G_{rec}) and ligand (G_{lig}) as following:

$$\Delta G_{bind} = G_{cpx} - (G_{rec} + G_{lig}) \quad (2)$$

The molecular structures used in the free energy calculations were obtained from the aforementioned energy-minimized systems. The binding free energy (ΔG_{bind}) was evaluated as a sum of the changes in the MM gas-phase binding energy (ΔE_{MM}), solvation free energy shift (ΔG_{solv}), and entropy contribution ($-T\Delta S$). The MM binding energies were calculated by using the Sander module of Amber8 program without using the cutoff following the energy minimization with the cutoff, as we did in our recent MM–PBSA calculations on other receptor-ligand binding systems.[11–13]

$$\Delta G_{bind} = \Delta E_{bind} - T\Delta S \quad (3)$$

$$\Delta E_{bind} = \Delta E_{MM} + \Delta G_{solv} \quad (4)$$

$$\Delta E_{MM} = \Delta E_{ele} + \Delta E_{vdw} \quad (5)$$

We note that the ΔE_{MM} value calculated by using Eq. (5) is actually the gas phase internal change in the standard thermodynamics. ΔH_{bind} (gas) = ΔE_{MM} when the binding process does not change the volume under constant temperature (T) and pressure (P). ΔE_{MM} is expected to be very close to ΔH_{bind} (gas) as the volume change during the binding process should be negligible. In addition, $\Delta G_{solv} \approx \Delta E_{solv}$ under the usual standard states (without any change on the temperature, pressure, or concentrations). The electrostatic solvation free energies used to evaluate ΔG_{PB} were calculated with the finite-difference solution to the Poisson–Boltzmann (PB) equation as implemented in the Delphi program.[14,15] The default van der Waals radii were used for all atoms, except for Mg^{2+} ion. The missing van der Waals radius for Mg^{2+} was set to 1.55 Å in all of our solvation calculations. The dielectric constants used in the solvation calculations are 1 for the solute and 80 for the solvent water. Further, the entropy contribution, $-T\Delta S$, to the binding free energy was calculated according to the empirical method developed by Bardi etc.[16]

The energy minimizations were performed on a HP Superdome, at the Center for Computational Sciences, University of Kentucky. The other computations were carried out on SGI Fuel workstations and a 34-processors IBM × 335 Linux cluster in our own lab.

Our modeled structures provide more detailed binding information. The previous X-ray crystal structures of PDHc E1 binding with ThDP, ThTDP, and PLThDP, despite of the fact that the positions of the hydrogen atoms were not available due to the limitation of the X-ray diffraction approach, demonstrated some important hydrogen bonds between PDHc E1 and the ligand (ThDP, ThTDP, or PLThDP).[5–7] Not surprisingly, our energy-minimized microscopic binding structures of the all-atom systems (including the coordination sphere of Mg^{2+}) are all consistent with the corresponding X-ray crystal structures. In addition, the energy-minimized structures also help us to more clearly observe all of the hydrogen bonds between PDHc E1 and its ligand, including some hydrogen bonds (with His640 and Val192) that have not been mentioned in previous publications.[5–7] In both the PDHc E1-ThDP and PDHc E1-ThTDP binding complexes (Fig. 3), the protonated N atom of His640 side chain forms a hydrogen bond with $N^{4'}$ of the ligand and the carbonyl oxygen of Val192 backbone forms a hydrogen bond with $N^{4'}$ to $H^{4'}$ of the ligand. Our energy-minimized structure of the PDHc E1-ThTTDP binding complex is very similar to the X-ray crystal structure of the PDHc E1-ThTDP binding complex. The only difference is that a water molecule formed a hydrogen bond with the carbonyl oxygen of ThTDP in the PDHc E1-ThTDP complex. Such a hydrogen bond is lost when the carbonyl oxygen in ThTDP is replaced by a sulfur atom in ThTTDP.

Summarized in Table 1 are the energetic results obtained from the MM–PBSA calculations, in comparison with the available experimental data. As seen in Table 1, the individual ΔE_{MM} and ΔG_{solv} values calculated for PDHc E1 binding with different ligands are quite different. However, the sum of these two terms always gives a ΔE_{bind} value ranging from −19.68 to −24.70 kcal/mol. So, the gas phase interaction energies are balanced with the corresponding solvation free energy energies. Further including the entropy contribution to the binding free energy, the calculated ΔG_{bind} values are −9.0, −12.5, −9.5, and −7.7 kcal/mol for the PDHc E1 binding with ThDP, ThTDP, ThTTDP, and PLThDP, respectively. The calculated binding free energies are in good agreement

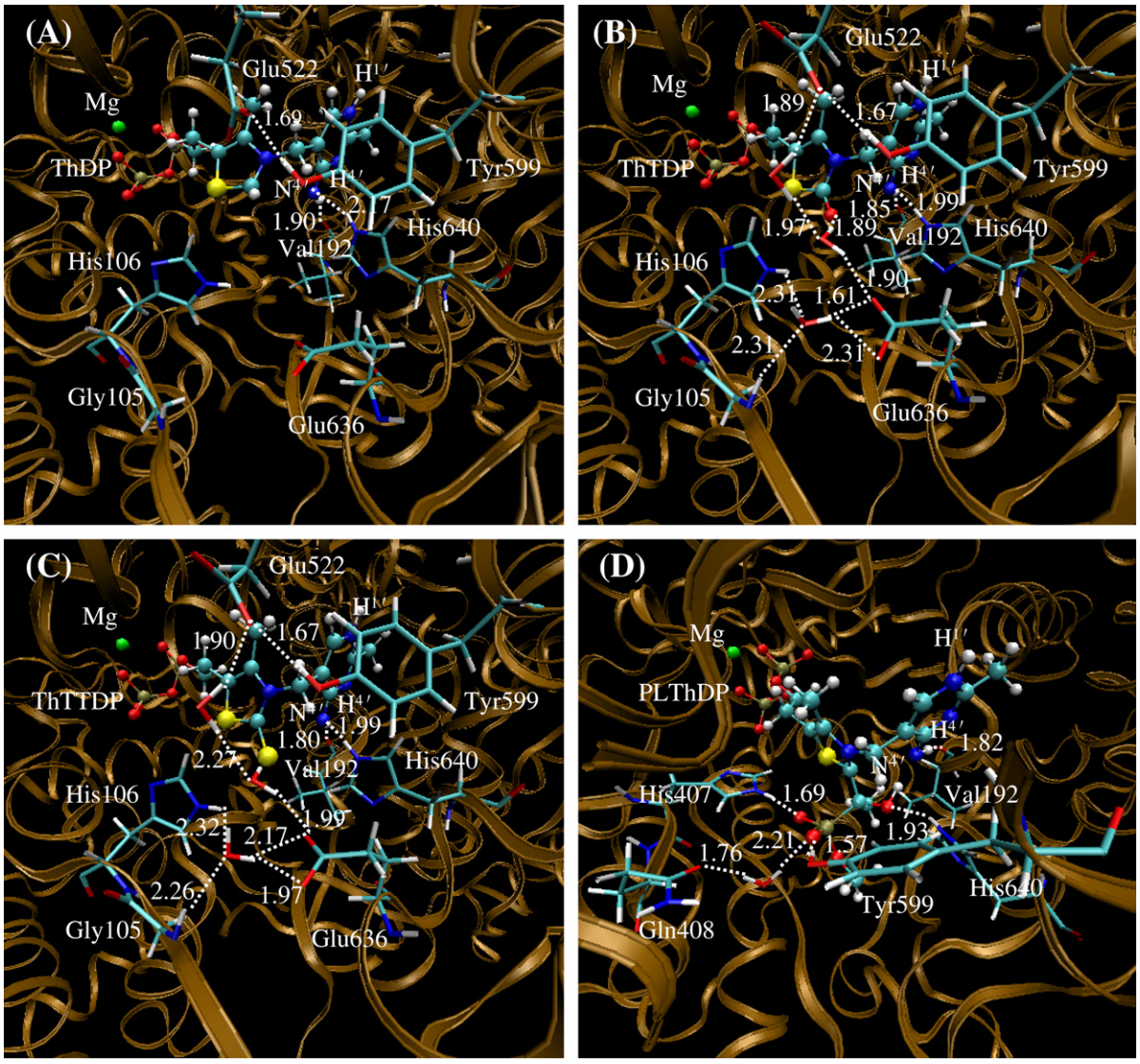

Figure 3. The energy-minimized geometries of the PDHc E1 binding with ligands. The ligand and Mg^{2+} are represented by the balls. The sticks refer to the key residues interacting with the ligand. The residues coordinating with Mg^{2+} and the residues that have common hydrogen bonds with the $P_2O_6^{2-}$ group or $H^{1'}$ atom in all of the four complexes are not shown in order to make other important interactions clear. (A) PDHc E1-ThDP complex; (B) PDHc E1-ThTDP complex; (C) PDHc E1-ThTTDP complex; (D) PDHc E1-PLThDP complex.

Table 1. Binding free energies (kcal/mol) calculated by using the MM–PBSA method for PDHc E1 binding with its ligands in comparison with available experimental data

	ThDP	ThTDP	ThTTDP	PLThDP
ΔE_{MM}[a]	−154.48	197.52	213.13	−80.06
ΔG_{solv}[a]	134.8	−222.22	−235.80	59.48
ΔE_{bind}[a]	−19.68	−24.70	−22.68	−20.59
$-T\Delta S$[a]	10.67	12.18	13.16	12.89
ΔG_{bind}[a]	−9.0	−12.5	−9.5	−7.7
Expt. ΔG_{bind}[b]	−7.9	−11.6	−9.8	−7.1
Expt. K_M or K_i[b] (μM)	1.58	0.003	0.064	6.69

[a] The results determined by the MM–PBSA calculations.
[b] The experimental ΔG_{bind} values were derived from the experimental K_M and K_i values reported in Refs. 3 and 4.

with the corresponding experimentally-derived binding free energies, −7.9, −11.6, −9.8, and −7.1 kcal/mol for the PDHc E1 binding with ThDP, ThTDP, ThTTDP, and PLThDP, respectively. Qualitatively, no matter whether the calculated or experimental binding free energies are used, the order of the binding affinities of the ligands with the enzyme is always ThTDP > ThTTDP > ThDP > PLThDP (from the highest binding affinity to the lowest). Quantitatively, the average absolute deviation of the calculated binding free energies from the corresponding experimental values is ~0.7 kcal/mol. The good agreement between the computational results and the experimental data suggests that the computational protocol tested in this study may be valuable in future rational design of new, more potent inhibitors of PDHc E1.

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