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Theoretical Study of the Methyl Transfer in Guanidinoacetate Methyltransferase

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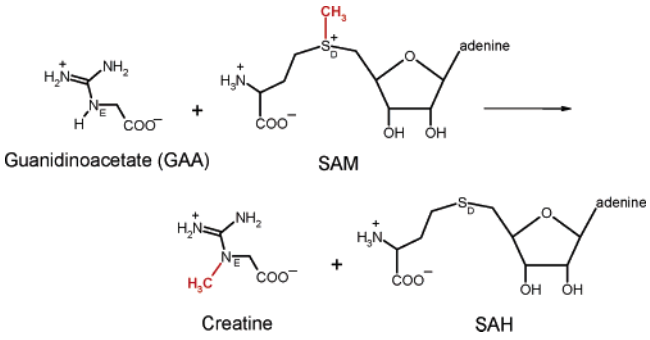
The reaction mechanism of the guanidinoacetate methyltransferase (GAMT) enzyme has been investigated by means of density functional theory using the B3LYP hybrid functional. GAMT catalyzes the *S*-adenosyl-L-methionine (SAM)-dependent methylation of guanidinoacetate (GAA) to form creatine. A quantum chemical model was built on the basis of the recent crystal structure of GAMT complexed with *S*-adenosylhomocysteine (SAH) and GAA. The methyl group transfer from SAM to N_E of GAA is shown to occur concertedly with a proton transfer from N_E to the neighboring O_{D1} of Asp134. Good agreement is found between the calculated barrier and the experimental rate.

I. Introduction

Guanidinoacetate methyltransferase (GAMT) catalyzes the last step of creatine biosynthesis by converting guanidinoacetate (GAA) and *S*-adenosylmethionine (SAM) into creatine and *S*-adenosylhomocysteine (SAH), Scheme 1. In humans, GAMT is found mainly in the liver and the pancreas. GAMT deficiency is an inborn error of creatine synthesis which leads to creatine deficiency and accumulation of guanidinoacetate in tissues and body fluids. This can cause mental retardation, speech delay, and epilepsy.

SCHEME 1: Reaction Catalyzed by GAMT



GAMT is a monomeric protein with a relative molecular mass of 26 000−31 000.[1,2] Recently, the intact GAMT was crystallized with SAH and GAA, and the structure was determined at 2.0 Å resolution.[3] Figure 1 shows the main features of the active site of this structure. The GAA substrate was found to be tightly bound to the enzyme by a number of strong hydrogen bonds. The guanidino group of GAA forms two pairs of hydrogen bonds to the carboxylate groups of Glu45 and Asp134, and the carboxylate part of GAA forms hydrogen bonds to the backbone amide groups of Leu170 and Thr171 and also to the O_G of Thr171. In this GAMT:(SAH + GAA) structure, the distance between the sulfur center of SAH (S_D) and the N_E of GAA is found to be ca. 3.9 Å. When the C_E−methyl group of SAM was added manually to SAH, the C_E−N_E became ca. 2.2 Å and the S_D−C_E−N_E centers were almost linear.[3]

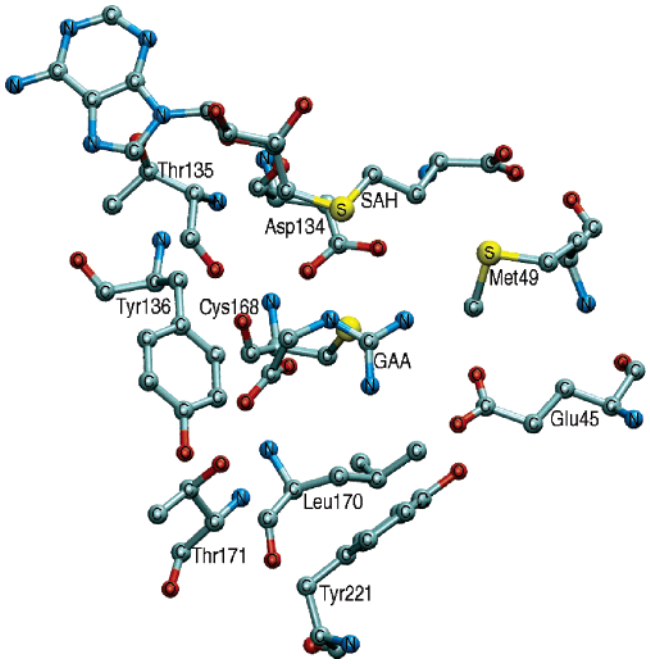


Figure 1. X-ray crystal structure of the active site of GAMT.[3]

On the basis of the GAMT:(SAH + GAA) structure and earlier biochemical experiments,[4−9] the following mechanism has been proposed for GAMT (Scheme 2).[3] The reaction starts with the binding of SAM and the GAA substrate. Next, the O_{D1} of Asp134 abstracts a proton from N_E of GAA. The protein environment is suggested to raise the pK_a of Asp134, making this step possible. The strong nucleophile generated on the deprotonated N_E of GAA is now able to abstract the methyl group from the positively charged S_D of SAM in an S_N2

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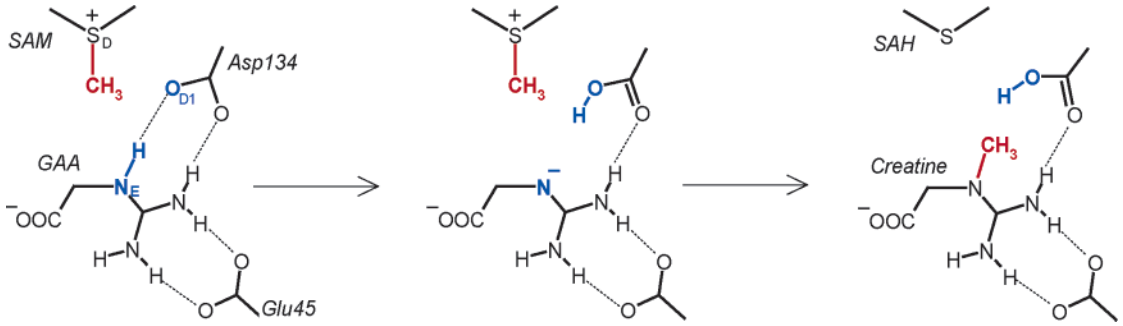
SCHEME 2: Proposed Steps for the Methyl Transfer Reaction in GAMT[3]



reaction, yielding the creatine product. Finally, the methylation of GAA breaks the hydrogen bond to Asp134 and causes the release of the creatine followed by deprotonation of Asp134 to the solvent.

In the present study, we have used the hybrid density functional theory (DFT) method B3LYP[10] to examine this suggested mechanism. We have previously used the same method and a similar active site model to study the methyl transfer step in the related enzyme glycine *N*-methyltransferase (GNMT).[11] That investigation could establish the adequacy of the approach to study this kind of reactions.

II. Computational Details

All geometries and energies presented in the present study are computed using the B3LYP[10] density functional theory method as implemented in the *Gaussian03* program package.[12] Geometry optimizations were performed using the 6-31G(d,p) basis set. On the basis of these geometries, single-point calculations with the larger basis set 6-311+G(2d,2p) were done to obtain more accurate energies. Solvation energies were added as single-point calculations using the conductor-like solvation model COSMO[13] at the B3LYP/6-31G(d,p) level. In this model, a cavity around the system is surrounded by polarizable dielectric continuum. The dielectric constant chosen is $\epsilon = 4$, which is the standard value used to model protein surrounding. Hessians were calculated at the B3LYP/6-31G(d,p) level to confirm the nature of the stationary points, with no negative eigenvalues for minima and only one negative eigenvalue for transition states. The Hessians were also used to calculate zero-point vibrational effects. Some centers were kept fixed to their X-ray positions in the geometry optimization. This procedure gives rise to a few small imaginary frequencies, typically on the order of 10i−30i cm^{-1}. These frequencies do not contribute significantly to the zero-point energies and can thus be tolerated.

III. Quantum Chemical Model

A model of the active site of GAMT was built on the basis of the GAMT:(SAH + GAA) X-ray crystal structure (PDB code 1XCJ).[3] The quantum chemical model employed comprises 92 atoms and consists of the following parts (see Figure 2):

(i) The full guanidinoacetate substrate.

(ii) The full Asp134 residue, including the peptide bond to the next amino acid, Thr135. This long chain ensures the flexibility needed to accept a proton and to accommodate methylation of the substrate, which requires breaking of the hydrogen bond to the substrate.

(iii) An acetate to model Glu45. This group forms hydrogen bonds to the guanidino group of the substrate and helps stabilize the positive charge.

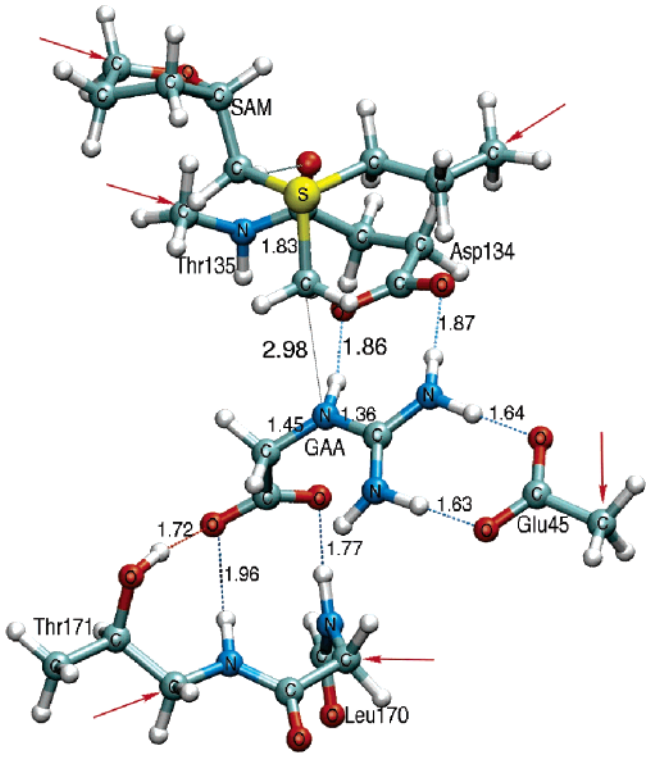


Figure 2. Optimized structure of the reactant. Distances are given in angströms. Arrows indicate atoms that are fixed to their X-ray positions.

(iv) Parts of Leu170 and Thr171. The amide groups and the hydroxyl moiety of these groups form hydrogen bonds to the acetate of GAA.

(v) A model of SAM based on the SAH structure, to which a methyl group was added at the S_D position. SAM was truncated in one side after the ribose ring and in the other three carbons away from S_D. This is sufficient to reproduce the properties of the S_D-C_E bond and to grant flexibility to the SAM model, especially since an additional methyl is added.

With all these parts, the total charge of this model is −1. In the geometry optimizations, certain atoms, typically where the truncation is done, were kept frozen to their X-ray positions. This approach is used to keep the various groups in place to resemble the crystal structure as much as possible. These fixed positions are indicated by arrows in the figures.

IV. Results and Discussion

As seen from the optimized geometry of the reactant species (Figure 2), the hydrogen-bonding network around the substrate orients the GAA substrate such that there is a nearly straight line between the sulfur center of SAM (S_D), the methyl group to be transferred (C_E), and the nitrogen of GAA (N_E). The

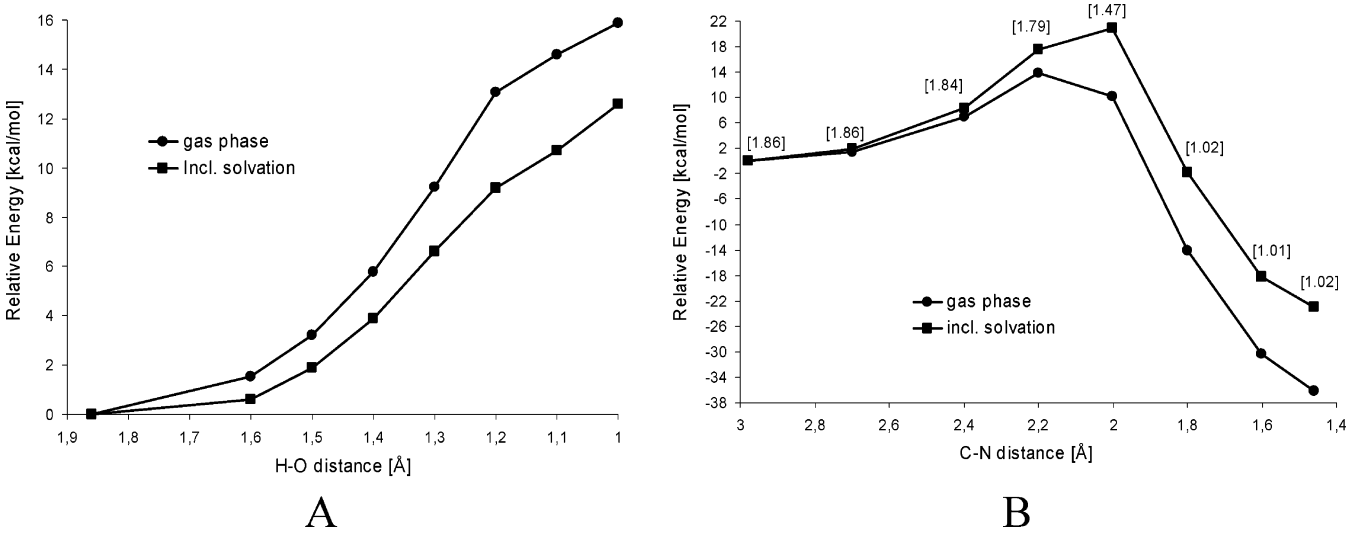


Figure 3. Potential energy curves for the following: (A) moving the proton from N_E of the substrate to O_G of Asp134 and (B) moving the methyl group from SAM to N_E of the GAA substrate. The solvation calculations are performed using the cavity model with $\epsilon = 4$. In (B), the H−O_G distance is given in brackets.

distance between C_E and N_E in this model is calculated to be 2.98 Å, and the $\angle S_D-C_E-N_E$ angle is found to be 167.7°. With this model, the distance between O_{D1} of Asp134 and the hydrogen atom bonded to N_E of GAA is 1.86 Å. The interaction between the N_E−H and O_{D1} of Asp134 causes the hydrogen atom to be slightly displaced from the sp^2 hybridization plane, tilting somewhat toward the oxygen atom.

The first step in the proposed mechanism is the proton transfer from N_E of the substrate to Asp134. To test this, we performed a linear transit scan in which the H−O_{D1} distance was kept fixed in steps between 1.86 Å (the distance at the reactant) and 1.00 Å, while all other degrees of freedom were optimized. As seen from Figure 3A, no energy minimum could be found corresponding to an intermediate where the proton is transferred to the aspartate; the energy increases monotonically. The energy at d(O−H) = 1.0 Å can still give an estimate of the energy required to move the proton from GAA to Asp134. In the gas phase, the energy cost is calculated to be 15.9 kcal/mol, while employing a model of the protein environment using a cavity model with $\epsilon = 4$ yields the somewhat lower value of 12.6 kcal/mol. This energy difference corresponds to a pK_a difference of ca. 9 units, which is too large to be a plausible first step of the reaction.

Instead, we used a similar linear transit scheme to move the methyl group from SAM to GAA. The C_E−N_E distance was kept fixed in steps starting from 2.98 Å, which is the distance in the reactant, to 1.46 Å, which is the distance of the product (energy scan shown in Figure 3B). As the methyl approaches the nitrogen center, the energy increases up to a distance of 2.2−2.0 Å, after which it starts to drop. The N_E−H proton moves toward Asp134, and the nitrogen center becomes more pyramidal. At a C_E−N_E distance of 1.8 Å, the proton has transferred completely. The methyl transfer and the proton transfer seem to be coupled.

We have managed to locate the exact unconstrained transition state for this reaction. The optimized structure is shown in Figure 4. It turns out that the methyl transfer and the proton transfer take place in one concerted asynchronous step. At the transition state (TS), the critical S_D−C_E and C_E−N_E bond distances are calculated to be 2.29 and 2.16 Å, respectively, and the N_E−H proton has barely started to move toward the aspartate (N_E−H and H−O_{D1} distances are 1.05 and 1.73 Å, respectively).

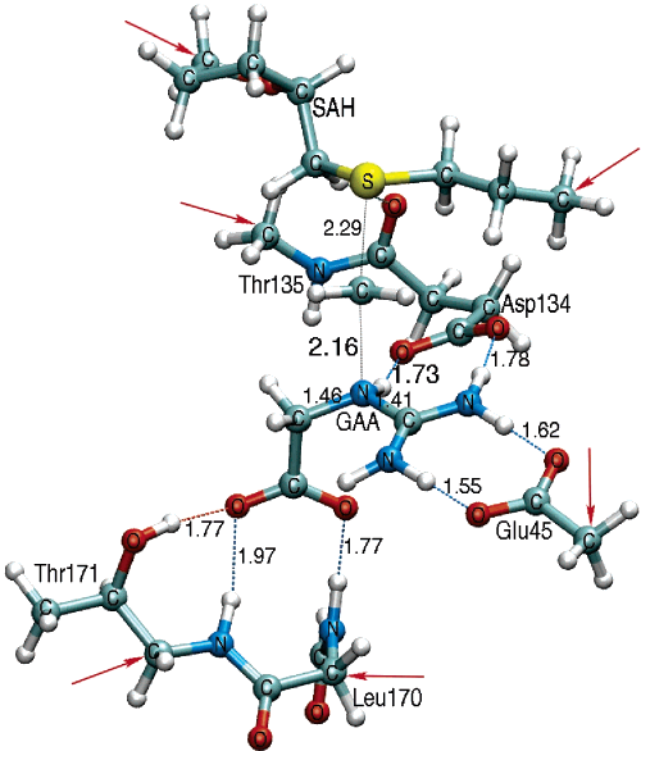


Figure 4. Optimized transition-state structure.

Frequency calculations confirmed the nature of the transition state with only one imaginary frequency of −446i cm^{-1}. The activation barrier for this process was calculated to be 14.9 kcal/mol in the gas phase and 19.7 kcal/mol using $\epsilon = 4$. This is in good agreement with the experimental rate constant measured for this reaction (3.8 ± 0.2 min^{-1})[3], which corresponds to ca. 19 kcal/mol. The reaction was calculated to be exergonic by as much as 36.2 kcal/mol in the gas phase and 24.0 kcal/mol using $\epsilon = 4$.

This large difference between the gas phase and $\epsilon = 4$ energies can be understood if we note that SAM, which is changing its charge state from cationic to neutral during the reaction, is located at the edge of the quantum chemical model. This will result in different solvation energies between the reactant and the product.

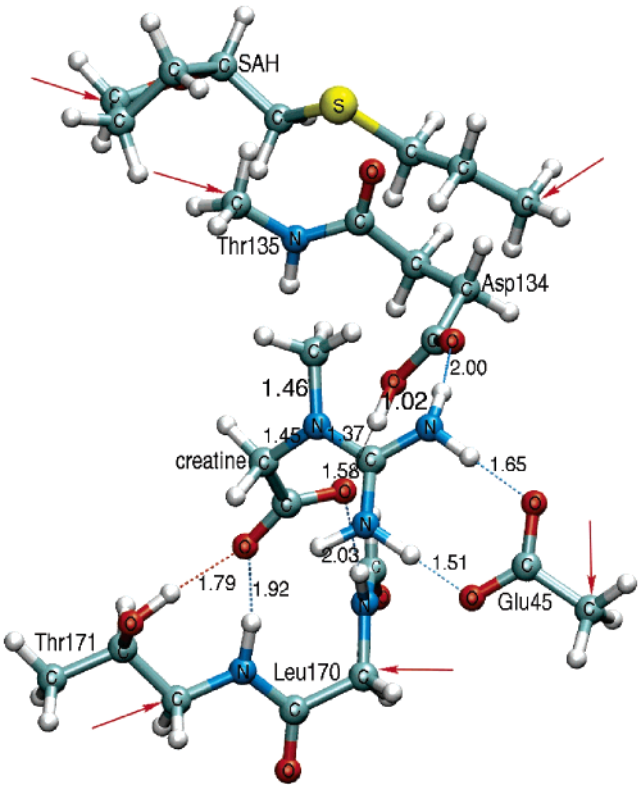


Figure 5. Optimized structure of the product.

We made several attempts to locate an intermediate structure in which the substrate is methylated but the proton has not transferred to Asp134, i.e., corresponding to a stepwise mechanism, but without any success. By this, we can of course not completely rule out this possibility, but the results indicate that, if such an intermediate exists, it is going to be kinetically insignificant.

The optimized structure of the product of the reaction is displayed in Figure 5. Upon transfer of the methyl group, the N_E returns to its original sp^2 hybridization, which causes the H-bond to Asp134 to break. This in turn results in rotation of the protonated Asp134 residue such that the carboxylic group hydrogen binds to the acetate part of GAA. One can speculate that the carboxylic proton of Asp134 can be picked up by the carboxylate of GAA when the creatine product leaves the active site, hence restoring the protonation state of the Asp134.

V. Conclusions

We have in this paper reported a quantum chemical examination of the methyl transfer reaction in guanidinoacetate methyltransferase. The calculations show that the methyl transfer from SAM to GAA is coupled with the proton transfer from GAA to Asp134. A concerted asynchronous transition state was located, and the barrier was calculated to be 19.7 kcal/mol, a value that is in good agreement with the experimental rate constant.

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Supporting Information Available: Cartesian coordinates of the reactant, transition state, and product, shown in Figures 2, 4, and 5, respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

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