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Computer-assisted study on the reaction between pyruvate and ylide in the pathway leading to lactyl–ThDP

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Abstract In this study the formation of the lactyl–thiamin diphosphate intermediate (L–ThDP) is addressed using density functional theory calculations at X3LYP/6-31++G(d,p) level of theory. The study includes potential energy surface scans, transition state search, and intrinsic reaction coordinate calculations. Reactivity is analyzed in terms of Fukui functions. The results allow to conclude that the reaction leading to the formation of L–ThDP occurs via a concerted mechanism, and during the nucleophilic attack on the pyruvate molecule, the ylide is in its AP form. The calculated activation barrier for the reaction is 19.2 kcal/mol, in agreement with the experimental reported value.

Keywords L–ThDP · Mechanism · DFT · Fukui · Activation barrier

Introduction

Thiamin diphosphate (ThDP) is an important coenzyme in a variety of enzymes involved in the decarboxylation of α-keto acids in sugar metabolism. It is composed of two aromatic rings, a 4-aminopyrimidine ring and a thiazolium ring bridged by a methylene group [1, 2]. During the catalysis by ThDP enzymes, the 4′-aminopyrimidine moiety can interconvert among four ionization/tautomeric states: the 4′-aminopyrimidine (AP), the N1′-protonated 4′-amino pyrimidium (APH^+), 1′,4′-iminopyrimidine (IP), and the C2-ionizated ylide (Y1). In all ThDP-dependent enzymes, the catalytic cycle is initiated with the attack of the C2 atom of the ylide on the Cα of a pyruvate molecule to form the lactyl–ThDP (L–ThDP) intermediate, which then undergoes decarboxylation to form the hydroxyethylthiamin diphosphate (HEThDP) enamine/carbanion. Then, HEThDP reacts with a second molecule of an α-keto acid to form the intermediate AHA–ThDP, which finally leads to the product release and the ylide recover (Fig. 1).

Despite the number of articles published on the catalytic cycle of ThDP-dependent enzymes, still there exist some aspects which remain unknown or controversial [3–6]. Specifically on the L–ThDP intermediate, there are some issues that stay unclear, namely, the protonation states of the N1′ and N4′ atoms, during the attack of C2 on the Cα of pyruvate, since not all of the ionization/tautomeric forms have been clearly characterized [2]. On the other hand, the manner the reaction occurs, i.e., via a stepwise or concerted mechanism, has not been clearly elucidated and remains as a controversial issue [7].

In this study the formation of the L–ThDP intermediate is addressed using high level density functional theory calculations, X3LYP/6-31++G(d,p). The study includes potential energy surface (PES) scans in order to identify and characterize critical points on it, transition state search, and intrinsic reaction coordinate calculations. Reactivity is analyzed in terms of Fukui functions.

Electronic supplementary material The online version of this article (doi:10.1007/s10822-012-9589-3) contains supplementary material, which is available to authorized users.

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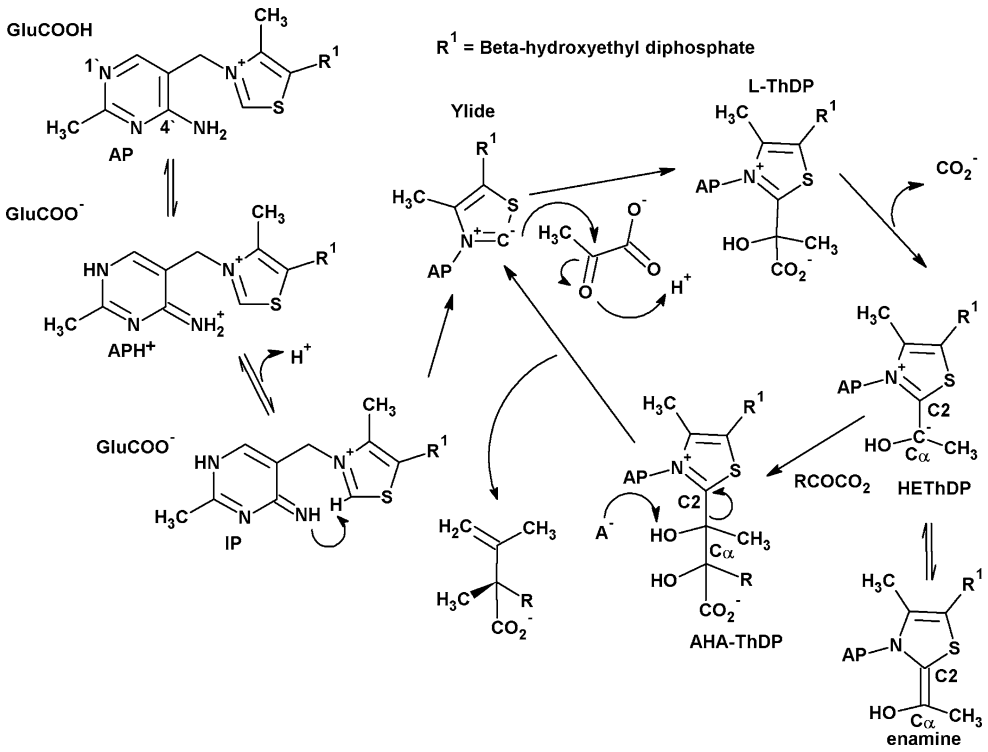

Fig. 1 Catalytic cycle of ThDP-dependent enzymes

Computational methods

The quantum chemical calculations were performed considering a reduced system consisting of ThDP, the conserved side chain of glutamic acid interacting with the N1′ atom of ThDP, and the pyruvate molecule. In order to simplify the calculations the diphosphate group of ThDP was replaced with –OH, since its primary function is to anchor the cofactor and it is not involved in the catalytic mechanism; and the side chains of the protein were ignored except for the above mentioned glutamic which was replaced by acetic acid. The geometries of all structures were optimized in gas phase using the same level of theory X3LYP/6-31++G(d,p). This functional has been reported to give very good geometries of hydrogen-bonded complex [8, 9].

The PES was explored defining two reaction coordinates, namely, the distance C2–Cα, defined between the C2 atom of the pyrimidyl ring of the ylide and the Cα of the pyruvate molecule; and the distance O–H, defined between the carbonyl oxygen atom on Cα, in the pyruvate molecule, and the proton in the 4′-amino group of ThDP (Fig. 2). The first reaction coordinate corresponds to the attack of C2 on the Cα of pyruvate, and the second one corresponds to the proton transfer from the N4′atom to the the carbonyl oxygen of the pyruvate molecule. Critical points on the PES were characterized by vibrational frequency calculations in order to identify local minima or saddle points.

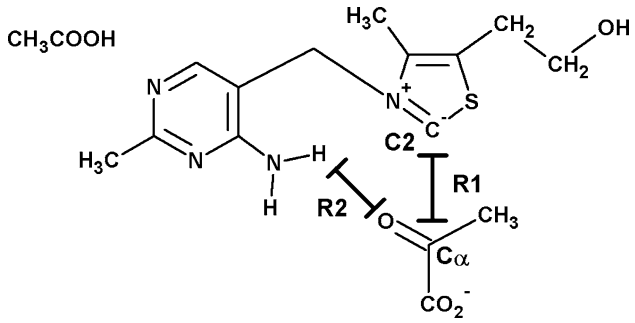

Fig. 2 Definition of the reaction coordinates

Intrinsic reaction coordinate (IRC) calculations were carried out at the same level of theory to confirm the optimized transition state geometry. All quantum chemical calculations in this study were carried out using the Jaguar 7.0 suite of programs [10].

Results and discussion

Prior to the exploration of the PES, the protonation state of the pyrimidyl ring, upon the attack of the ylide on the pyruvate molecule, was investigated in terms of reactivity as expressed by the Fukui functions. The results show that in the case of the APH^+ form, the accepted form in literature [11–13], the most important nucleophile centers are the oxygen atoms of the carboxylate group interacting with N1′ atom, and not the C2 atom as required for a

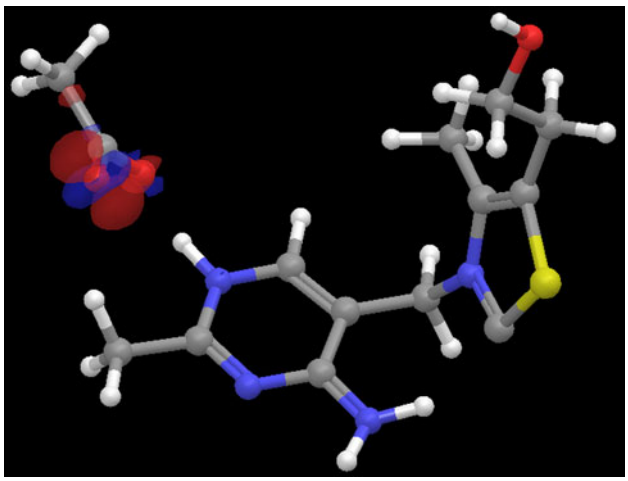

Fig. 3 Nucleophilic character of the ylide in its APH^+ form as expressed by the f^- Fukui function (*red cloud*)

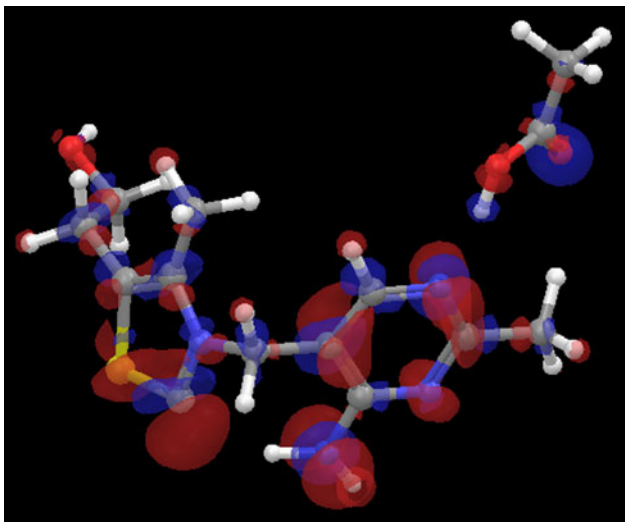

Fig. 4 Nucleophilic character of the ylide in its AP form as expressed by the f^- Fukui function (*red cloud*)

nucleophilic attack on the Cα atom of the pyruvate molecule (Fig. 3). This suggests that the carboxylic group should be protonated, as expected from the values of the physiological pH and the pK_a of glutamic acid in the enzymatic ambient. The AP form, instead, shows the C2 atom as the most important nucleophile center, as expected (Fig. 4). The calculated atomic f_{C2}^- Fukui index on the C2 atom, for the AP and APH^+ forms, are 0.26 and 0.00, respectively, corroborating the strong nucleophilic character of the C2 atom in the AP form, versus its negligible nucleophilic tendency in the APH^+ form. These results suggest that the nucleophilic attack of the C2 atom on the Cα of pyruvate requires the ylide in the AP form, ylide (AP). This finding has not been reported earlier and represent a new approach of the reaction mechanism.

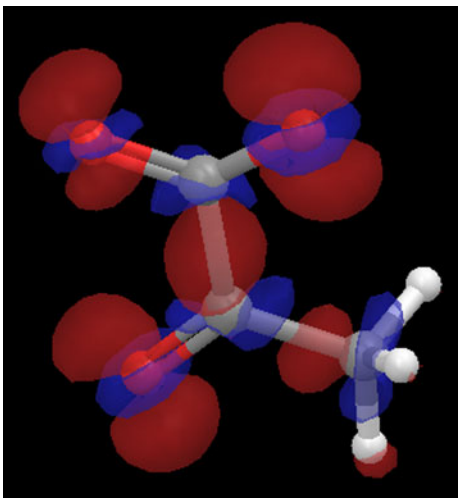

Fig. 5 Nucleophilic character of the isolated pyruvate molecule as expressed by the f^- Fukui function (*red cloud*)

On the other hand, the reactivity analysis of the pyruvate molecule shows important reactivity as nucleophile of the oxygen atoms, whereas the Cα atom appears to be an electrophile center (Fig. 5). The respective condensed-to-atom Fukui indices for the Cα and carbonyl oxygen atoms are $f_{C\alpha}^+ = 0.50$ and $f_O^- = 0.24$. These complimentary chacteristics between the ylide and pyruvate assure that the reaction be feasible, therefore the exploration of the PES was carried out considering the ylide (AP) form.

The PES shows three critical points that are associated to the reactants (R), transition state (TS) and product (L–ThDP) (Figs. 6, 7). The topology of the surface suggests that the reaction occurs via a concerted mechanism, i.e., the C2 atom gradually attacks the carbonyl oxygen of

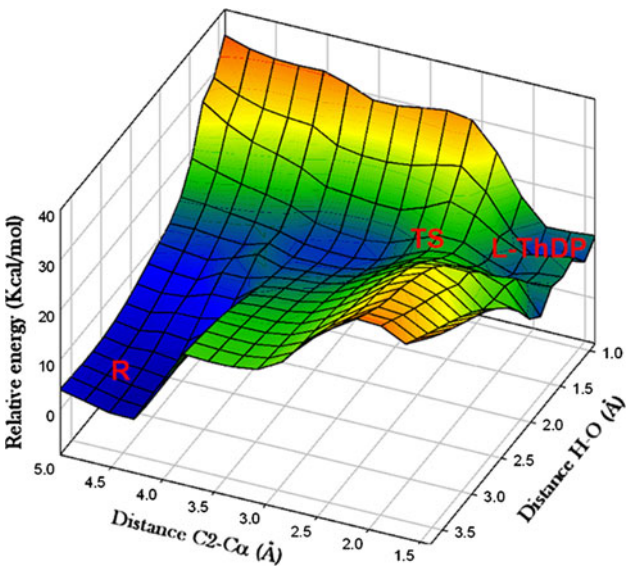

Fig. 6 3-D view of the potential energy surface (PES)

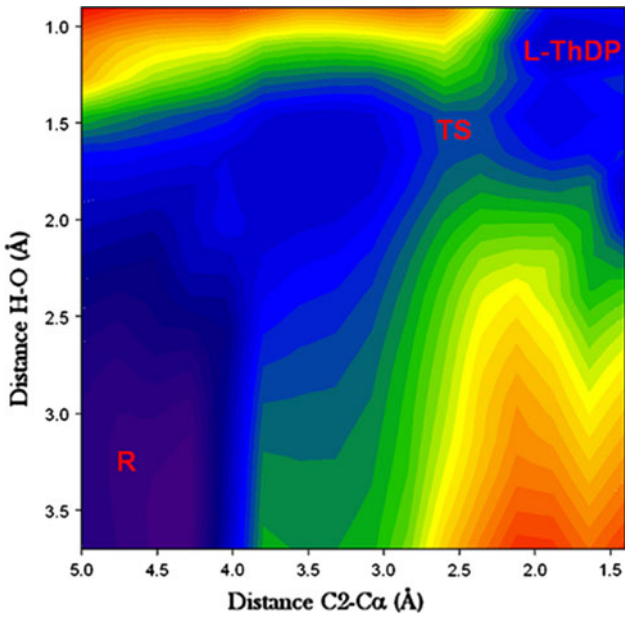

Fig. 7 2-D view of the potential energy surface (PES)

pyruvate, while the proton on the N4′ atom is gradually transferred to the carbonyl oxygen of pyruvate. The existence of one saddle point is also observed in the reaction pathway.

The optimized structure of the reactant state, coordinates ~4.5, 3.5 on the PES, is shown in Fig. 8. The dihedral angles ϕ_T and ϕ_P for the ylide are 98° and –84°, respectively. Even though in the ylide (AP) the N1′ atom is not protonated, the proton of the carboxylic group is in close proximity, 1.67 Å, forming a strong hydrogen bond between them.

The TS search was carried out using a quasi-Newton method as implemented in Jaguar. The method searches for the TS nearest to the initial geometry guess. The initial TS geometry was estimated from the location of the observed saddle point on the PES. The search led to a structure having one and only one negative frequency, $-219.68\ cm^{-1}$, as required to confirm the presence of a TS. This frequency corresponds to the stretching of the C2 ←→ Cα and O ←→ H bonds, supporting the concerted character of the mechanism insinuated from the PES. The optimized structure of the TS is shown in Fig. 9 and the respective Cartesian coordinates are given as supplementary information. In this postulated TS, with the N1′ atom deprotonated, the atomic Fukui index on the carbonyl oxygen of pyruvate reaches the maximum value of 0.27, compared to the value of 0.24 for the isolated pyruvate molecule, indicating the increase of its nucleophilic character as the Cα atom is attacked by the C2 atom of the ylide, as expected. Figure 10 shows the f^- Fukui function for the TS. It is observed that the f^- function isosurface, the red lobe, of the C2 atom is pointing to the Cα of pyruvate, evidencing that the nucleophilic attack is in progress. On the other hand, for pyruvate the f^- function of the carbonyl oxygen is pointing to the proton of the 4′ amino group, denoting the proton transfer from the 4′-amino group is on the way.

Intrinsic reaction coordinate (IRC) calculations were carried out to confirm that the observed TS corresponds to the reaction of interest. The experiments done in forward

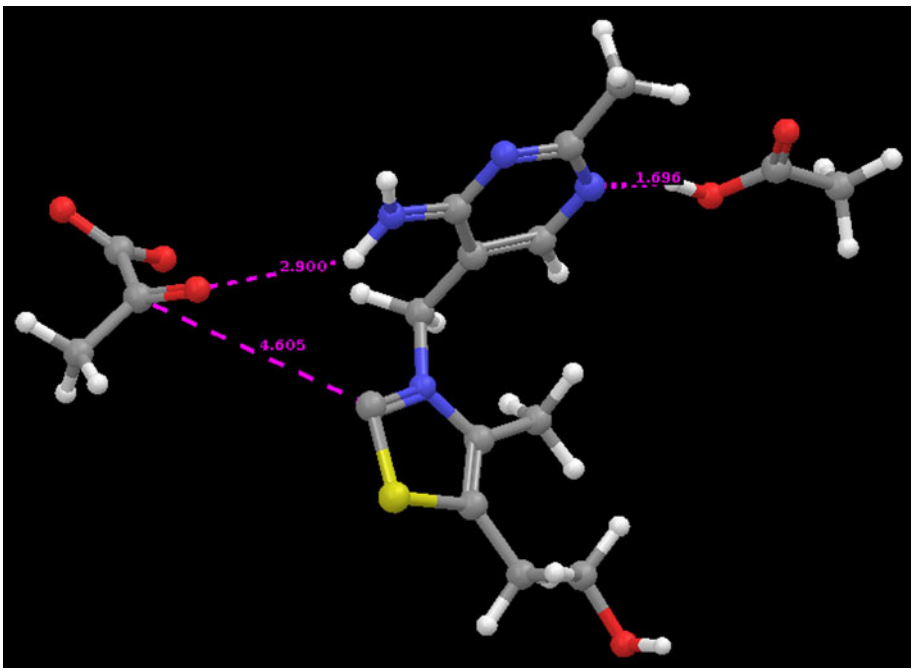

Fig. 8 Optimized structure of the reactant state

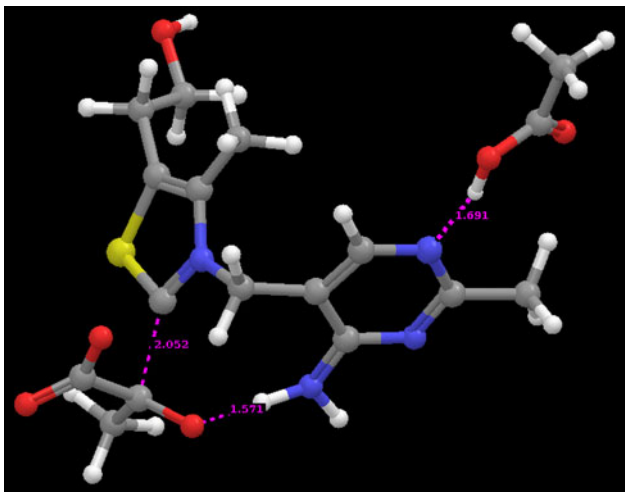

Fig. 9 Optimized structure of the transition state

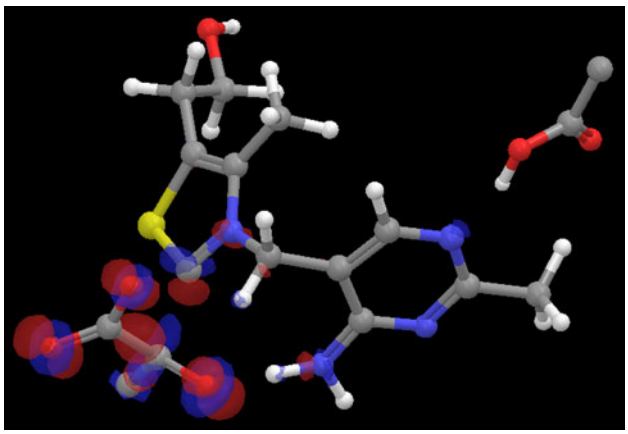

Fig. 10 Nucleophilic character of the transition state as expressed by the f^- Fukui function (*red cloud*)

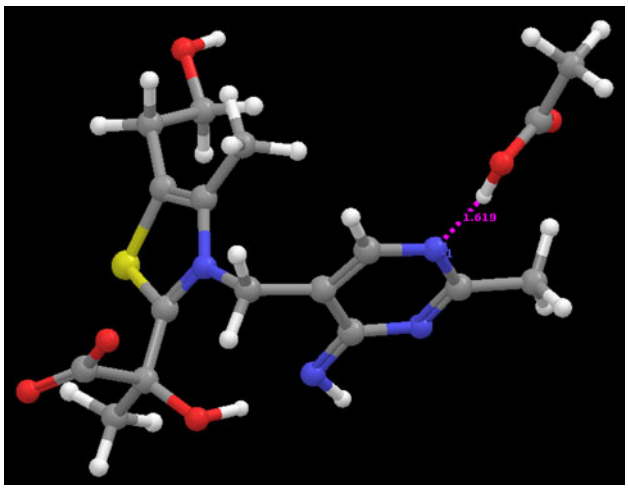

Fig. 11 Optimized structure of the product L–ThDP

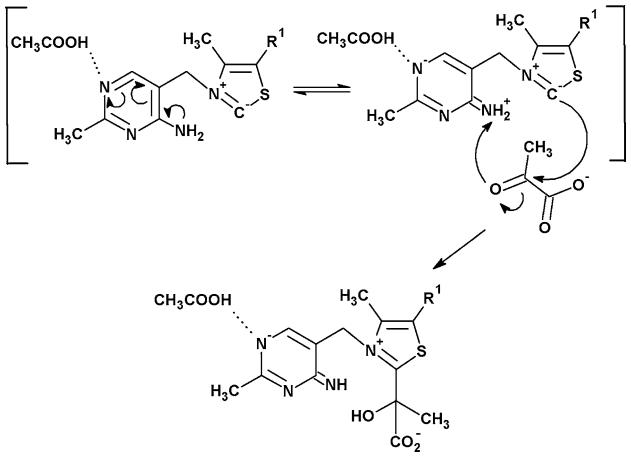

Fig. 12 Proposed mechanism for the formation of the intermediate L–ThDP

and reverse directions led to the expected species, L–ThDP and R, respectively. The calculated activation barrier for the product formation results to be 19.3 kcal/mol, in agreement with reported experimental value of 16.2 kcal/mol [5]. On the other hand, if the TS is considered to be in its usual reported APH^+ form, i.e., the N1′ atom protonated, the activation barrier increases reaching the value of about 28 kcal/mol. This change in the activation barriers is caused by differences in stability, in the first case the negative charge is delocalized in the pyrimidine ring giving stability to the system, whereas in the second case the negative charge is lying on the two oxygen atoms of the carboxylate group solely. In addition, in the present postulated TS the carbonyl oxygen of pyruvate is in close proximity to the proton of the 4′-amino group of the thiazolium ring, 1.57 Å, favoring the transference. Figure 11 shows the optimized structure for the product L–ThDP, it is observed that it exists in a tautomeric form in which the N1′ atom is deprotonated and the N4′ atom is in its imino form; the respective dihedral angles ϕ_T and ϕ_P are 108.1 and −61.9. The respective cartesian coordinates are given as supplementary information. This structure gives a strong basic character to the 4′-imino group, consequently we conjecture that it could detach a proton from the environment to regenerate the 4′-amino group.

Regarding the thermodynamics, the results show that the proposed reaction is slightly exergonic ($\Delta G^0 = -2.0$ kcal/mol). However, as many biochemical processes, this reaction is followed by an highly exergonic reaction, the decarboxylation of the L–ThDP, resulting in a highly thermodynamic favored process.

Finally, it is necessary, however, to mention that the definition of other reaction coordinates may alter slightly the results and the derived conclusions of this study.

Conclusions

The main conclusions of this study can be summarized as follows: (1) the reaction leading to the formation of L–ThDP occurs via a concerted mechanism, i.e., the carboligation C2–Cα and the proton transfer from the 4′amino group to the carbonyl oxygen of the pyruvate molecule occur simultaneously, (2) during the reaction, the N1′atom of the pyrimidyl ring is deprotonated, (3) the optimized structure of L–ThDP shows the N4′ atom in its imino form and the N1′ atom deprotonated. In light of the above results, we propose the mechanism depicted in Fig. 12 for the formation of the intermediate L–ThDP. Consequently, it may be concluded that the participation of the pyrimidyl ring in the APH^+ form, in the reaction between pyruvate and the ylide, is not supported by the quantum chemical calculations of this study. In closing, we can mention that these new findings should be valuable for rational design of novel herbicides and antibacterial drugs, as it was stressed in a recent paper [7].

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