# Quantum Chemical Study of Ester Aminolysis Catalyzed by a Single Adenine: A Reference Reaction for the Ribosomal Peptide Synthesis

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**Abstract:** Herein we report the results of a HF/6-31+G** and B3LYP/6-31+G** computational investigation of the title reaction for the peptide bond synthesis catalyzed by a single adenine. This system constitutes a reference reaction to study the basic chemical events that have been proposed to occur at the peptidyl transferase active site of ribosomes on the basis of structural determinations (*Science* **2000**, *239*, 920−931). Thus, the analysis of the geometry, charge distribution, and energetics of the critical structures involved in this title reaction yields insight into the catalytic mode of action of RNA molecules. Our computational results give further support to the hypotheses that the activated nucleotide A2451 in the ribosome acts as a base catalyst and that this role is similar to that of the His residue in the catalytic triad of serine proteases.

## Introduction

Very recently, the structure of the 50*S* ribosomal subunit from the *H. marismortui* bacteria and its complexes with substrate analogues has been reported at atomic resolution (2.4 Å).[1,2] This structural breakthrough has unequivocally established that the ribosome is a ribozyme since the peptidyl transferase site, located in the 23*S* subunit, is entirely built from rRNA nucleotides. The conserved A2451 residue (using the *E. coli* numbering) is the only nucleotide that is positioned to participate in the catalytic mechanism. This is in agreement with the unusual p$K_a$ of A2451 ($7.6 \pm 0.2$) which has been measured in the *E. coli* ribosome by examining its reactivity with dimethyl sulfate as a function of pH.[3] The neutral p$K_a$ of A2451, which has been assigned to its N3 atom,[2] appears related to the observed hydrogen bonding of A2451 with the conserved G2447 nucleotide which, in turn, interacts with the buried phosphate of A2450 and a potassium ion. Thus, it has been proposed[2] that some of the negative charge of the phosphate group could be relayed to the N3 atom of A2451, thereby stabilizing its imino tautomer and increasing its p$K_a$ so that, at physiological pH, a significant fraction of A2451 could function as a general base catalyst (see Scheme 1).

On the basis of the experimental results and the nature of the ester aminolysis reaction involved in peptide synthesis, a chemical mechanism for ribosome catalysis has been proposed in which the initially unprotonated N3 atom of A2451 abstracts a proton from the α-amino group of the aminoacyl tRNA in the A-site as this nucleophilic group attacks the carbonyl C atom attached to the peptidyl-tRNA in the P-site.[2,3] In this mechanism, the nucleophilic α-amino group is in its neutral form since its p$K_a$ could be lowered when the aminoacyl tRNA is bound to the ribosome. It has also been noted that this mechanism is quite similar to the reverse of the acylation step in serine proteases where the essential His residue abstracts a proton from the α-amino group of the peptide hydrolysis product as it attacks the acyl-enzyme intermediate. Thus, both RNA and protein enzymes may use similar chemical principles for catalysis.[2,4] Other authors, however, have noted that a definitive assignment of A2451 as the catalytic nucleotide should be treated with caution.[5]

Clearly, the mechanism for ribosome catalysis deserves much theoretical and experimental attention. Herein, we report a quantum-chemical investigation of the ester-aminolysis between methylamine and methyl acetate as models of the reactive amino (A-site) and ester (P-site) groups, respectively (see Scheme 2). The reaction is catalyzed by an adenine anion, which represents the basic imino form of the crucial A2451 nucleotide as experimentally proposed. The process sketched in Scheme 2 can be considered as a reference reaction for the base-catalyzed mechanism in the actual ribosome active site. The structure and energetics of the transition states (TSs) and intermediates located for this model system can give further insight into the catalytic mode of action of RNA molecules.

## Computational Methods

Ab initio calculations were carried out with the Gaussian 98 suite of programs.[6] All the optimizations were done in the gas phase with no constraints. Geometries were optimized at the HF/6-31+G** and B3LYP/6-31+G** levels of theory[7,8] and further characterized by analytic computation of harmonic frequencies at the HF/6-31+G** level. Electronic energies were also recalculated at the B3LYP/6-31+G** level using the HF/6-31+G** geometries. Atomic charges were computed for all structures with the Natural Population Analysis[9] (NPA) using the corresponding HF/6-31+G** and B3LYP/6-31+G** density matrices.

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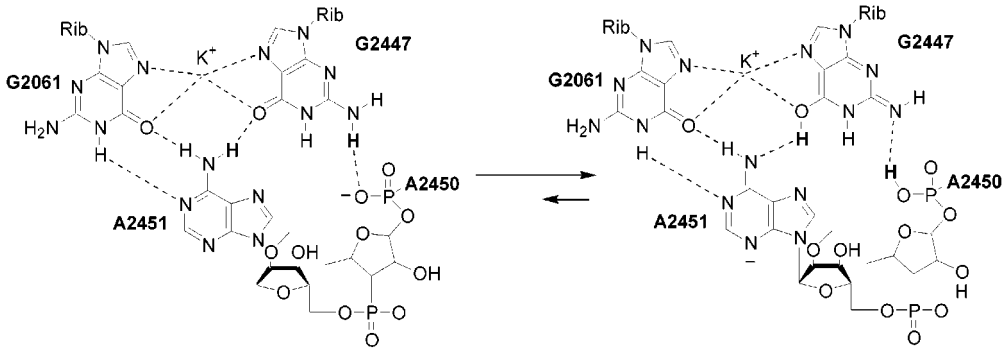
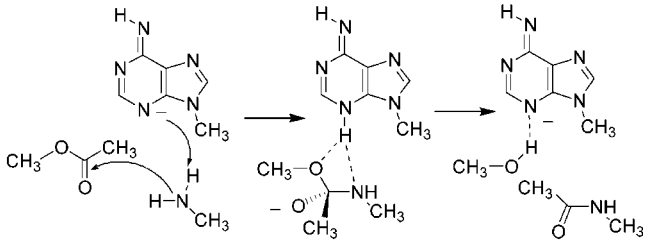
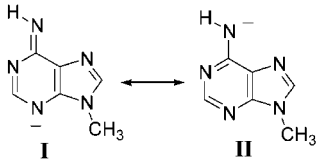
**Scheme 1**




**Scheme 2**



**Scheme 3**




## Results and Discussion

We first examined the structure and charge distribution of the anionic imino form of the adenine base and compared it with those of the neutral amino form (see Figure 1). We found that the two resonance structures shown in Scheme 3 are required to represent the anionic form. For example, with respect to the neutral amino form, the pyrimidine ring and the exocyclic imino group bear ∼0.50 e and ∼0.40 e, respectively, of the global negative charge while the N3 atom acquires only ∼0.10 e on going from the neutral amino to the anionic imino form. The total charge placed on the N3 atom amounts to 0.76 (HF) and 0.65 e (B3LYP) in the anionic imino form. We note that the contribution of the resonance structure **II**, which partially preserves the aromaticity of the pyrimidine ring, could be reinforced in the ribosome by means of the A2451−G2447 interaction observed in the crystal structure. The mechanistic details of the ester aminolysis reaction with base catalysis exerted by the adenine anion are illustrated by the critical structures located at the HF/6-31+G** and B3LYP/6-31+G** levels of theory (see Figure 2). The corresponding relative energies included in Table 1 are given with respect to the prereactive complex formed between the adenine anion and the mehylamine−methyl acetate dimer (**C** in Figure 2). In the prereactive complex (**C**), the relative orientation of the reactive groups is compatible with the structural findings reported for the ribosome−inhibitor complexes.[2] From **C**, the nucleophilic attack of the amino group proceeds via a transition state $\mathbf{TS_1}$ in

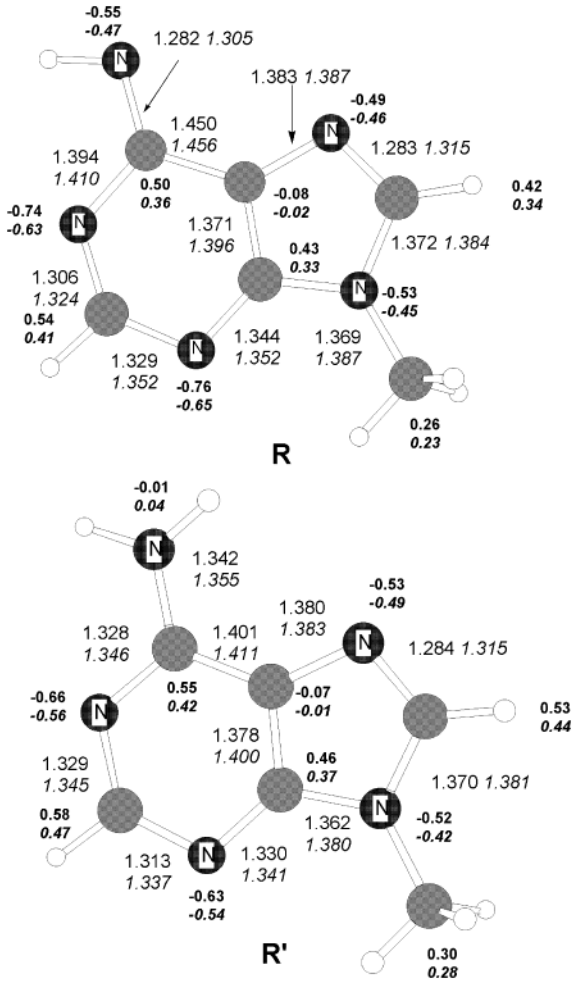



**Figure 1.** HF/6-31+G** and *B3LYP/6-31+G*** optimized geometries of the anionic imino form (**R**) and neutral amino form (**R′**) of the adenine base studied in this work. Distances are given in angstroms. NPA atomic charges with hydrogens summed into heavy atoms are also indicated in bold characters.

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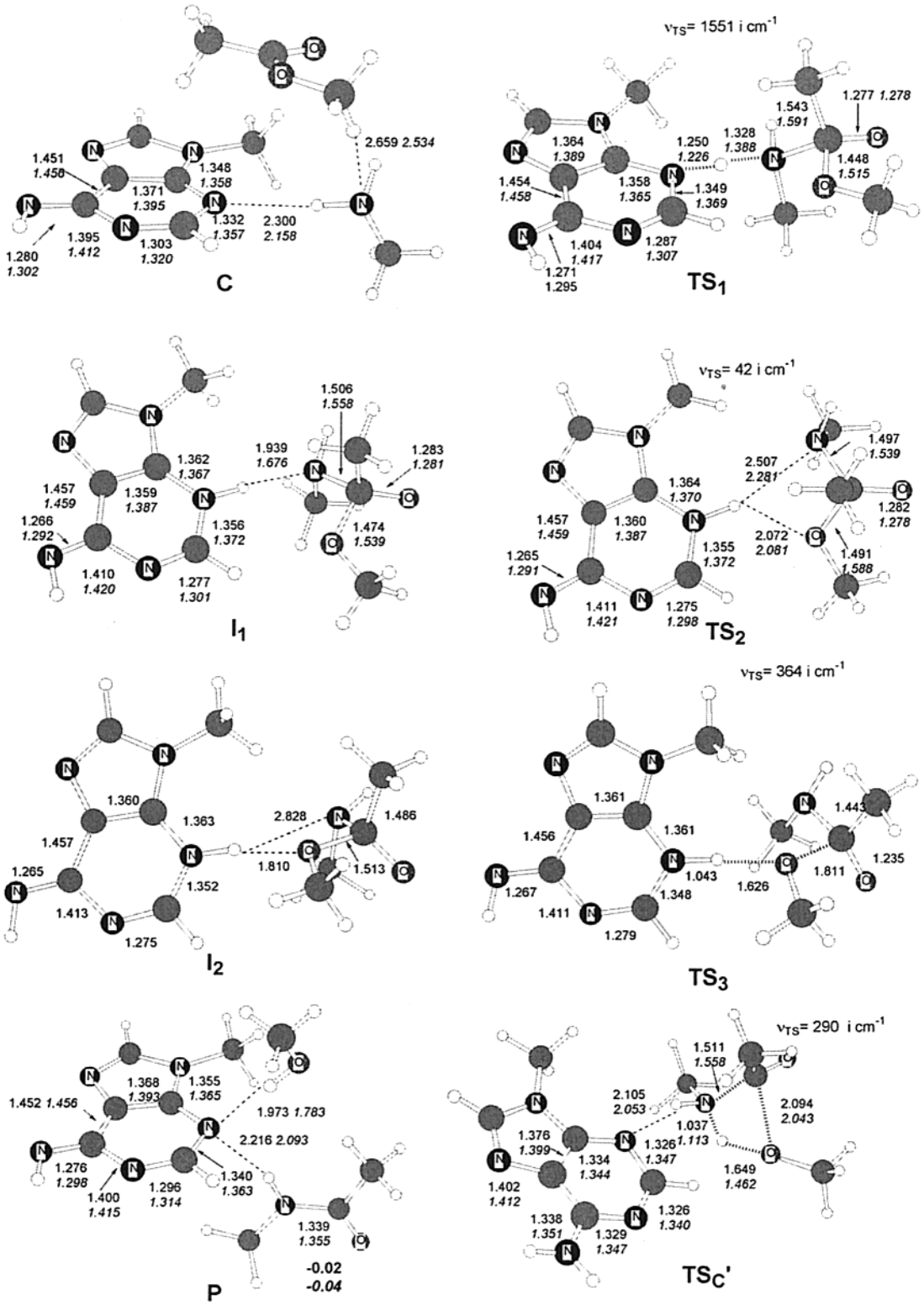


**Figure 2.** HF/6-31+G** and *B3LYP/6-31+G*** optimized structures for the ester aminolysis reaction between methylamine and methyl acetate. The series of structures (**C** → **TS$_1$** → **I$_1$** → **TS$_2$** → **I$_2$** → **TS$_3$** → **P**) describe the reaction path catalyzed by the adenine anion. **TS$_C$′** corresponds to the rate-determining transition state for a nonassisted pathway (see text for details). Distances are given in angstroms. HF/6-31+G** imaginary frequencies are also indicated.

which the forming C−N bond is quite advanced (∼1.6 Å) while the transition vector is dominated by the proton transfer to the N3 atom of the adenine moiety. Note that the formation of the C−N bond at **TS$_1$** facilitates the proton abstraction from the destabilized amino group by the adenine. From **TS$_1$**, an anionic tetrahedral intermediate (**I$_1$**) is formed which is bound to the neutral adenine through a short N3−H···N contact. For the leaving O atom to accept a proton from the protonated N3 atom, the N3−H···N interaction must be broken. This process takes place through a transition state **TS$_2$** that is characterized by a bifurcated hydrogen bond between the N3 atom of adenine with the attacking N atom and the leaving O atom. At the HF/6-31+G** level, we also located an intermediate **I$_2$** that hydrogen bonds with the leaving O atom directly. The collapse of this

**Table 1.** Relative Energies (kcal/mol) of the Critical Points for the Ester Aminolysis Reaction between Methylamine and Methyl Acetate Catalyzed by a Single Adenine Anion[a]

| structures | HF | B3LYP//HF | B3LYP |
|---|---|---|---|
| **C** | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) |
| **$TS_1$** | 37.8 (36.8) | 23.4 (22.4) | 23.8 (22.8) |
| **$I_1$** | 31.9 (33.5) | 23.9 (25.5) | 23.0 (24.6) |
| **$TS_2$** | 33.0 (34.5) | 25.4 (26.9) | 25.3 (26.8) |
| **$I_2$** | 32.0 (33.6) | 23.1 (24.7) | - |
| **$TS_3$** | 34.8 (34.7) | 20.8 (20.7) | - |
| **P** | −12.3 (−11.9) | −15.4 (−15.0) | −15.5 (−15.1) |

[a] All the calculations were done with the 6-31+G** basis set. Values in parentheses include the ZPVE correction obtained from HF/6-31+G** analytical frequencies.

tetrahedral intermediate can occur readily through the elongation of the C−O bond followed by the N3−H(adenine) → methoxy proton transfer (**$TS_3$**). We note that the **$I_2$** and **$TS_3$** structures were not stable structures on the B3LYP/6-31+G** Potential Energy Surface (PES) so that, at this level of theory, **$TS_2$** would lead directly to the product complex **P**.

Inspection of the bond distances and the atomic charges (Figure 2 and Table S1 in the Supporting Information) reveals that neutralization of the adenine base during catalysis affects the pyrimidine ring whereas the exocyclic imino group retains much of its negative charge (∼0.40−0.50 e). On the other hand, the series of structures involved in the proton-transfer steps and the rupture of the C−O bond (**$TS_1$** → **$I_1$** → **$TS_2$** → **$I_2$** → **$TS_3$**) show a similar charge distribution, the global negative charge being distributed among the electronegative atoms in the tetrahedral species. In agreement with their geometrical and electronic similarity, these structures are also very close in electronic energy (see Table 1). Moreover, inclusion of electron correlation effects at the B3LYP/6-31+G** level suggest that the collapse of the tetrahedral species accompanied by proton transfer from adenine to the leaving O atom could be barrierless once the N3−H···N interaction is lost at **$TS_2$**. Similarly, addition of the ZPVE corrections to the B3LYP/6-31+G** energies predicts that **$TS_1$** is not a true transition state since **$TS_1$** is computed to be 1.8 kcal/mol more stable than **$I_1$**. Therefore, along the ZPVE-corrected B3LYP/6-31+G** energy profile, **$TS_2$** is the rate-determining structure for the base-catalyzed ester aminolysis reaction. This unstable structure, which represents the high-energy tetrahedral intermediate interacting with the protonated adenine through a bifurcated hydrogen-bond interaction, gives an overall energy barrier of 26.8 kcal/mol for the model reaction.

To find out if the adenine-assisted mechanism is catalytically relevant, we also studied the ester aminolysis reaction in the presence of the neutral amino form of the adenine base. However, we found that the series of structures (**$TS_1$** → **$I_1$** → **$TS_2$** → **$I_2$** → **$TS_3$**) are no longer stable when the adenine base becomes neutralized, that is, the neutral adenine base does not play a direct role in assisting the proton-transfer events in consonance with its poor basicity. Alternatively, we studied a concerted reaction pathway[7] in which the C−O bond cleaves at the same time as the C−N bond is formed and the proton transfer is at its initial stages. In the transition structure for this mechanism (**$TS_C'$**), the neutral adenine base plays only a passive role by stabilizing the attacking amino group via a N3···H−N hydrogen bond. The energy barrier for **$TS_C$** amounts to 37.4 kcal/mol at the B3LYP/6-31+G** level including the HF ZPVE correction. This value is 10.6 kcal/mol above that of **$TS_2$**, Therefore, we confirm that a single adenine anion could efficiently catalyze the ester aminolysis reaction involved in ribosomal peptide synthesis.

In summary, our computational results delineate structural and energetic characteristics of base catalysis exerted by a single adenine anion. Most interestingly, the N3 atom in the catalytic anion accumulates only a small negative charge with respect to the neutral noncatalytic adenine while the exocyclic imino group maintains an important negative charge of ∼0.40 e during catalysis. The charge distribution of the pyrimidine ring results in its considerable catalytic activity favoring the proton-transfer events. The tetrahedral intermediate is located in a very shallow region of the PES and, therefore, we expect that tetrahedral intermediates would not accumulate during the course of the reaction. Indeed the absence of a stable tetrahedral species is a typical feature of serine proteases catalysis.[11] Overall, these results give further support to the previous proposal[2] that the chemical role of the activated A2451 in the ribosome resembles that of the essential His residue in the catalytic triad of serine proteases.

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**Note Added in Proof:** According to very recent in vitro experiments,[12] large ribosomal subunits with mutated A2451 showed significant activity in several independent assays using peptidyl analogues (P-site) and puromycin, a structural analogue of the reactive amino group (A-site). The authors concluded that ribosome catalyzes peptidyl transfer primarily by fixing the proper position of the reaction substrates although they do not rule out a possible contribution of chemical catalysis. These experimental results suggest that the mechanisms studied in this work in the presence of the anionic and neutral forms of adenine should be competitive in the peptidyl transferase site. Alternatively, it might also be possible that a buried water molecule acts as a bifunctional catalyst.

**Supporting Information Available:** Tables containing atomic charges and Cartesian coordinates for the series of structures **C** → **$TS_1$** → **$I_1$** → **$TS_2$** → **$I_2$** → **$TS_3$** → **P** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Other stepwise processes with a similar stability to that of the concerted route could be possible for the nonassisted reaction. See, for example: Zipse, H.; Wang, L.; Houk, K. N. *Liebigs Ann.* **1996**, 1511−1522.

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