J Mol Model (2013) 19:3799–3803
DOI 10.1007/s00894-013-1908-7

ORIGINAL PAPER

Electron density reactivity indexes of the tautomeric/ionization forms of thiamin diphosphate

Gonzalo A. Jaña · Eduardo J. Delgado

Received: 22 October 2012 / Accepted: 3 June 2013 / Published online: 23 June 2013

Abstract The generation of the highly reactive ylide in thiamin diphosphate catalysis is analyzed in terms of the nucleophilicity of key atoms, by means of density functional calculations at X3LYP/6–31++G(d,p) level of theory. The Fukui functions of all tautomeric/ionization forms are calculated in order to assess their reactivity. The results allow to conclude that the highly conserved glutamic residue does not protonate the N1′ atom of the pyrimidyl ring, but it participates in a strong hydrogen bonding, stabilizing the eventual negative charge on the nitrogen, in all forms involved in the ylide generation. This condition provides the necessary reactivity on key atoms, N4′ and C2, to carry out the formation of the ylide required to initiate the catalytic cycle of ThDP-dependent enzymes. This study represents a new approach for the ylide formation in ThDP catalysis.

Keywords DFT · Reactivity indexes · ThDP · Ylide

Introduction

Thiamin diphosphate (ThDP) is a cofactor that assists in the catalysis of carbon-carbon bond-forming and bond-breaking reaction in sugar metabolism. It is composed of two aromatic rings, one 4′ aminopyrimidine ring and one thiazolium ring bridged by a methylene group. According to the literature, in the catalytic cycle the 4′-aminopyrimidine ring can interconvert among four ionization/tautomeric states, Fig. 1, the 4′-aminopyrimidine (AP), the N1′-protonated 4′-aminopyrimidium (APH^+), 1′,4′-iminopyrimidine (IP), and the C2-ionizated ylide (Y1), whose formation is believed to activate the ThDP to initiate the catalytic cycle in thiamin dependent enzymes [1–6]. The general catalytic cycle for ThDP-dependent enzymes has been extensively studied in literature, both empirically [7–14] and theoretically [15–19]

In a recent paper [20], we reported a theoretical study on the reaction between pyruvate and ylide leading to the formation of the intermediate lactyl-ThDP. In this study it is found that the participation of ThDP with the pyrimidyl ring in its APH^+ form is not supported by the quantum chemical results. This finding motivated us to revisit the accepted pathway leading to the activation of ThDP, formation of the ylide, from the point of view of the nucleophilicity of the key atoms involved in the formation of the ylide.

In this article, continuing with the theoretical studies on ThDP catalysis, the nucleophilicity of the several species involved in the activation of ThDP is addressed using density functional theory (DFT) calculations at X3LYP/6–31++G(d,p) level of theory. The study includes calculation of Fukui functions and condensed-to-atom Fukui indices as a means to assess the electrophilic and nucleophilic character of key atoms in the pathway leading to the formation of the ylide. Hybrid QM/MM calculations were also carried out for the first stage.

Computational methods

The quantum chemical calculations were performed considering a reduced model consisting of ThDP and the conserved chain of glutamic acid interacting with the N1′ atom of the pyrimidyl ring. In order to simplify the calculations the

G. A. Jaña
Departamento de Ciencias Químicas,
Facultad de Ciencias Exactas, Sede Concepción,
Universidad Andrés Bello, Santiago, Chile

E. J. Delgado (✉)
Computational Biological Chemistry Group (QBC),
Faculty of Chemical Sciences, Universidad de Concepción,
Concepción, Chile
e-mail: edelgado@udec.cl

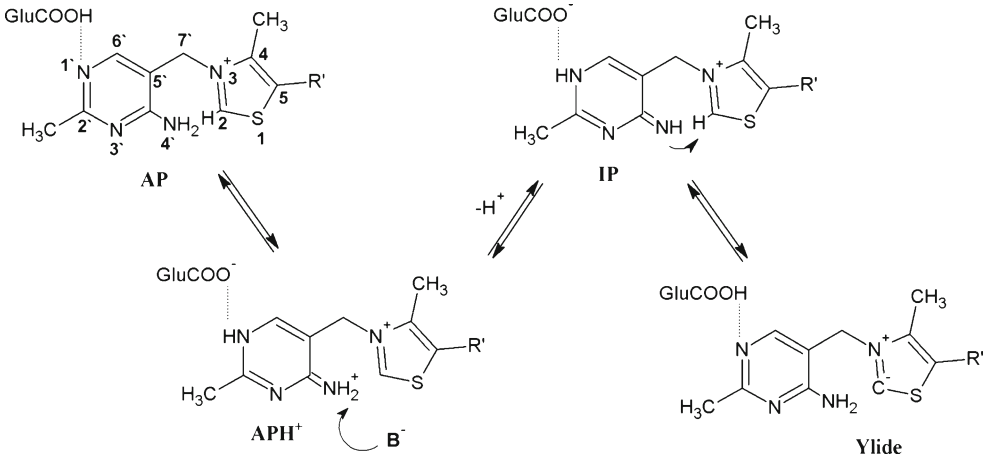

Fig. 1 Activation of ThDP according to the literature

diphosphate group of ThDP was replaced with a OH group, since its primary function is to anchor the cofactor and it is not involved in the catalytic mechanism; in addition the side chains of the protein were ignored except for the above mentioned glutamic residue 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 complexes. All quantum chemical calculations in this study were carried out using Jaguar 7.0 suite of programs [21]. Hybrid QM/MM calculations were carried out using QSite suite of programs [22]. In these calculations ThDP was considered in the QM region, while the rest of the protein in the MM region. The OPLS-2001 force field was used in the molecular mechanics part of the calculation.

Global Fukui function of a molecule as a whole measures the response of its ground-state electron density per unit shift in the system number of electrons for constant external potential due to the nuclei. The Fukui function and related hardness/softness characteristics constitute important reactivity criteria and give rise to corresponding contributions to the interaction energy between reactants. Several calculations schemes have been formulated in order to obtain the Fukui function within the molecular-orbital framework. Within the finite difference approximation, for example the Fukui function is calculated in terms of finite density differences between charged species [23]. Differential and variational approaches have also been reported [24, 25]. In Jaguar the regional Fukui functions $f^{+}(r)$ and $f^{-}(r)$ are calculated as differences in the electron density between the N-electron system on one hand, and the N+δ and N-δ systems on the other hand. A script runs all three calculations and generates the electron difference densities, which can be visualized as an isosurface.

Atomic Fukui indices, on the other hand, are an attempt to quantify the anticipated reactivity of a molecule in various types of reactions. These indices, based on Fukui functions, are calculated in Jaguar within the frozen-orbital approximation. In this approach the Fukui functions may be simply calculated from the frontier molecular orbital coefficients and overlap matrix. The condensed-to-atom index is readily obtained by summing over the atomic orbitals associated with each atomic center in the molecule [26, 27].

Results and discussion

According to the literature the sequence of reactions leading to the formation of the ylide begins with the protonation of the N1′ atom of the pyrimidyl ring, by the highly conserved glutamic acid residue to form the 4′-amino pyrimidium (APH^{+}) intermediate.

In order to investigate the reactivity of the N1′ atom, the Fukui $f^{-}(r)$ function as well as the atomic Fukui index on this atom were calculated. The $f^{-}(r)$ function is considered as a measurement of the nucleophilic character of the atom. The results, shown in Fig. 2, indicate the negligible nucleophilic character of the N1′ atom; on the other hand, the respective atomic Fukui index f_{N1}^{-} is just 0.01. Both results indicate that the protonation of the N1′ atom by the carboxylic group should not proceed, in agreement with the expected protonation state of glutamic acid from the values of its pK_a and the physiological pH. On the other hand, the optimization of the APH^{+} structure without constraints leads to the AP structure because of the high nucleophilic character of the carboxylic oxygens, Fig. 3. Energetically the AP structure is stabilized in about 23 kcal mol^{-1} respect to the APH^{+} structure. Similar computations considering the enzymatic ambient, QM/MM calculations, for the first stage do not show significant changes in the values of the atomic Fukui indices. These results support the hypothesis that the deprotonation of glutamic acid does not occur,

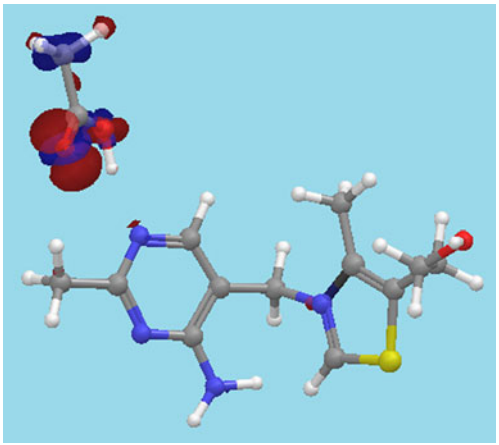

Fig. 2 Nucleophilic character of the AP form as expressed by the f^- Fukui function (red lobe)

instead the carboxylic hydrogen participates in a strong hydrogen bond with the N1′ atom located at a distance of about 1.7 Å.

The imino form, IP, is formed by the abstraction of a proton from the 4′-amino group of the pyrimidyl ring by a Lewis base whose identity is quite unknown so far. In the case of PDC, the residue glutamate 473 has been suggested as the possible base able to abstract this proton. However, in other ThDP-dependent enzymes, namely acetohydroxyacid synthase (AHAS), such residue is not present and no species has been suggested to play this role. Summarizing, the reaction of formation of IP remains as a chemical mystery and therefore it can not be addressed further.

The generation of the ylide requires the proton abstraction from the C2 atom by the N4′ atom of the IP form. Therefore,

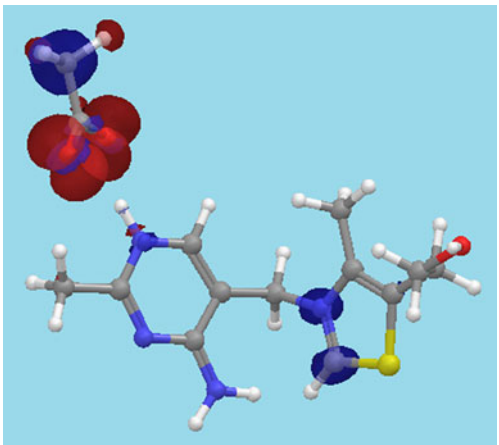

Fig. 3 Nucleophilic character of the APH^+ form as expressed by the f^- Fukui function (red lobe)

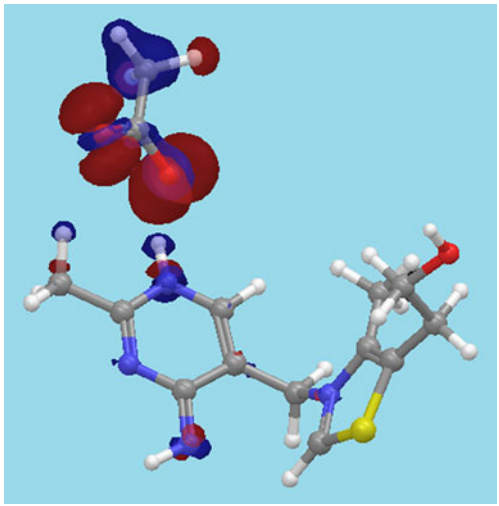

Fig. 4 Nucleophilic character of the N1′-protonated IP form as expressed by the f^- Fukui function.

the nucleophilic character of the N4′ atom plays a fundamental role in the formation of the ylide. In order to address the proton transference, the Fukui function and the atomic Fukui indices on this atom were calculated for two alternative forms of IP, those showing the N1′ atom protonated and deprotonated, respectively. The nucleophilic character of the N4′ atom as expressed by f^- Fukui functions is showed in Figs. 4 and 5. It is observed that the f^- isosurface is negligible in the structure having the N1′ atom protonated; while the other form, N1′ atom deprotonated, shows an important nucleophilic character as required for the proton

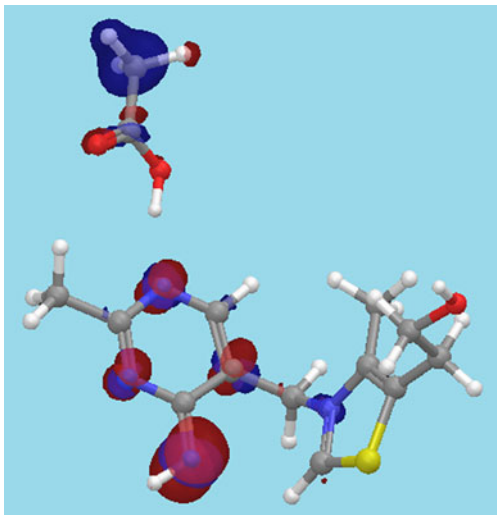

Fig. 5 Nucleophilic character of the N1′-deprotonated IP form as expressed by the f^- Fukui function

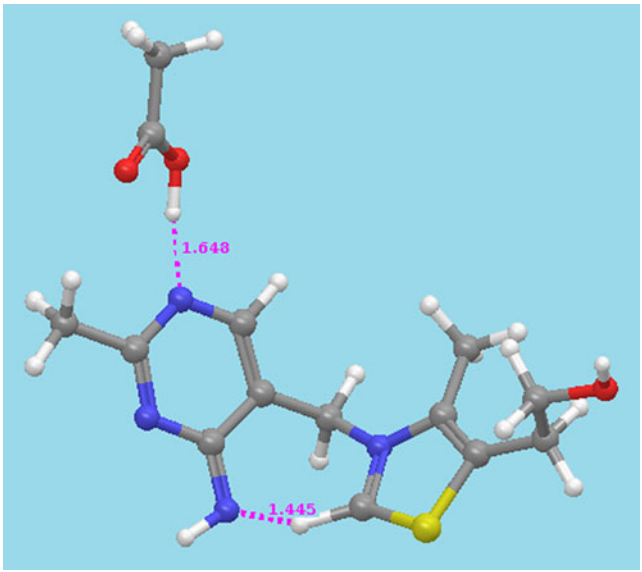

Fig. 6 Optimized structure of the transition state for the proton abstraction from the C2 atom

abstraction. The respective condensed–to-atom Fukui indices are 0.00 and 0.41, respectively. These results suggest that the imino form should be with the N1′ atom deprotonated in order to favor the proton abstraction. The optimized structures of both IP forms show the N4′ atom and the H-C2 atom at close distance, 2.39 Å. The search of the transition sate for this transference led to a structure, Fig. 6, having one and only one imaginary frequency, 893.2 cm^{-1}, corresponding to the stretching of the H←→ C2 bond. The respective ϕ_t and ϕ_p dihedral angles are 67.1 and −70.4. In this distorted V structure the N4′ atom is just at only 1.45 Å from the proton, which in turn is bonded to the C2 atom by means of a rather long bond, 1.25 Å, accounting for the proton transfer in progress. The calculated activation barrier is just 0.7 kcal mol^{-1}, as expected for a rapid proton transference. The results also show

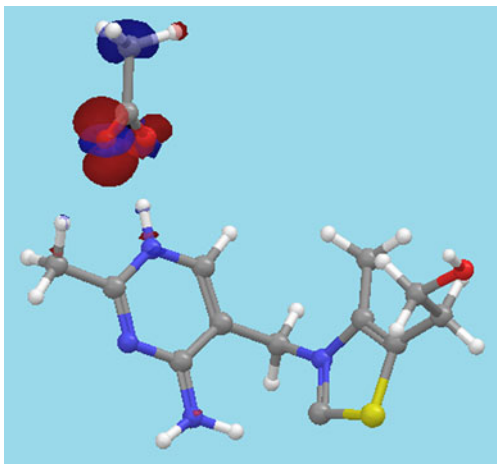

Fig. 7 Nucleophilic character of the N1′-protonated ylide form as expressed by the f^- Fukui function

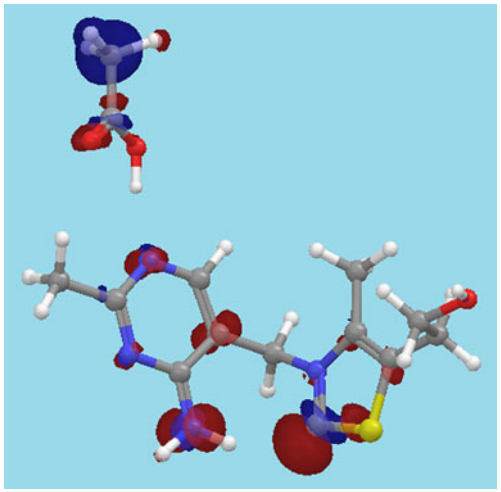

Fig. 8 Nucleophilic character of the N1′-deprotonated ylide form as expressed by the f^- Fukui function

that the reaction of formation of the ylide is exergonic with a standard free energy change of −35.19 kcal mol^{-1}.

The ylide so formed initiates the catalytic cycle with the nucleophilic attack on the Cα atom of the pyruvate molecule to form the intermediate lactyl-ThDP. The reaction is strongly dependent on the nucleophilic character of the C2 atom. To address this point the f^- Fukui functions on this atom were calculated. The results are shown in Figs. 7 and 8, it is observed that the ylide form having the N1′ atom deprotonated show an important nucleophilic character on the C2 atom, unlike the case in which the N1′ atom of the ylide is protonated, where the C2 atom does not show any tendency to carry out a nucleophilic attack. Instead, the most important nucleophilic reactivity is lying on the oxygen atoms of the carboxylate group, evidencing the stronger Lewis basicity of these atoms compared to the N1′ atom, and suggesting in this way that the N1′ atom should be deprotonated. The respective atomic Fukui indices on the C2 atom are 0.00 and 0.34 for the protonated and deprotonated N1′ atom forms, respectively.

Conclusions

The results of this study allow to conclude that the formation of the ylide requires an important nucleophilic character on the two key atoms, N4′ and C2; condition which is reached only when the N1′ atom is deprotonated, and whose eventual negative charge is stabilized by a strong hydrogen bonding with the carboxylic group, located at close distance. This status confers the N4′ atom with nucleophilic character necessary to abstract the proton from the C2 atom and in this way to form the ylide. On the other hand, it assists the C2 atom to perform the nucleophilic attack on the carbonyl atom of the pyruvate molecule to form the lactyl-ThDP intermediate. Summarizing, the results suggest that in all stages leading to

the formation of the ylide, the N1′ atom is deprotonated, in agreement to that expected from the pK_a of the glutamic residue in the proteic ambient and the physiologic pH. Hybrid QM/MM calculations for the first stage support the above DFT results for the reduced model. This finding has not been reported earlier and represents a new approach for the ylide formation.

Acknowledgments The financial support from Fondecyt, grant N^0 1130082, is gratefully acknowledged.

References

1. Kluger R, Tittmann K (2008) Thiamin diphosphate catalysis: enzymic and nonenzymic covalent intermediates. Chem Rev 108:1797–1833
2. Nemeria N, Korotchkina L, McLeish MJ, Kenyon GL, Patel MS, Jordan F (2007) Elucidation of the chemistry of enzyme-bound thiamin diphosphate prior to substrate binding: defining internal equilibria among tautomeric and ionization states. Biochemistry 46:10739–10744
3. Jordan F, Nemeria NS (2005) Experimental observation of thiamin diphosphate-bound intermediates on enzymes and mechanistic information derived from these observations. Bioorg Chem 33:190–215
4. Nemeria NS, Chakraborty B, Jordan F (2009) Reaction mechanisms of thiamin diphosphate enzymes: defining states of ionization and tautomerization of the cofactor at individual steps. FEBS J 276:2432–2446
5. Agyei-Owusu K, Leeper FJ (2009) Thiamin diphosphate in biological chemistry: analogues of thiamin diphosphate in studies of enzymes and ribowitches. FEBS J 276:2905–2916
6. Kern D, Kern G, Neef H, Tittmann K, Killenberg-Jabs M, Wikner C, Schneider G, Hübner G (1997) How thiamin diphosphate is activated in enzymes. Science 275:67–70
7. Zhang S, Liu M, Yan Y, Zhang Z, Jordan F (2004) C2-α-lactylthiamin diphosphate is an intermediate on the pathway of thiamin diphosphate-dependent pyruvate decarboxylation. J Biol Chem 279:54312–54318
8. Nemeria N, Chakraborty S, Baykal A, Korotchkine LG, Patel MS, Jordan F (2007) The 1′,4′-iminopyrimidine tautomer of thiamin diphosphate is poised for catalysis in asymmetric active centers on enzymes. Proc Natl Acad Sci USA 104:78–82
9. Tittmann K, Vyazmensky M, Hübner G, Barak Z, Chipman DM (2005) The carboligation reaction of acetohydroxyacid synthase II:steady-state intermediate distributions in wild type and mutants by NMR. Proc Natl Acad Sci USA 102:553–558
10. Balakrishnan A, Paramasivam S, Chakraborty S, Polenova T, Jordan F (2012) Solid-state nuclear magnetic resonance studies delineate the role of the protein in activation of both aromatic rings of thiamin. J Am Chem Soc 134:665–672
11. Tittmann K, Golbik R, Uhlemann K, Khailova L, Schneider G, Patel M, Jordan F, Chipman DM, Duggleby RG, Hübner G (2003) NMR analysis of covalent intermediates in thiamin diphos′hate enzymes. Biochemistry 42:7885–7891
12. Engel S, Vyazmensky M, Vinogradov M, Berkovich D, Bar-Ilan A, Qimron U, Rosiansky Y, Barak Z, Chipman DM (2004) Role of a conserved arginine in the mechanism of acetohydroxyacid synthase. J Biol Chem 279:24803–24812
13. Vyazmensky M, Steinmetz A, Meyer D, Golbik R, Barak Z, Tittmann K, Chipman DM (2011) Significant catalytic roles for Glu47 and Gln 110 in all four of the C-C bond-making and breaking steps of the reactions of acetohydroxyacid synthase II. Biochemistry 50:3250–3260
14. Blakrishnan A, Gao Y, Moorjani O, Nemeria NS, Tittmann K, Jordan F (2012) Bifunstionality of the thiamin diphosphate cofactor: assignment of tautomeric/ionization states of the 4′-aminopyrimidine ring when various intermediates occupy the active sites during the catalysis of yeast pyruvate decarboxylase. J Am Chem Soc 134:3873–3885
15. Friedemann R, Tittmann K, Golbik R, Hübner G (2004) DFT studies on key intermediates in thiamin catalysis. Int J Quantum Chem 99:109–114
16. Friedemann R, Tittmann K, Golbik R, Hübner G (2009) DFT and MP2 studies on the C2-C2α bond cleavage in thiamin catalysis. J. Mol. Catal. B: Enzymatic 61:36–38
17. Paramasivam S, Balakhrishnan A, Dimitrenko O, Godert A, Begley TP, Jordan F, Polenova T (2011) Solid-state NMR and density functional theory studies of ionization states of thiamin. J Phys Chem B 115:730–736
18. Xiong Y, Liu J, Yang GF, Zhan CG (2009) Computational determination of fundamental pathway and activation barriers for acetohydroxyacid synthase-catalyzedcondensation reactions of α-keto acids. J Comput Chem 31:1592–1602
19. Alstrup-Lie M, Schiott B (2008) A DFT study of solvation effects on the tautomeric equilibrium and catalytic ylide generation of thiamin models. J Comput Chem 29:1037–1047
20. Alvarado O, Jaña G, Delgado EJ (2012) Computer-assisted study on the reaction between pyruvate and ylide in the pathway leading to lactyl-ThDP. J Comput Aided Mol Des 26:977–982
21. Jaguar (2007) version 7.0, Schrodinger, LLC, New York, NY
22. QSite (2010) version 5.6, Schrodinger, LLC, New York, NY
23. Yang W, Mortier WJ (1986) The use of global and local molecular parameters for the analysis of the gas-phase basicity of amines. J Am Chem Soc 108:5708–5711
24. Michalak A, De Proft F, Geerlings P, Nalewajski RF (1999) Fukui functions from the relaxed kohn-Sham orbitals. J Phys Chem A 103:762–771
25. Chattaraj PK, Cedillo A, Parr RG (1995) Variational method for determining the Fukui function and chemical hardness of an electronic system. J Chem Phys 103:7645–7646
26. Contreras RR, Fuentealba P, Galvan M, Perez P (1999) A direct evaluation of regional Fukui functions in molecules. Chem Phys Lett 304:405–413
27. Chamorro E, Perez P (2005) Condensed-to-atoms electronic Fukui functions within the framework of spin-polarized density-functional theory. J Chem Phys 123:114107