ELSEVIER

Biophysical Chemistry 110 (2004) 259–266

Biophysical Chemistry

www.elsevier.com/locate/bpc

A quantum chemical study of the water-assisted mechanism in one-carbon unit transfer reaction catalyzed by glycinamide ribonucleotide transformylase

Qing An Qiao[a], Zheng Ting Cai[a,*], Da Cheng Feng[a], Yuan Sheng Jiang[a,b]

[a] *Institute of Theoretical Chemistry, Shandong University, Jinan 250100, China*
[b] *College of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China*

Received 15 January 2004; received in revised form 28 February 2004; accepted 3 March 2004
Available online 27 April 2004

Abstract

A theoretical study for the water-assisted mechanism in one-carbon unit transfer reaction catalyzed by glycinamide ribonucleotide transformylase (GAR Tfase) is investigated in which the proton transfers in an indirect way and the energy barrier for each transition state has been lowered about 80–100 kJ/mol when compared with the corresponding one in a no-water-involved mechanism. There are two possible pathways in each mechanism: one is concerted and the other is stepwise. Our results have verified the presumption from experiments that one water molecule can assist to achieve the whole reaction. Because the addition of this water molecule in the transition states can relax the strong strain in the unstable system and greatly lowered the energy barrier. The water-assisted paths are preferable to the no-water-involved ones and the bulk solvent effect of water is also discussed.

Keywords: One-carbon unit transfer; Water-assisted mechanism; Density Function Theory (DFT)

1. Introduction

The de novo purine biosynthetic consists of 10 enzymatic reactions that convert 5-phosphoribosyl-1-pyrophosphate to inosine monophosphate (IMP), which can then be converted to adenosine monophosphate and guanine monophosphate [1,2]. This pathway is used by virtually all organisms to produce purine nucleotides that are essential for many cellular processes. Consequently, this pathway has attracted considerable attention for the development of inhibitors, in particular for cancer chemotherapy since rapidly dividing cells require large amounts of purine. Glycinamide ribonucleotide transformylase (GAR Tfase, EC 2.1.2.2) is one of the two enzymes in the de novo purine biosynthetic pathway that use N10-formyl-5,6,7,8-tetrahydrofolic acid (10f-THF) as a cofactor for formyl transfer, the other being extensively studied in this pathway is 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (AICAR Tfase, EC 2.1.2.3). GAR Tfase catalyzes the conversion of β-glycinamide ribonucleotide (GAR) to formyl-β-glycinamide ribonucleotide (f-GAR) (Fig. 1). Because of its association with DNA synthesis, this enzyme has

* Corresponding author. Tel.: +86-531-8365748; fax: +86-531-8564464.
E-mail address: zhtcai@sdu.edu.cn (Z.T. Cai).

0301-4622/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2004.03.003

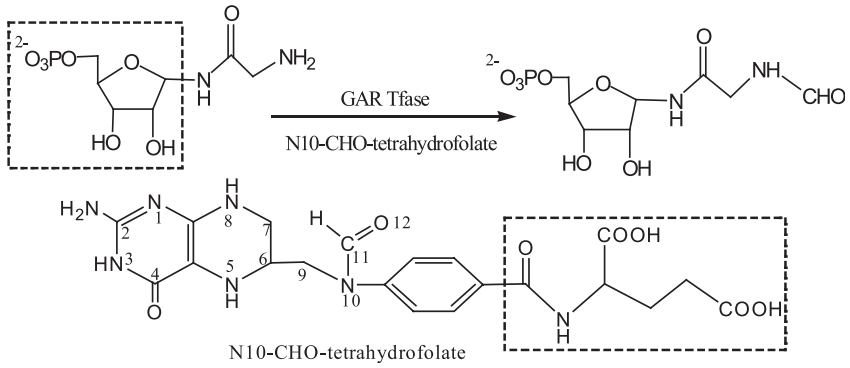

Fig. 1. The GAR Tfase catalyzed reaction and the molecular structure of 10f-THF.

become the target of anti-neoplastic agents. Much has been done on the catalytic mechanism of GAR Tfase and more and more important information has been achieved from experiments [2–8]. The required chemical steps in this reaction are the transfer of a formyl group, the one-carbon unit, from 10f-THF to the amino group of GAR, and a removal of one proton from the amino group of GAR to N10 atom of 10f-THF. Much effort has been done on the catalytic mechanism of GAR Tfase, but it is still unknown whether these steps occur concertedly or not [9], and a new mechanism needs to be invoked to explain the spectroscopic and crystallographic findings.

Water is one of the most common solvents in chemical reactions as well as in the enzymatic reactions. Of course, the solvent effect is an important factor to effect reactions, and a number of theoretical methods have been developed to give a reasonable consideration of it, such as the Onsager model [10], the Polarized Continuum Model (PCM) [11] and the (static) Isodensity Surface Polarized Continuum Model (IPCM) [12]. All these models have a common character, that is, the solvent molecules and the solute molecules are absolutely divided into two parts, and the electrostatic interaction of the solid can be included in the solute's Hamiltonian allowing the inclusion of polarization effects. In other words, all the solvent molecules serve as a whole polar environment and none of them can go into the solute system (which is the reactive area). But in actual systems, it is possible for the solute molecules to join the reaction system and assist to help to complete the whole process. Such phenomena have already been reported on hydrolyses of the β-lactam model system [13], in which a water molecule assisted in both the neutral and the alkaline hydrolyses. The results in Ref. [13] show that the water-assisted mechanism is different from solvent effect and it is much preferable.

Klein et al. [4] had proposed a water-assisted mechanism for GAR Tfase, and considered that the site of this water molecule might be fixed by a hydrogen bond with the carbonyl group of residue Asp 144. According to their opinion, such a structure could enhance the stability of the transition state complex and calibrate the proton transfer process. But the transition state cannot be located from experiments, and the presumption above cannot be confirmed by X-ray structure of GAR Tfase. Therefore, a precise theoretical description is quite necessary. The present paper has two purposes: (i) to give a detailed description of the water-assisted mechanism in one-carbon unit transfer reactions, and (ii) give a full comparison of the water-assisted mechanism with the no-water-involved one.

In the following calculations, only the GAR molecule and the N10-formyl-THF are included. It is a reasonable approximation because no other residues have been reported to be directly involved in the catalysis. Furthermore, the ribonucleotide part of GAR molecule and the glutamic acid residue (which are cycled by the dashed line rectangle in Fig. 1) in N10-formyl-THF are replaced with hydrogen atoms, as they do not directly participate in the one-carbon unit transfer process and both are more than 5 Å away from the formyl group in the X-ray crystal structure [14]. Therefore, the model system is a reasonable approximation of the real system.

In the model system, the one-carbon unit transfer can be achieved via two possible mechanisms: one is

the no-water-involved mechanism, referred as Mech. 1, the other is the water-assisted mechanism called Mech. 2. There are two possible reaction pathways in each mechanism. The first (path a, path a′) are concerted and the second (path b, path b′) are stepwise (Fig. 2). In the following, we will discuss Mech. 1 first.

2. Methodology

The full catalytic mechanisms of this model system are examined by the hybrid density functional theory (DFT) B3LYP [15] as implemented in Gaussian98 program package [16], a method which has previously

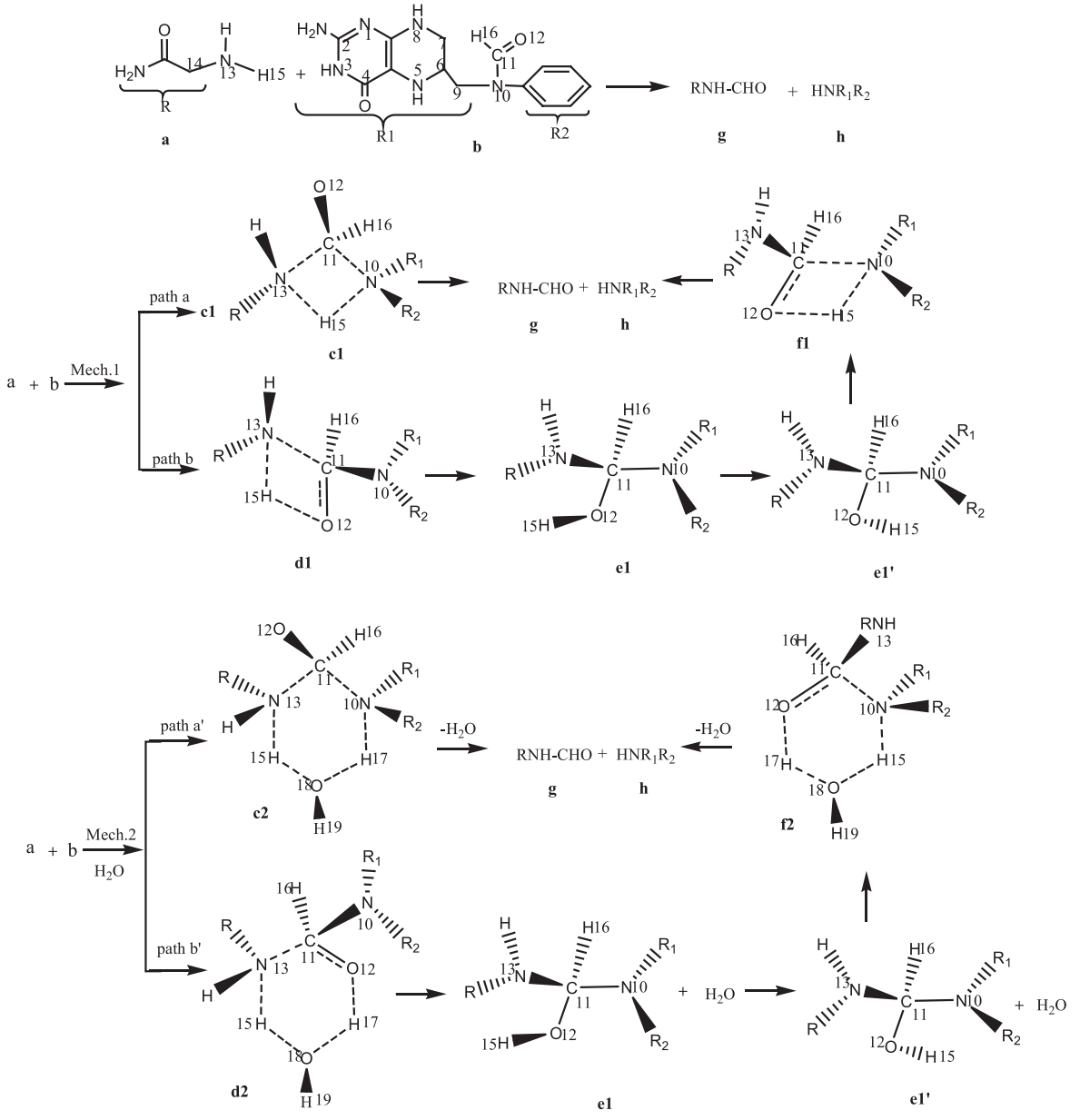

Fig. 2. The different reaction paths, together with the structures of all transition states and intermediates.

been successfully applied to study a number of enzyme mechanisms [17]. The inherent accuracy of the B3LYP method can be estimated from benchmark tests [18], in which the average error in the atomization energies of the G2 set, consisting of 55 small first- and second-row molecules, is found to be 2.2 kcal/mol. When the 6−31G* and 6−311+G (3df, 2p) basis sets were used, the B3LYP hybrid functional was much preferred to Hartree–Fock and MP2 methods. The average deviation for bond lengths is less than 0.02 Å, and for angles and dihedrals less than 1°.

The structures of all reactants, intermediates, transition states and products are fully optimized by B3 LYP method at 6−31G* basis level. The most stable conformations as well as their energies at every equilibration and transition states have been figured out. Frequency calculations have been performed at all stationary points. The transition states have been identified by analyzing their vibrational modes and each has only one imaginary frequency. Solvent effect calibration (SEC) has also been carried out by means of the Onsager model [10] implemented in Gaussian98 package, in which the liquid is assimilated as a continuum characterized by its dielectric constant (78.39 for water).

3. Results and discussion

3.1. No-water-involved mechanism: Mech. 1

In Mech. 1, there is no water molecule involved through the whole process. The Natural Population Analysis (NPA) for reactant b suggests that N10 and O12 are electronegative (−0.493 and −0.595, respectively) and both of them can be easily attacked by a proton. As a result, the one-carbon unit transfer reaction has two different pathways: path a and path b. In the former, the migration of CHO group from N10 to N13 and transfer of H15 to N10 will occur at the same time. But in path b, the one-carbon unit transfer will be finished via two following steps: firstly, the proton H15 migrates to O12 to generate intermediate e1 via transition state d1, then it will experience a second migration from O12 to N10 atom via transition state f1 to form the products. The main structure data of all the reactants, intermediates, transition states and products have been listed in Table 1.

3.1.1. Path a

In the concerted reaction path a, c1 is the only transition state to connect the reactants and products. The bond length of C10−N11 has been elongated from an equilibrium value of 1.383 Å−1.881 Å and is near breaking. C11 atom begins to show some characters of sp3 hybridization when nucleophilic attacked by N13 atom. At the same time, the double bond of C11−O12 is stretched, increasing its single bond character. The distance between N13 and C11 is 1.661 Å, which is close to the normal length of a C−N single bond. The two reactants have their orbits overlapped with each other through a weak covalent bond. From Table 1, one can easily find out that H15, N10, N13 and C11 atoms have formed a four-membered ring in c1's structure. The break in this ring will lead to the products. The dihedral of H15N10C11N13 is nearly zero, suggesting a coplanar character of the ring, and the small angle degrees of about 80° for C11N13H15 and C11N10H15 show strong strain in this system. The only imaginary frequency of c1 is 1332.5i cm^{-1}, and the normal vibrational modes analysis showed that H15 is the most reactive atom in c1. The energy barrier for c1 is as high as 214.9 kJ/mol (Fig. 3) and the case does not change much after the SEC for the stationary points.

3.1.2. Path b

Transition state d1 is produced in the first migration of proton H15 from N13 to O12 atom. There are now four groups linked to N13 atom, and the system becomes unstable. As well as c1, there is a four-membered ring in d1's structure composed of C11, N13, H15, and O12 atoms. As O12 is attacked by the H15 atom, the bond length of C11−O12 becomes longer, increasing its single bond character. The NPA for d1 shows high negative charge on O12 atom (−0.837), while the nature charge population on H15 is 0.494, and they are inclined to form a covalent bond with each other.

Intermediate e1 is located after the first migration of H15. The double bond of C11012 has converted to a single bond completely. The relative energy of e1 is a little higher than the energy sum of the two reactants, indicating its instability. The orientation change of H15 results in another intermediate e1'. It is a conformational isomer of e1 and both of them are local minimums on the potential energy surface. The

Table 1
The main structure data of the stationary points together with their relative energies[a]

	Reactants[b]	c1	d1	e1	e1′	f1	c2	d2	f2	Products[b]
Relative energy (kJ/mol)	0.0	214.9	142.6	16.7	23.9	161.9	113.3	54.2	55.2	−27.5
	(0.0)	(225.9)	(145.0)	(24.6)	(34.3)	(172.7)	(145.8)	(66.4)	(82.6)	(−42.3)
Bond length (Å)										
N13H15	1.009	1.208	1.243	–	–		1.180	1.180	–	–
H15N10	–	1.401	–	–	–	1.532	–		1.682	1.016
N13C11	–	1.611	1.651	1.462	1.432	1.329	1.585	1.579	1.330	1.361
N10C11	1.383	1.881	1.445	1.475	1.485	2.196	1.669	1.453	2.337	–
H15O12	–	–	1.325	0.971	0.987	1.095	–		–	
C11O12	1.210	1.217	1.338	1.418	1.404	1.315	1.251	1.348	1.298	1.210
H15O18							1.368	1.377	1.028	
O18H17							1.362	1.256	1.435	
H17N10							1.187		–	
H17O12								1.195	1.067	
Bond angle (degree)										
C11N13H15		81.6	69.6	–	–	–	103.1	98.1	–	
C11N10(O12)H15		67.5	78.5	104.0	105.03	52.33	–	–	–	
N10C11N13		83.7	114.2	116.2	114.8	117.6	97.8	112.0	109.2	
C11N10H17(H15)							100.5	–	81.5	
N13H15O18							150.9	153.3	–	
N10(O12)H17O18							155.7	157.9	160.0	
Dihedral (degree)										
H16C11O12N10(N13)	0.0	−125.1	−132.7	−115.3	−115.7	160.2	−119.8	−124.4	156.8	0.0
H15N10(O12)C11N13		−0.5	−2.9	−0.7	−174.5	−123.2	–	–	–	
N10C11N13C14		−121.8	−114.0	−111.6	−52.8	−81.4	−172.8	−69.5	−67.5	

[a] The energy sum of the reactants was taken as zero. The values in brackets are the relative energies with SEC.
[b] The values in the two columns were obtained from separated calculation results of a, b, g and h.

alteration is necessary for the second proton transfer. The structure of e1' is much closer to the structure of transition state f1. The formyl group has changed to a hydroxymethyl group in both intermediates.

f1 is the transition state for the second migration of H15, which connects e1' with the products. Due to the removal of H15, the bond length of C11–O12 becomes shorter and tends to reform a double bond. At the same time, C11 is separated from the N10 atom and is inclined to return to its sp2 hybridization state. The bond length of C11–N10 is 2.196 Å, suggesting a very weak covalence between them, and the four-membered ring composed of C11, O12, H15, and N10 atoms is easy to rupture. The dihedral of H16C11O12N13 is 160°, suggesting that the hydroxymethyl group will reform a formyl group soon. Because there are two rings around N10 atom (the pterin ring and the benzene ring), the steric hindrance is an important factor in the proton transfer. This is why the relative energy for f1 is a little higher than d1.

In path b, the energy barriers for d1 and f1 are much lower than the one of c1 in path a. Obviously, path b is much favored to path a in the competitive reaction, and as is still the case after the SEC to all the stationary points. The bulk solvent (of water) effect is relatively very weak in the whole reaction when compared with the high-energy barriers.

3.2. Water-assisted mechanism: Mech. 2

Based on previous studies [13] and Klein et al.'s [4] presumption from experiments, we have given a detailed study of the water-assisted mechanism in the model system. The water molecule can accept a proton from the donor and then give a proton of itself

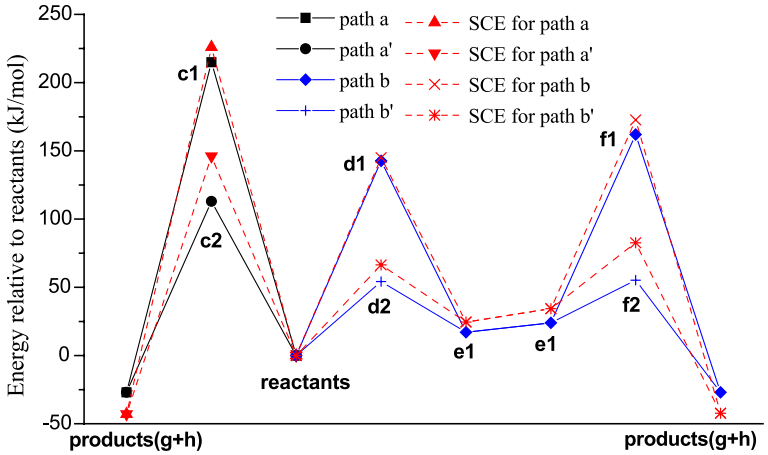

Fig. 3. The energy profiles of all the reaction paths.

to the acceptor simultaneously so as to assist to complete the one-carbon unit transfer reaction.

Similar to Mech. 1, there are also two different pathways for Mech. 2 referred as path a′ and path b′. The former is concerted and is similar to path a, while the latter is a stepwise one like path b.

3.2.1. Path a′

c2 is the only transition state in path a′ in which the H15 atom is not directly migrating from N13 to N10 as in path a, but attacking the O18 atom of the water molecule. The most obvious differences between c1 and c2 result from their structures. There is a six-membered ring (but not a four-membered ring) in c2's structure including C11, N13, H15, O18, H17, and N10 atoms. This ring is nearly planar but not a regular hexagon. O18 atom is 1.368 Å away from H15, and the O18–H17 bond orientates to the N10 atom with a bond length of 1.187 Å. The bond of O18–H19 is bending outward of the hexagon. In principle, c2 can form both endo- and exo- conformations due to the different orientations of O18–H19. In this work, we select the former to discuss. The removal of proton H17 has increased the negative charge on H18 to −1.051 which is easy to be attacked by another proton H15 to reform a new O-H bond. The bond angle N10C11N13 is 103.1°, which is about 20° larger than the one in c1's structure. What's more, the bond angles of N13H15O18 and N10H17O18 are both more than 150°. Most of the energy savings has been achieved because of the loosen system. The hybridization state of C11 and the interactions between C11 and its neighbor atoms are similar to those in c1. The only imaginary frequency for c2 is 1022.0i cm^{-1}, and the two protons, H15 and H17, vibrated the most intensively. Transition state c2 has an energy barrier of 113.3 kJ/mol in gas phase which is 101.6 kJ/mol lower than that of c1. The joint of the water molecule has made the molecular structure greatly relaxed so that the one-carbon unit transfer process can be easily achieved.

3.2.2. Path b′

Path b′ is a stepwise reaction like path b, and there are two sequential transition states referred as d2 and f2 in it. Both d2 and f2 have a six-membered ring in their structures, and there are also two relative large angles more than 150° in the ring as well as in transition state c2. Transition state d2 results from the nucleophilic attack process between the water molecule and the two reactants. The proton H15 attacks O18 from the left side while H17 leaves O18 from the right concertedly. The joint of the water molecule has changed the direct way of proton transfer into an indirect one. The energy barrier for d2 is 54.2 kJ/mol in gas phase, which is 88.4 kJ/mol lower than that of d1. The difference between them changes to 78.6 kJ/mol after solvent effect calibration. Obviously, the additional water molecule stabilizes the transition state and therefore lowered its relative energy.

In the second transition state f2, N10C11 bond has been stretched to 2.337 Å, which indicates that the interaction between them is very weak and the covalence is very easy to rupture. The negative charge on O12 atom has increased as a result of H17's removal, and C11 atom is almost returning to its sp2 hybridization state to generate a formyl group. The relative energy of f2 has been reduced to 55.2 kJ/mol which is 106.7 kJ/mol than that of f1 in gaseous phase. After SEC, the energy difference between them changes to 90.1 kJ/mol.

In Mech. 2, the stepwise path b′ is much preferable to the concerted path a′ due to its lower energy barriers. The effects of the bulk water solvent to both pathways are very minimal. One water molecule can mediate the process by serving as a 'tunnel' that connects the donor and the acceptor sites for proton transfer. One proton is coming into the 'tunnel' while the other is leaving it at the same time. Though the one left behind is not the one that entered, we can get the same products. The joint of the water molecule can stabilize the transition states and substantially lower the energy barrier.

Our calculations have verified the presumption of Klein et al. [4], and proved that a water-assisted mechanism in the one-carbon unit transfer reaction catalyzed by GAR Tfase does exist. Path b′ is the favorable pathway for formyl group transfer.

4. Conclusions

The following conclusions can be drawn from our calculations for the model system of GAR Tfase:

(1) For both mechanisms, the energy barriers for the stepwise paths (path b and path b′) are lower than the concerted ones (path a and path a′), which indicates that the stepwise paths are much favored. The effect of bulk water solvent cannot affect the relative energy very much, and could not change the priority of pathways either.
(2) All the transition states in Mech. 1 have a four-membered ring in their reactive sites, and the proton to be transferred (H15) is the most reactive atom. But in Mech. 2, the four-membered ring has changed to a six-membered ring, and there are two protons (H15 and H17) that are the most reactive. H15 and h17 will be transferred concertedly. The energy barriers in Mech. 2 are much lower than the corresponding ones in Mech. 1, both in gas phase and in water solvent. The energy reduction varies in a range of 80–100 kJ/mol.

The results show that the presumption of Ref. [4] is quite conceivable. The participation of a water molecule in the transition state can loosen its structure and give a considerable relaxation to the unstable system. If the water molecule is removed, the higher energy barrier will make it hard to achieve the target products. It should be noticed that the effect of the residues in this reaction are out of consideration in the model system. In fact, in an actual system, the whole reaction may be much easier to proceed, and the energy barriers may be even lower than what we have calculated in path b′ . Our results in this paper have well explained the presumption from experiments [4], and might be a valuable reference for further research in such a field.

Acknowledgements

This work was supported by the National Natural Scientific Foundation of China (No. 20173032 and No. 29973021), the Foundation for Invited Professor of Shandong University and PhD Special Foundation of the Chinese Education Department.

References

[1] J. Aimi, H. Qiu, J. Williams, H. Zalkin, J.E. Dixon, De novo purine nucleotide biosynthesis: cloning of human and avian cDNAs encoding the trifunctional glycinamide ribonucleotide synthetase-aminoimidazole ribonucleotide synthetase-glycinamide ribonucleotide transformylase by functional complementation in *E. coli*, Nucleic Acids Res. 18 (1990) 6665–6672.
[2] P. Chen, U. Schulz-Gahmen, E.A. Stura, J. Inglese, D.L. Johnson, A. Marolewski, S.J. Benkovic, I.A. Wilson, Crystal structure of glycinamide ribonucleotide transformylase from *Escherichia coli* at 3.0 Å resolution, J. Mol. Biol. 227 (1992) 283–292.
[3] R.J. Almassy, C.A. Janson, C. Kan, Z. Hostomska, Structures of apo and complexed *Escherichia coli* glycinamide ribonucleotide transformylase, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 6114–6118.

[4] C. Klein, P. Chen, J.H. Arevalo, E.A. Stura, A. Marolewski, M.S. Warren, S.J. Benkovic, I.A. Wilson, Towards structure-based drug design: crystal structure of a multisubstrate adduct complex of glycinamide ribonucleotide transformylase at 1.96 Å resolution, J. Mol. Biol. 249 (1) (1995) 153–175.

[5] J.H. Shim, S.J. Benkovic, Catalytic mechanism of *Escherichia coli* glycinamide ribonucleotide transformylase probed by site-directed mutagenesis and pH-dependent studies, Biochemistry 38 (1999) 10024–10031.

[6] M.S. Warren, K.M. Mattia, A.E. Marolewski, S.J. Benkovic, The transformylase enzymes of de novo purine biosynthesis, Pure Appl. Chem. 68 (11) (1996) 2029–2036.

[7] J. Inglese, J.M. Smith, S.J. Benkovic, Active-site mapping and site-specific mutagenesis of glycinamide ribonucleotide transformylase from *Escherichia coli*, Biochemistry 29 (1990) 6678–6687.

[8] P.L. Nagy, A. Marolewski, S.J. Benkovic, H. Zalkin, Formyltetrahydrofolate hydrolase, a regulatory enzyme that functions to balance pools of tetrahydrofolate and one-carbon tetrahydrofolate adducts in *Escherichia coli*, J. Bacteriol. 177 (5) (1995) 1292–1298.

[9] M.S. Warren, A.E. Marolewski, S.J. Benkovic, A rapid screen of active site mutants in glycinamide ribonucleotide transformylase, Biochemistry 35 (1996) 8855–8862.

[10] M.W. Wong, M.J. Frisch, K.B. Wiberg, Solvent effects: 1. The mediation of electrostatic effects by solvents, J. Am. Chem. Soc. 113 (1991) 4776–4782.

[11] M. Cossi, V. Barone, R. Cammi, J. Tomasi, Ab initio study of solvated molecules: a new implementation of the polarizable continuum model, Chem. Phys. Lett. 255 (1996) 327–335.

[12] J.B. Foresman, T.A. Keith, K.B. Wiberg, J. Snoonian, M.J. Frisch, Solvent effects: 5. Influence of cavity shape, truncation of electrostatics, and electron correlation on ab initio reaction field calculations, J. Phys. Chem. 100 (1996) 16098–16104.

[13] J. Pitarch, M.F. Ruiz-López, E. Silla, J.-L.P. Ahuir, I. Tuñón, Neutral and alkaline hydrolyses of model β-lactam antibiotics. An ab initio study of water catalysis, J. Am. Chem. Soc. 120 (1998) 2146–2155.

[14] Y. Su, M.M. Yamashita, S.E. Greasley, C.A. Mullen, J.H. Shim, P.A. Jennings, S.J. Benkovic, I.A. Wilson, A pH-dependent stabilization of an active site loop observed from low and high pH crystal structures of mutant monomeric glycinamide ribonucleotide transformylase at 1.8 to 1.9 Å, J. Mol. Biol. 281 (1998) 485–499.

[15] (a) C. Lee, W. Yang, R.G. Parr, Development of the Colle–Salvetti correlation-energy formula into a functional of the electron density, Phys. Rev., B 37 (1988) 785–789;
(b) A.D. Becke, A new mixing of Hartree–Fock and local density-functional theories, J. Chem. Phys. 98 (1993) 1372–1377;
(c) A.D. Becke, Density-functional thermochemistry: III. The role of exact exchange, J. Chem. Phys. 98 (1993) 5648–5652.

[16] Gaussian 98, Revision A.9, Gaussian Inc., Pittsburgh, PA, 1998.

[17] P.E.M. Siegbahn, R.A.M. Blomberg, Transition-metal systems in biochemistry studied by high-accuracy quantum chemical methods, Chem. Rev. 100 (2000) 421–437 (and references therein).

[18] (a) C.W. Bauschlicher Jr., A comparison of the accuracy of different functionals, Chem. Phys. Lett. 246 (1995) 40–44;
(b) C.W. Bauschlicher Jr., A. Ricca, H. Partridge, S.R. Langhoff. In: Recent Advances in Density Functional Methods, Part II, World Scientific Publishing, Singapore, 1997, p. 165.