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Molecule dynamics and combined QM/MM study on one-carbon unit transfer reaction catalyzed by GAR transformylase

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Abstract: Both a molecule dynamic study and a combined quantum mechanics and molecule mechanics (QM/MM) study on Glycinamide ribonucleotide transformylase (GAR Tfase) catalytic mechanism are presented. The results indicate a direct one-carbon unit transfer process but not a stepwise mechanism in this reaction. The residues near the active center can fix the cofactor (N10-formyltetrahydrofolate) and GAR in proper relative positions by a H-bond network. The transition state and the minimum energy pathway are located on the potential energy surface. After all the residues (including H_2O molecules) are removed from the system the activation energy has increased from 145.1 kJ/mol to 243.3 kJ/mol, and the formyl transfer reaction is very hard to achieve. The interactions between coenzyme, GAR and residues near the reactive center are discussed as well.

Keywords: GAR Tfase, QM/MM method, formyl transfer

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1 Introduction

Glycinamide ribonucleotide transformylase (GAR Tfase, EC 2.1.2.2) [1] is a folate-dependent enzyme central to the *de novo* purine biosynthetic pathway. GAR Tfase utilizes the cofactor N10-formyltetrahydrofolate (10f-THF) to transfer a formyl group to the primary amine of its substrate, glycinamide ribonucleotide (GAR, Fig. 1). This one-carbon transfer process incorporates the C-8 carbon of the purines, and is the first of two formyl transfer reactions; the other widely investigated is aminoimidazole carboxamide ribonucleotide transformylase (AICAR Tfase). Because of its association with DNA synthesis, GAR Tfase has become the target of anti-neoplasitc agents and much effort has been done on its catalytic mechanism [2-11].

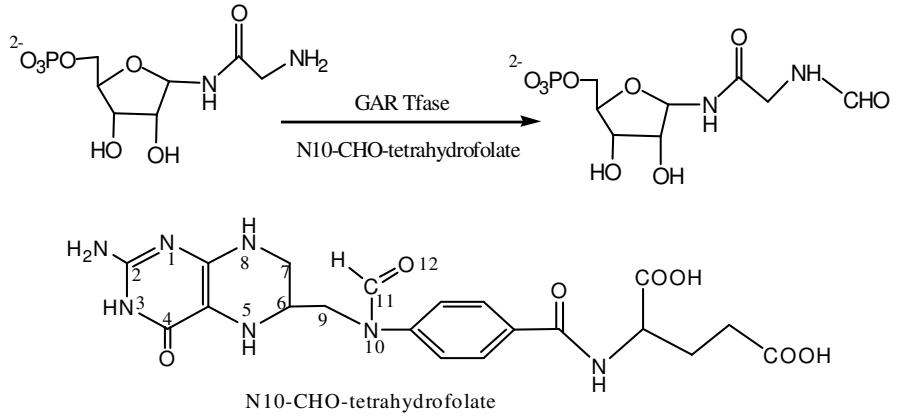

Fig. 1 The GAR Tfase catalyzed reaction and the molecular structure of 10f-THF.

In the catalytic system, the apoenzyme crystal structure has revealed a proposed binding pocket lined by side chains of 13 strictly conserved residues [2]. The results from Almasy, et al.[3] has confirmed this idea and also indicated that three of these residues, His108, Asn106 and Asp144, are positioned such that they may play a key role in the reaction. But the results from site-directed mutagenesis [6] suggested that none of the polar residues close to the catalytic center of the enzyme are irreplaceable, although several of them, namely, His108, Asn106, Ser135 and Asp144, are important for full activity. Further study by Jae Hoon Shim[6] revealed that His108 acts in salt bridge with Asp144 as a general acid catalysis. But this conclusion is not enough to explain the inconsistency of the results above. How the binding pocket acts in the catalysis process and how the residues interact with the cofactor and GAR still need further research both experimentally and theoretically. In this paper we present a molecular dynamic process followed by a QM/MM study to explain the catalytic mechanism of GAR Tfase. The transition state and the minimum energy pathway are also located on the potential energy surface.

2 Computational details

Of the many crystallographic structures of GAR Tfase, we selected the one named 1C3E [12] from Research Collaboratory for Structural Bioinformatics (RCSB) databases [13]. This structure is an inhibitor designed to form an enzyme-assembled multi-substrate adduct with the substrate GAR. Necessary modifications are made on certain residues, in particular the nitrogen atoms at 5-, 8- and 10- sites were replaced by carbon atoms, so N10-formyl-5, 8, 10-trideaza-tetrahydrogen folic acid was replaced by 10f-THF. Due to the fact that X-ray structures do not determine the orientation of all the hydrogen atoms in the protein, H atoms and atom-centered charges were added to the amino acid residues. Missing from the X-ray data are various terminal amino acid residues far removed from the sites of interest within the protein, so no attempt is made to replace these missing groups and the chain ends are also capped with H atoms (according to valency). Then, chain A and 104 crystallographic water molecules within a distance of 10 Å to the backbone were selected to do molecular dynamics. For all water atoms, TIP3P charges [14] were assigned and the steepest descent method was applied. The waters were reoriented until the root-mean-square gradient reached a value that was less than 0.1 kcal mol^{-1} $Å^{-1}$.

In the first step, the polypeptide backbone and the oxygen atoms in the crystallographic water molecules were initially fixed during the addition of hydrogen atoms. In the second step, the crystallographic water molecules were allowed to move while the backbone was strongly constrained (100 kcal mol^{-1} $Å^{-1}$) to keep the tertiary; the protein backbone was then slightly constrained (20 kcal mol^{-1} $Å^{-1}$) for 3000 steps in order to allow the gradual relaxation of the crystallographic structure; at last, all the constraints were removed and the entire system was minimized to convergence (0.02 kcal mol^{-1} $Å^{-1}$).

The molecular dynamics simulation of the whole system was done by means of CFF (Consistent Force Field) [15] method embedded in Cerius2 (version 4.6) [16] program package.

As to GAR Tfase, the most disputable question focused on the catalytic mechanism and the function of the 'binding pocket' residues. For this purpose, after the dynamic process, the cofactor, the substrate and the residues in the 'binding pocket' (Gly11, Ser12, Asn13, Arg64, Phe88, Ile91, Leu92, Asn106, Ile107, His108, Ser135, His137, Thr140, Asp144, Glu173) were selected as a simplified system to serve as reactant (**R1**) in a QM/MM calculation (Fig. 2). Such treatments have been done on many enzymatic systems [17] and proved to be efficient. The simplified system contained 365 atoms in all, in which the cofactor, 10f-THF, and the substrate GAR were calculated by Density Functional Theory (B3LYP) [18] at 6-31G* basis level [19], while a MM method DREIDING [20] was used to deal with the residues. A number of enzymatic systems have been calculated [21-22] in which the residues around the substrates are replaced and estimated by very small molecules (eg. water, formaldehyde or formate). Compared with them, our simplified model system was much closer to an actual one. After the transition state (**TS1**) and the product (**P1**) were located, the potential energy surface was scanned to get the minimum energy pathway. All the QM/MM calculations have been carried out

by Gaussian03 package [23].

3 Results and discussion

After the molecule dynamics process, the relative positions of 10f-THF and GAR molecules before the one-carbon unit transfer process were determined and displayed as ball and stick model in Fig. 2 (while the residues were shown as stick model). A QM/MM calculation was done on GAR, 10f-THF and the residues in the 'binding pocket'. In this simplified system, one-carbon unit transfer reaction would take place in an 'organic' environment but not in a gaseous phase. The structures of reactant (**R1**), transition state (**TS1**) and product (**P1**) are fully optimized by ONIOM [24] (B3LYP/6-31G*: DREIDING) method (see Table 1). The most stable conformations as well as their energies at every equilibration and transition state have been calculated. B3LYP/6-31G* calculations were also done on GAR and 10f-THF reaction system in gas phase, in order to investigate the function of the residues.

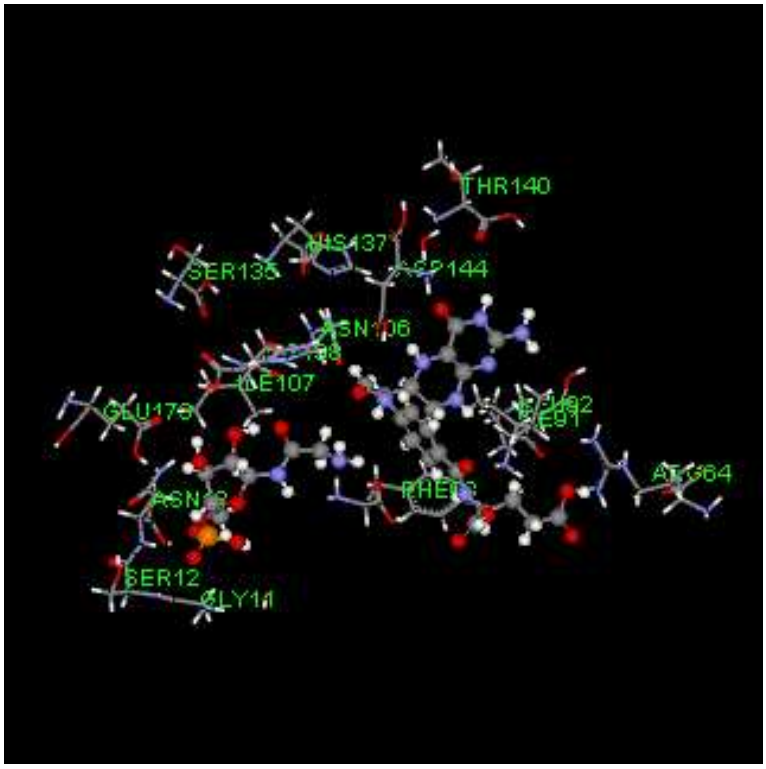

Fig. 2 The QM/MM calculation model system.

In the optimized structure of the 'binding pocket' (Fig. 2), His108, Asn106 and Asp 144 were strictly kept in the active center. From the data in Table 1, one can easily find that there is a salt bridge between His108 and the carboxylate of Asp144, which is consistent with the experimental results from Ref [6]. To describe the system in detail, a new sequence number was given to every atom as shown in Fig. 3. Our calculations indicated that the –CHO group transfer process is concerted with H atom migration, and both steps occurred directly as Ref. [7] and Ref. [25] pointed out. **TS1** is the only

	R1	**TS1**	**P1**	**R2**	**TS2**	**P2**
Relative energy (kJ/mol)	0	145.1	-30.1	0	243.3	-22.3
Bond length(Å)						
N42H46	1.033	1.195		1.031	1.212	
N12H46		1.264	1.029		1.272	1.021
N42C13		1.642	1.449		1.686	1.430
N12C13	1.393	1.744		1.390	1.718	
C13O23	1.201	1.230	1.204	1.202	1.225	1.117
O23H231	3.357	2.718	3.119			
O329H33	2.829	2.775	2.738			
H14O224(N258)	2.905	2.907	2.774			
Bond angle (degree)						
H46N42C13	122.8	77.6	103.4	123.1	75.9	107.1
N42C13N12	97.3	80.5		97.7	82.1	
C13N12H46	71.6	71.4			73.4	
N12H46N42		127.7	71.0		128.4	67.2
Dihedral (degree)						
H46N42C13N12		-5.1			-2.3	

Table 1 Optimized structure data of reactants, products and transition states together with their relative energies in the one-carbon unit transfer reaction.

transition state in the reaction. The bond length of C13-N12 has been elongated from an equilibrium value of 1.393 Å to 1.744 Å and is about to break. C13 atom begins to show some characters of sp3 hybridization when nucleophilic attacked by N42 atom. At the same time, the double bond of C13-O23 is stretched, increasing its single-bond character. The distance between N42 and C13 is 1.642Å, which is close to the normal length of a C-N single bond. The cofactor and GAR molecule have their orbits overlapped with each other through a weak covalent bond. From Table 1, it can be easily found out that H46, N12, N42 and C13 atoms have formed a four-membered ring. The breaking of this ring will direct to the products. The dihedral of H46N42C13N12 is nearly zero suggesting a coplanar character of the ring, and the small angle degrees of about 80^o for H46N42C13 and N42C13N12 imply strong strain in this system. The normal vibrational modes analysis showed that H46 is the most reactive atom in **TS1**, and the energy barrier for **TS1** is as high as 145.1 kJ/mol.

O23 atom can form H-bond with H231 of residue **Asn106** in the reaction. They are getting closer to each other in **TS1** than in **R1**, indicating stronger interaction in the transition state. When the one-carbon unit transfer process successfully completed, they are inclined to depart from each other. The main function of **Asn106** is to adjust the relative position of the formyl group to a proper site so as to achieve the –CHO transfer. H-bond is also found between H14 atom (which is 'behind' C13 in Fig. 3) and the carbonyl oxygen of **His108**, which contributes much to constrain the relative positions of the reactants to form the transition state. Other residues in the system are a

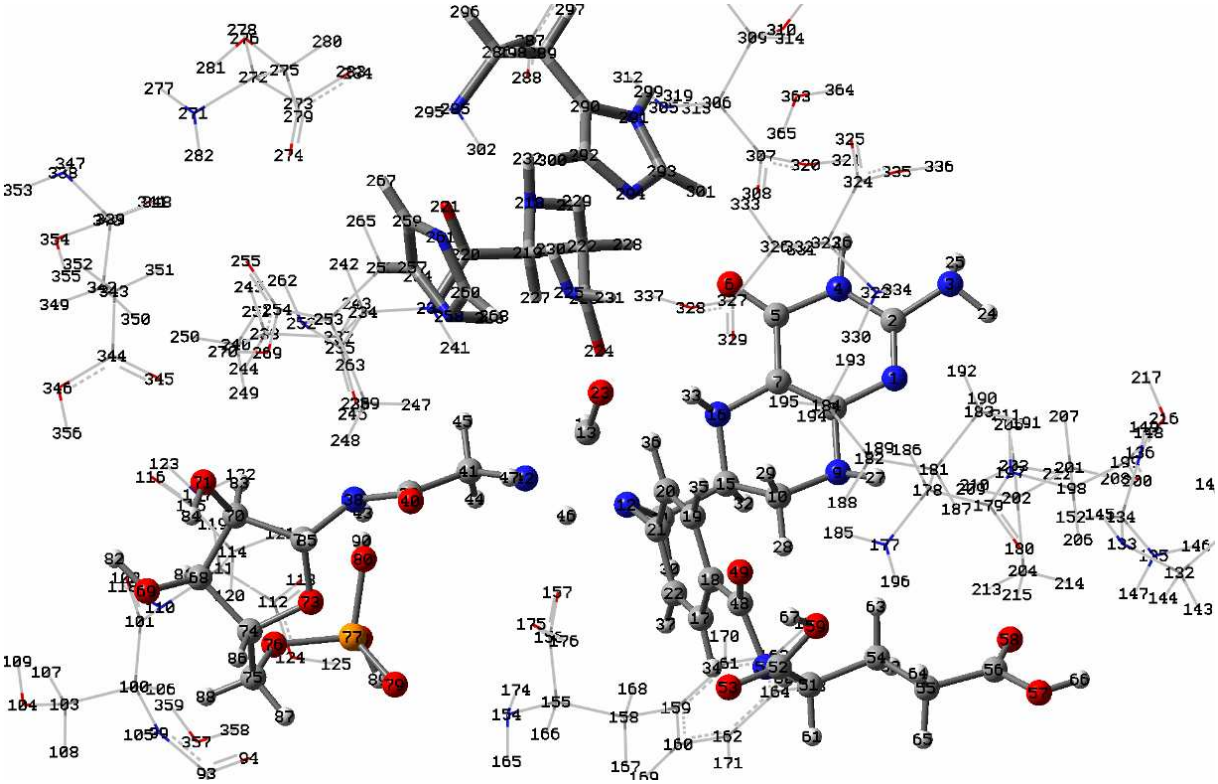

Fig. 3 The three-dimensional structure of **TS1**.

little far from the active center, but they can form a number of H-bonds with GAR or the cofactor. The 'network' composed of H-bonds will fix the cofactor and GAR molecules in proper positions where they are apt to form a transition state with lowest energy barrier.

We have scanned the potential energy surface of this system by AM1 method with two bond lengths (N42H46 and N12H46) as the variables. The minimum energy pathway (MEP) is showed as a dashed line in Fig. 4 (energy unit in Hartree and the energy of **R1** was taken as zero), in which **R1**, **TS1** and **P1** are three stationary points. The reaction pathway showed in the potential energy surface is consistent with our calculation.

For comparison, further calculations were done on the system with no residues or water molecules but only the cofactor 10f-THF and GAR molecules. The only transition state is described as **TS2** in Table 1, and reactants and products were denoted as **P2 and R2** respectively. To **TS2**, The bond length, bond angle and dihedral around the reactive center are similar to those of **TS1**'s. But the activation energy of **TS2** (243.3 kJ/mol) is about 98.2 kJ/mol greater than that of **TS1**. The main reason resulted from the most constrained freedoms by H-bond network in the generation of **TS1**, which led to limited structure variations between **P1** and **TS1**. Most of the energy savings has been achieved in this way, and the formyl group transfer progress will be completed successfully. But when there were no residues around, the structure differences between **TS2** and **R2** are considerable large, especially the glutamic 'tail' of cofactor and the ribonucleotide part of GAR molecule. In other words, to form **TS2**, more energy is needed than **TS1**, and

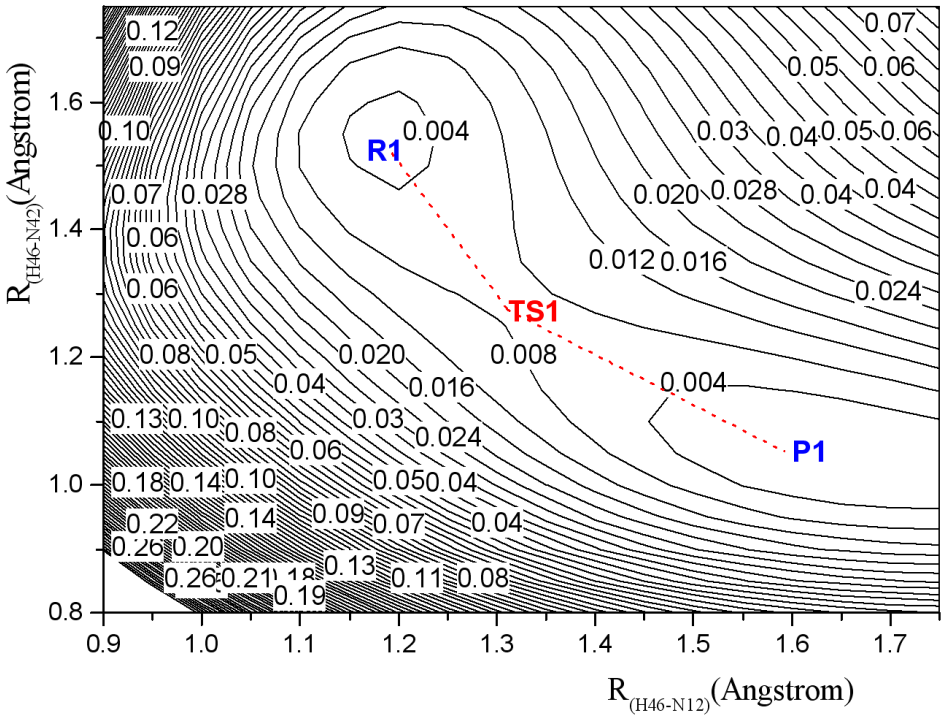

Fig. 4 The potential energy surface and the minimum energy pathway (the unit of contour is 0.004Hartree).

it will be much hard to get the target products.

4 Conclusion

The one-carbon unit transfer catalyzed by GAR Tfase experienced a concerted migration process of H atom and –CHO group. The residues in the 'binding pocket' could form a H-bond 'network', which will direct to the target products with relative low activation energy. The residues of His108, Asn106 and Asp144 were strictly conserved in the reaction and contribute much to lead favorite relative positions of the cofactor and GAR. After the residues and H_2O molecules were all removed from the system but only the cofactor 10f-THF and GAR molecules kept, the activation energy becomes much higher, so it will be more difficult to achieve the one-carbon unit transfer reaction. Of course, due to the participation of other medium, the reactions in actual reaction may be easier than what was investigated in this paper. Our calculation is consistent with experimental results [7, 25], and properly imitates the enzymatic one-carbon unit transfer reaction in organisms. This work might provide a valuable reference for further study of such fields.

Acknowledgment

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