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Quantum Chemical Study on a New Mechanism of One-carbon Unit Transfer Reaction: The Water-assisted Mechanism

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It is a theoretical study on the water-assisted mechanism of one-carbon unit transfer reaction, in which the energy barrier for each transition state lowered by about 80—100 kJ/mol when compared with the one in no-water-involved mechanism. The water-assisted path 4 is the favorite reaction way. Our results well explained the presumption from experiments.

Keywords one-carbon unit transfer, water-assisted mechanism, energy barrier

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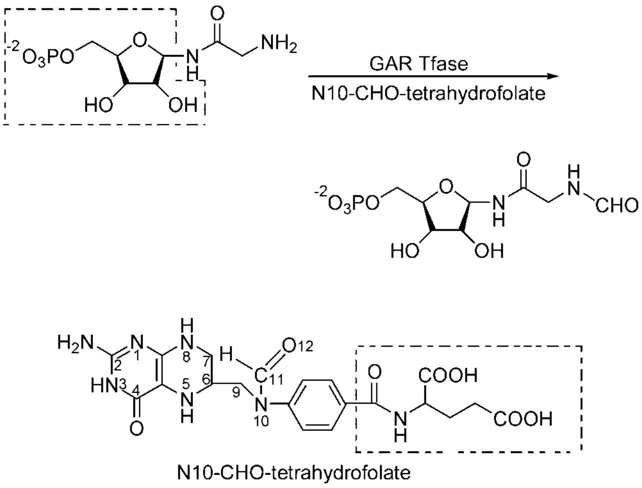

Figure 1 Reaction catalyzed by GAR Tfase and the structure of N10-formyl-THF molecule.

Enzymatic one-carbon unit transfer is a very important process in biosyntheses and metabolism.[1,2] Glycinamide ribonucleotide transformylase (GAR Tfase, EC 2.1.2.2) catalyzes the first of two formyl transfer reactions in the *de novo* purine biosynthetic process (Figure 1).[3] This reaction has attracted considerable attention for the development of cancer chemotherapy inhibitors, which requires a large amount of purine to divide cells rapidly. The required chemical steps in this reaction are the transfer of a formyl group, the one-carbon unit, from N10-formyl-tetrahydro-folate (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 made on the catalytic mechanism of GAR Tfase,[4,5] but it is still unknown whether these steps occur concertedly or not,[6,7] and a new mechanism needs to be invoked to explain the spectroscopic and crystallographic findings.

Because no other residues have been reported to be directly involved in the catalytic reaction, only the GAR molecule and the N10-formyl-THF are included in our calculations. Furthermore, to limit the computational time and test a number of different pathways, we also replaced the ribonucleotide part of GAR molecule and the glutamic acid part in 10f-THF (that are cycled by the dashed line rectangle in Figure 1) by hydrogen atoms, as they did not participate in the formyl group transfer process and the main function of them is to bind the substrates and the enzyme together to achieve the one-carbon unit transfer process. Therefore, the removal of them did not change the reaction mechanisms, *i.e.*, the reaction channels.

In this work, we examined the full catalytic mechanism of GAR Tfase by means of a hybrid density functional theory (DFT) B3LYP at 6-31G* basis level. The structures of all stationary points were fully optimized, and the most stable conformations as well as their energies at every equilibration and transition states were figured out. Frequency calculations of all stationary points were performed, and all transition states were identified by analyzing their vibrational modes because each has only one imaginary frequency. The bulk solvent effect of water was also calibrated for all stationary points by Onsager model at the same basis level. All the calculations were carried out by Gaussian 98 package.[8]

There are four different paths for this reaction, which belongs to two different mechanisms (Figure 2). One is the no-water-involved mechanism (Mech. 1) and the other is water-assisted mechanism (Mech. 2). Our calculations indicate that all the four paths are possible to complete the one-carbon unit transfer reaction. The main structure data of stationary points are listed in Table 1.

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The most obvious difference between Mech. 1 and Mech. 2 is the corresponding structures of transition states. In Mech. 1, there is a four-membered ring in **c1**, **d1** and **f1**, which indicates that there are strong strains in the transition states. In such a structure, the proton migration goes through a direct transfer from the donor atom to the acceptor one. If a water molecule assists in the reaction as shown in Mech. 2, the four-membered ring changes to a six-membered ring which can relax the strong strain in the transition states and make them more stable. Now, the proton does not transfer directly to the target atom, but migrates in an indirect manner. It will migrate to the oxygen atom of the water molecule and the other hydrogen atom of the water molecule will transfer to the target atom at the same time. In this process, one of the O—H bonds of the water molecule will elongate while the other remains nearly unchanged, and this is a common character for all the three transition states of **c2**, **d2** and **f2** in Mech. 2.

In Mech. 1, the energy barriers for all the three transition states are relatively high and their values change very little after the calibration of solvent effect very little after the calibration of solvent effect (the values in brackets in Figure 2). The relative energy of **c1** is more than 200 kJ/mol, which means the reaction is very difficult to proceed through path 1 at room temperature whether in vacuum or in water. The case is slightly changed in path 2, because both the energy barriers for **d1** and **f1** are relatively lower than **c1**, which means that in Mech. 1, path 2 is much favored to path 1 both in vacuum and in water. The two intermediates **e1** and **e1'** are local minimums in the potential energy surface and the main difference between them is the orientation change of H(15) atom.

In the water-assisted mechanism (Mech. 2), the cases are quite different. In principle, each of the reactants can form a complex with the water molecule named **a'** and **b'**, respectively. **a'** is located on the energy surface but we have not obtained **b'**. The main reason is that the steric hindrance around N(10) is larger than that of N(13). The energy barriers for all the three transition states, **c2**, **d2** and **f2**, are greatly lowered in different degrees when compared to the ones in Mech. 1. The

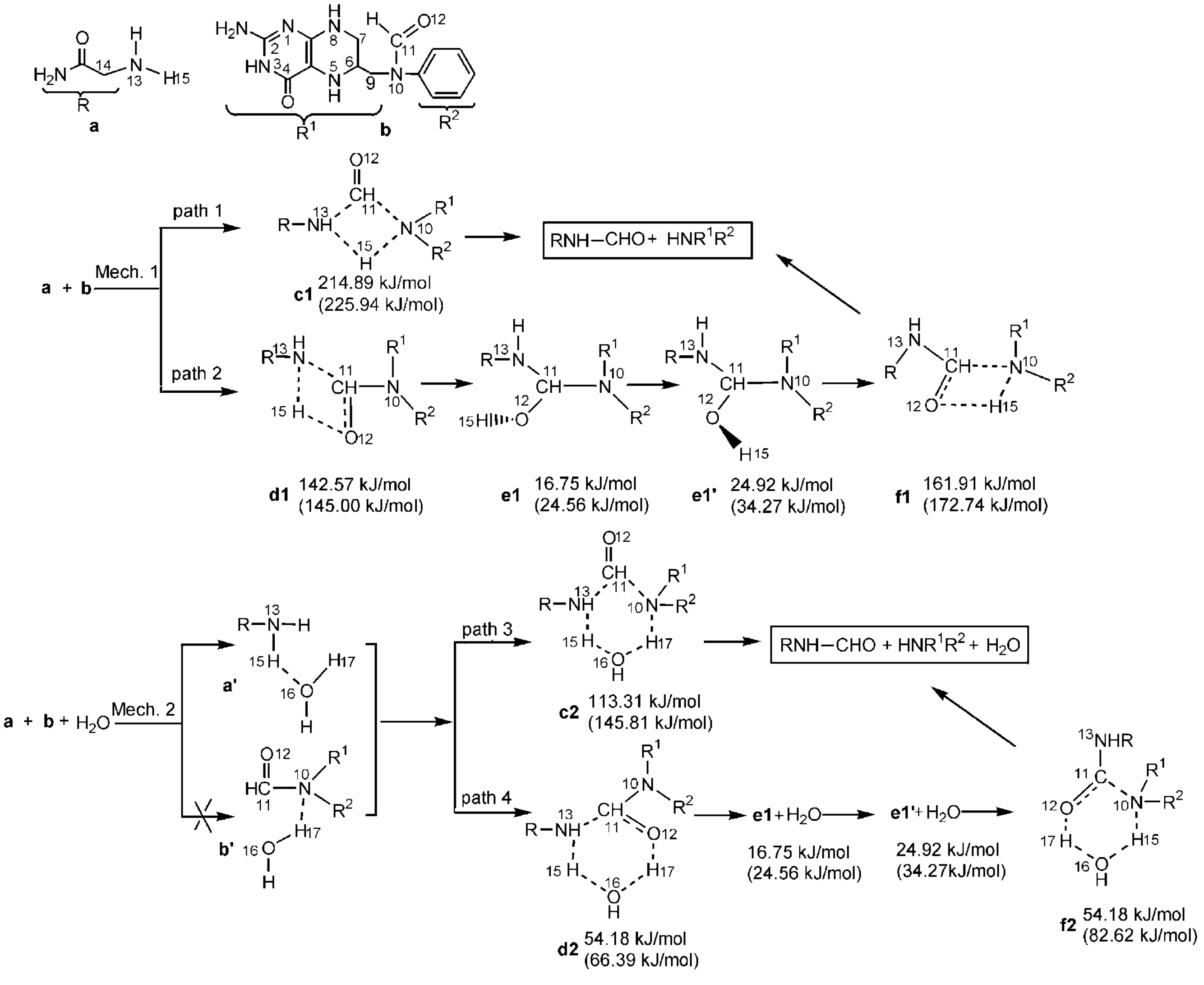

Figure 2 Mechanisms of one-carbon unit transfer reaction and the relative energies of all stationary points (the energy sum of the reactants is taken as zero).

Table 1 Main bond lengths (nm) and bond angles (°) of stationary points

Bond length	**c1**	**d1**	**e1**	**e1´**	**f1**	**c2**	**d2**	**f2**
N(13)—H(15)	0.121	0.124				0.118	0.118	
H(15)—N(10)	0.140				0.153			0.168
N(13)—C(11)	0.161	0.165	0.146	0.143	0.133	0.159	0.158	
N(10)—C(11)	0.188	0.145	0.148	0.149	0.220	0.167		0.234
H(15)—O(12)		0.133	0.097	0.099	0.110			
C(11)—O(12)		0.134	0.142	0.140	0.132		0.135	0.130
H(15)—O(16)						0.137	0.138	0.103
O(16)—H(17)						0.136	0.126	0.144
H(17)—N(10)[O(12)]						0.119	0.120	0.107
Bond angle								
N(13)-H(15)-N(10)[O(12)]	127.2	117.7			131.8[a]			
N(13)[N(10)]-H(15)-O(16)						150.9	153.3	156.2
N(10)[O(12)]-H(17)-O(16)						155.7	157.9	160.0
H(15)-O(16)-H(17)						80.9	80.4	87.2

[a] The bond angle of N(10)H(15)O(12).

energy barrier for **c2** is more than 100 kJ/mol lower than that for **c1** in vacuum and 80 kJ/mol lower in water, and the cases are nearly the same as **d2** and **f2**. We are surprised to find that the relative energies for transition state **d2** and **f2** are so low that the one-carbon unit transfer reaction could be completed at room temperature via path 4. Similar to Mech. 1, the energy barriers change very little after the calibration of solvent effect. Klein *et al*.[3] 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 residue Asp 144. Our calculations confirmed their presumption, that is, this mechanism does exist and if a water molecule participates in the reaction but not only serves as environment, the one-carbon unit transfer reaction will easily proceed due to the lower energy barrier.

In conclusion, there does exist a water-assisted mechanism for one-carbon unit transfer reaction, in which the active sites of the transition states change to a six-membered ring. The strong strain in the transition states can be relaxed in such a structure, and the transition states are more stable than the ones in no-water-involved mechanism, furthermore, the energy barriers of them lowers greatly in a range of 80—100 kJ/mol. In both mechanisms, the stepwise channels (path 4 and path 2) are preferable to the concerted ones (path 3 and path 1). Among the four channels, path 4 is the most favored reaction path. But the energy calibration of solvent effect is very little to all paths. Our results confirmed the presumption from experiments and may be a valuable reference for further studies in this field.

References

1 Blakely, R. L. *The Biochemistry of Folate Acid and Related Pteridines*, North-Holland, Amsterdam, **1969**.

2 Kang, C.-M.; Qi, C.-S.; Feng, D.-C.; Cai, Z.-T. *Acta Chim. Sinica* **2001**, *59*, 1031 (in Chinese).

3 Klein, C.; Chen, P.; Arivalo, J. H.; Stura, E. A.; Marolewski, A.; Warren, M. S.; Stephen, J. B.; Wilson, I. A. *J. Mol. Biol.* **1995**, *249*, 153.

4 Mark, S. W.; Karen, M. M.; Ariane, E. M.; Stephen, J. B. *Pure Appl. Chem*. **1996**, *68*, 2029.

5 Peter, L. N.; Ariane, M.; Stephen, J. B.; Howard, Z. J. *J. Bacteriol*. **1995**, *177*, 1292.

6 Mark, S. W.; Ariane E. M.; Stephen, J. B. *Biochemistry* **1996**, *35*, 8855.

7 Shim, J. H.; Stephen, J. B. *Biochemistry* **1999**, *38*, 10024.

8 Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, Jr., R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Rega, N.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, Revision A. 9, Gaussian, Inc., Pittsburgh PA, **1998**.

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