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DFT and MP2 studies on the C2–C2α bond cleavage in thiamin catalysis

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ABSTRACT

Thiamin diphosphate (ThDP), the biologically active derivative of vitamin B_1, is an important cofactor of several enzymes that catalyze the oxidative and non-oxidative conversion of α-keto acids. The final step of non-oxidative decarboxylation of pyruvate by pyruvate decarboxylase – the liberation of acetaldehyde – requires deprotonation of the α-hydroxyl group and cleavage of the C2–C2α bond of the transitory 2-(1-hydroxyethyl)-ThDP intermediate. It has been proposed that the cofactor 4′-amino/imino function is essentially involved in the deprotonation of the α-hydroxyl group. Proton transfer and C2–C2α cleavage may occur in a stepwise manner, or, alternatively in a concerted mechanism. Here, density functional theory (DFT) calculations as well as second order Møller–Plesset perturbation theory (MP2) studies were performed on a simple model for the enzyme using the program package Gaussian 03. Calculations favor a stepwise mechanism with initial formation of the C2α alkoxide, followed by C2–C2α bond cleavage.

1. Introduction

The general principles of enzymic and non-enzymic thiamin catalysis have already been revealed by many studies [1–5]. That notwithstanding, there still remain open questions in the mechanistic understanding of some elementary steps of catalysis taking place in the protein environment. X-ray structural analysis of thiamin diphosphate (ThDP)-dependent enzymes [6–8], ^{1}H NMR-based analysis of reaction intermediates [9], proton/deuterium exchange experiments [10], infrared difference spectroscopic [11] and mutagenesis data [12] have consistently supported a model in which the interaction of a strictly conserved glutamate (Glu) side chain with N1′ atom of ThDP triggers activation of the cofactor. Most notably in that concern, the reactivity of the exocyclic 4′-amino group is controlled by this protein-cofactor proton shuttle in a way that it can act both as proton donor (aminopyrimidine or aminopyrimidinium forms) and as proton acceptor (1′,4′-imino form) in the various steps of enzymatic catalysis. Here, we have performed density functional theory (DFT) calculations on the B3LYP/6-31G(d) and 6-311G(p,d) levels as well as second order Møller-Plesset perturbation theory (MP2) studies within a 6-31G(d) basis set using the program package Gaussian 03 [13]. The three methods include the effect of electron correlation on different levels. The consideration of this effect in calculations is crucial for describing chemical phenomena, e.g. linkage and cleavage of bonds. It should be verified by the comparative studies if the DFT method with the standard 6-31G(d) basis set describes the biochemical model systems in a sufficient way. This would be a justifiable argument to extend the studies on this level to larger systems including important amino acid residues of the protein environment with maintainable effort.

2. Results and discussion

Calculations were started from the N1′-deprotonated form of 1′,4′-imino 2-(1-hydroxyethyl)-ThDP (HEThDP), which is assumed to be a key-intermediate in the catalytic cycle of pyruvate decarboxylase, and will be generated from 1′,4′-imino HEThDP involving proton transfer to the conserved Glu in the active site (Fig. 1). Although we are aware that (i) deprotonation of N1′ will generate a high-energy N-based anion (a seemingly unlikely prospect), and that thus (ii) deprotonation of N1′ is likely coupled to protonation of N4′, we have – for the sake of clarity – decided not to include protein side chains in this first approach.

Since the early theoretical studies on thiamine model systems by Jordan [14], several independent quantum chemical studies on the electronic structure of thiamin and of related compounds have been performed [15–21]. So far, there are only scarce quantum chemical studies on key-intermediates employing more sophisticated methods including for instance electron correlation effects. Therefore, we have conducted systematic calculations including one- and two-fold potential energy surface (PES) scans in order to characterize microscopic steps of acetaldehyde liberation as being part of the catalytic sequence in pyruvate decarboxylase. From X-ray studies on enzyme-bound ThDP and functional investigations it is

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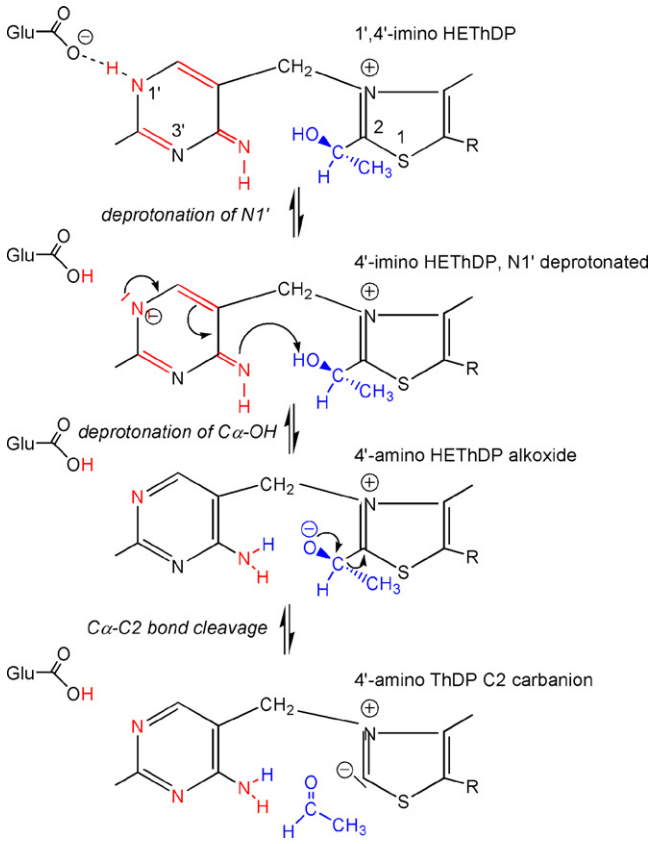

Fig. 1. Microscopic steps of acetaldehyde liberation in pyruvate decarboxylase catalysis.

established [22] that the diphosphate moiety has an anchor function for cofactor binding and is essentially not involved in catalysis. Therefore, we have restricted our model calculations to the corresponding thiamin (Th) systems to reduce computational effort. Moreover, we have modeled the influence of the apoenzyme environment in a very simple way by fixing the values of the characteristic torsion angles $\Phi_T = 93^\circ$ and $\Phi_P = -68^\circ$ as observed in the enzyme-bound state [7]. The enforced V-like conformation is a significant feature of enzyme-bound ThDP and fixes the amino/imino group in the immediate vicinity of the thiazolium C2. As stated above, no active site residues were included within the simplified

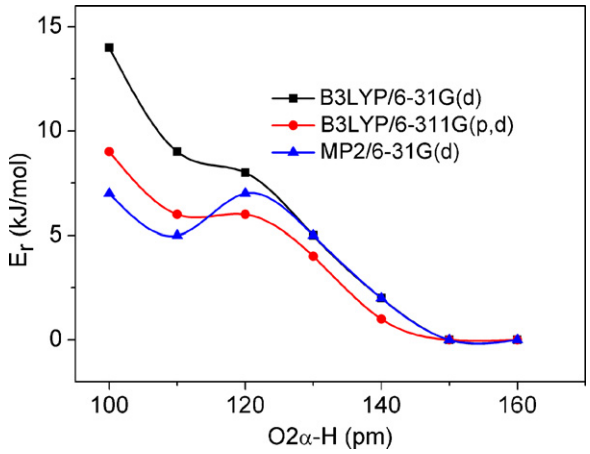

Fig. 2. Analysis of proton transfer from O2α–H···4′N in the N1′ deprotonated form of 1′,4′-imino HETh by different methods (constraints with respect to C2–C2α bond length).

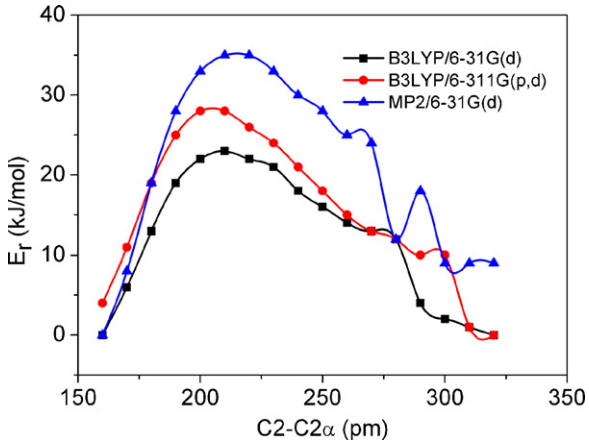

Fig. 3. Reaction coordinate for cleavage of the C2–C2α bond in the 4′-amino HETh alkoxide calculated by different methods.

model. In a first step, we have calculated the reaction coordinate of the O2α–H···4′N proton transfer from the tautomeric hydroxyethyl to the oxyethyl anion form of the 1′,4′-imino HETh anion with a constraint of 155 pm imposed on the C2–C2α bond. The results are illustrated in Fig. 2.

The calculations with all three methods consistently indicate that proton transfer is energetically favorable by about 7–15 kJ/mol. The pathways exhibit either no (DFT) or a small barrier (MP2). Next, cleavage of the C2–C2α bond was investigated starting from the final oxyethyl anion structure of the proton transfer as considered above. The results of the calculations are summarized in Fig. 3.

The three methods indicate significant activation barriers for the liberation of acetaldehyde, though slightly different barriers were calculated: 23 kJ/mol (B3LYP/6-31G(d)), 28 kJ/mol (B3LYP/6-311G(d,p)) and 35 kJ/mol (MP2/6-31G(d)), respectively. Major deviations in the shape of the curves were found for bond distances larger than 280 pm, especially evident for the DFT and MP2 data. Transition state searches embedded within the QST3 option in Gaussian 03 [13] were carried out for C2–C2α bond cleavage. This procedure optimizes a transition state (TS) based on the structures of the reactant, the product and an initial TS as input. These data are available from the results in Fig. 3. The three

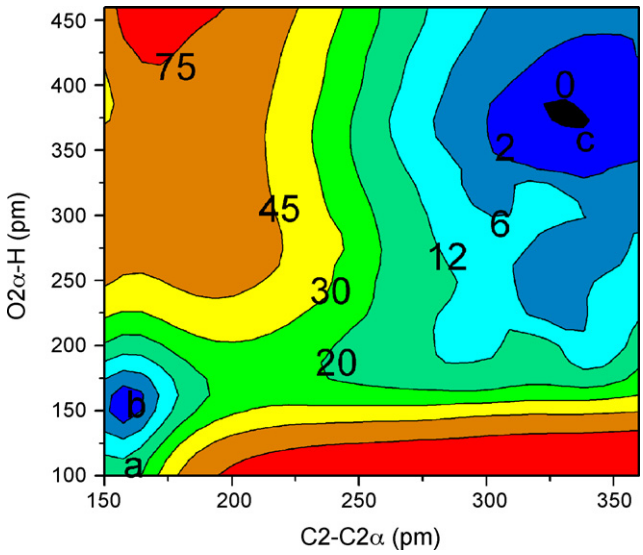

Fig. 4. Two-fold PES scan for the liberation of acetaldehyde in thiamin catalysis (**a** → **b** proton transfer, **b** → **c** C2–C2α cleavage, numbers of the lines with same relative energy in kJ/mol).

methods characterize the transition state structure by the following values for the C2–C2α bond length and activation barriers: 213 pm/16 kJ/mol (B3LYP/6-31G(d)), 208 pm/21 kJ/mol (B3LYP/6-311G(d,p)), and 215 pm/27 kJ/mol (MP2/6-31G(d)). The results are in good agreement with the findings presented in Fig. 3. A two-fold PES scan of the two-step mechanism was performed on the B3LYP/6-31G(d) level to elucidate the reaction pathway for the liberation of acetaldehyde in thiamin catalysis in a more complex way. The distances O2α-H (100–450 pm) and C2–C2α (150–350 pm) were stepwise changed in 10 pm increments. The findings of the partial optimization procedure with constraints to the O2α–H and C2–C2α bonds are shown in Fig. 4.

The starting point **a** corresponds to the N1′-deprotonated form of 1′,4′-imino HETh. The downhill O2α–H···4′N transfer yields the 4′-amino HETh alkoxide and is indicated as minimum **b** on the PES. The reaction coordinate of the C2–C2α cleavage goes from **b** over a saddle point of approximately 20 kJ/mol to the product state **c**. At this point the acetaldehyde is liberated with concomitant formation of the C2 carbanion of 4′-amino Th. The results of the reaction coordinates within the simple model thus suggest a two-step mechanism for the liberation of acetaldehyde in thiamin catalysis.

3. Conclusions

Both the DFT calculations with different basis sets and the MP2 studies indicate qualitatively comparable findings for the characterized transition states. The results of the reaction coordinates within the simple model suggest a two-step mechanism for the liberation of acetaldehyde in thiamin catalysis. For a further understanding of the mechanistic aspects more specific environmental effects will have to be included. Moreover, the findings foreshadow that this aim could be achieved on the B3LYP/6-31G(d) level with sufficient quality and maintainable effort. Finally, a comparison of the results obtained by the simple non-enzymic model with those of an enhanced one would pave the way for understanding specific effects of the enzyme environment on elementary steps in thiamin catalysis.

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