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# Unambiguous Evidence for Efficient Chemical Catalysis of Adenosine Ester Aminolysis by Its 2′/3′-OH

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Peptide bond formation by alkyl ester aminolysis is a very slow reaction and requires a proton transfer catalyst in order to proceed at a measurable rate.[1] As a three-molecular reaction, it involves a large entropy loss which accounts for the extremely slow rates. During protein biosynthesis,[2] this entropy problem is solved by binding of the two substrates and the catalyst in a proper configuration, which transforms the slow three-molecular reaction into a very fast intracomplex one. This could mean that the ribosome provides favorable configurational entropy for efficient catalysis by the proton transfer catalyst—the 3′-terminal adenosine 2′-OH of the peptidyl tRNA. The verification of this hypothesis requires studies of both the ribosome and model systems. The current mechanistic proposals are dominated by the idea of the ribosome being a template for entropic activation only without providing acid−base catalytic groups.[3] Here, we report on computational and experimental studies of intramolecular aminolysis reactions, catalyzed by a vicinal OH group. They were designed as model reactions (Chart 1) of the ribosome intracomplex aminolysis in order to find support for the crucial role of the 3′-terminal adenosine 2′-OH of the peptidyl tRNA as acid−base catalyst in the ribosome peptidyl transfer.

Quite recently[4] we reported a computational study of the mechanism and energetics of the simplest model of the reaction naturally catalyzed by the ribosome—the ammonolysis of 1-*O*-formyl 1,2-ethanediol **1** (Chart 1). The favored pathway predicted by calculations is a stepwise addition/elimination in which addition and elimination are coupled with *syn*-2-OH-assisted proton shuttling to maintain the neutrality of the tetrahedral intermediate formed. The model substrate **1** is designed to possess enantiotopic rather than diastereotopic re and si faces of the ester carbonyl and the amine attack produces R and S transition states/tetrahedral intermediates with the same energy and mirror-image structures. To model the intramolecularity of the ribosome aminolysis reaction, we now study the aminolysis of 1-*O*-*δ*-aminovaleryl 1,2-ethanediol **2**, in which the $NH_2$ group is an integral part of the substrate and attacks the pro-S face of the ester carbonyl (Chart 1) as does probably α-$NH_2$ of the aminoacyl tRNA during the ribosome reaction.[3d] The calculations were performed at the B3LYP level with the 6-31G(d,p) basis set.[4]

Our reference reaction is the ammonolysis of 1-*O*-formyl 1,2-ethanediol with an anti-oriented 2-OH **1a** (Chart 1), in which neither 2-OH nor *δ*-$NH_2$ can exert a proximity effect. The calculated reduction of the activation Gibbs free energy of the reference reaction $\Delta\Delta G^*$, caused by the proximity effects in the addition and elimination transition states **TS1** and **TS2** of the stepwise mechanism, is shown in Table 1. The design of the substrates allows us to calculate the individual contribution to $\Delta\Delta G^*$ of (a) the intramolecular attack of *syn*-*δ*-$NH_2$ **2a**, (b) the intramolecular catalysis by *syn*-2-OH **1b**, and (c) the combined action of *syn*-2-OH and *syn*-*δ*-$NH_2$ **2b** (Chart 1).

***Chart 1***

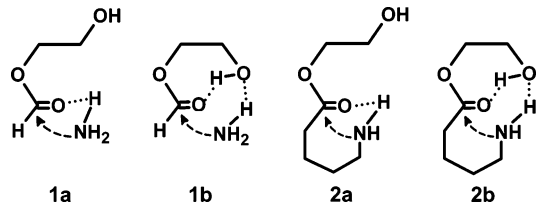


***Table 1.*** Calculated Reduction of $\Delta G^*$, $\Delta H^*$, and $T\Delta S^*$ of the Reference Aminolysis Reaction **1a** by an Intramolecular Pro-S Attack of *syn*-*δ*-$NH_2$ (**2a**), an Intramolecular Participation of *syn*-2-OH (**1b**), and Their Combined Effect (**2b**), Associated with the First, Second, and Rate-Determining Transition States **TS1**, **TS2**, and **RDTS**

| | TS1 | | | TS2 | | | RDTS | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\Delta\Delta G^*$ | $\Delta\Delta H^*$ | $T\Delta\Delta S^*$ | $\Delta\Delta G^*$ | $\Delta\Delta H^*$ | $T\Delta\Delta S^*$ | $\Delta\Delta G^*$ | $\Delta\Delta H^*$ | $T\Delta\Delta S^*$ |
| **1a** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **2a** | 6.5 | −1.0 | −7.5 | 11.0 | 3.3 | −7.7 | 6.5 | −1.0 | −7.5 |
| **1b** | 22.5 | 26.3 | 3.8 | 8.1 | 10.2 | 2.1 | 15.9 | 17.5 | 1.6 |
| **2b** | 32.8 | 29.2 | −3.6 | 16.9 | 11.8 | −5.1 | 24.7 | 19.1 | −5.6 |

Characteristically, in the first transition state **TS1**, the reduction of $\Delta\Delta G^*$ induced by *syn*-OH catalysis (22.5 kcal/mol for **1b**) is more than three times larger than the reduction caused by the intramolecularity of the *δ*-$NH_2$ attack (6.5 kcal/mol for **2a**). The combined proximity effect of the two groups is synergistic (32.8 kcal/mol for **2b**). On the contrary, the proximity effect of *syn*-*δ*-$NH_2$ in the second transition state **TS2** is even higher than that of *syn*-2-OH, and their combined effect is almost additive (Table 1).

Thus, the calculated variations in $\Delta\Delta G^*$ match the effect of the transition state proton transfer geometry,[4] suggesting a domination of *syn*-2-OH proton shuttling catalysis in the acceleration of the aminolysis. This conclusion is supported by the variation of the calculated enthalpic and entropic contributions $\Delta\Delta H^*$ and $T\Delta\Delta S^*$ (Table 1). Actually, the entropic contribution in both the first and second transition states of **2a** aminolysis exceeds the enthalpic contribution due to the unfavorable direct proton transfer in the absence of the *syn*-OH assistance.[4] On the contrary, the enthalpic contribution to the reduction of the free energy of the two transition states in **1b** and **2b** is 2−6 times larger than the entropic contribution (Table 1) due to the effective *syn*-2-OH-assisted proton shuttling.[4] Therefore, the driving force for the acceleration of the model aminolysis reaction is the proton shuttling catalysis by *syn*-2-OH.

The experimental determination of $\Delta H^*$ and $\Delta S^*$ requires dissection of $\Delta G^*$ into enthalpy and entropy contributions. However, this cannot be done for $\Delta G^*$ determined in aqueous solutions because of the enthalpy−entropy compensation in water:[5] the measured values contain contributions from $\Delta H_{sol}$ and $\Delta S_{sol}$ that cancel out in $\Delta G^*$ but may obscure measured $\Delta H^*$ and $\Delta S^*$. On



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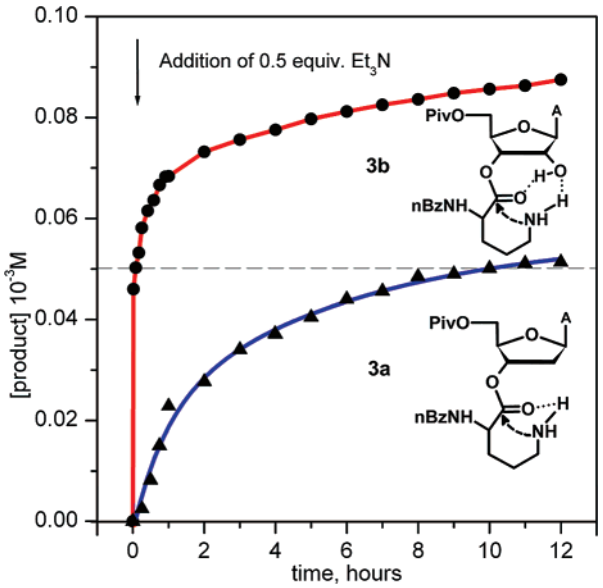


***Figure 1.*** Time course of the intramolecular aminolysis (lactamization) in acetonitrile at 25 °C of 0.1 mmol trifluoroacetate of 2′/3′-*O*-(α-*N*-*p*-nitrobenzoyl ornithinyl) 5′-*O*-pivaloyl adenosine **3b** and deoxyadenosine **3a** to α-*N*-*p*-nitrobenzoyl cyclic ornithine after addition of 1/2 equiv of $Et_3N$.

the basis of such experimental data, the ribosome has been considered as an entropy trap.[3a] On the other hand, the computer simulation approaches predict a larger contribution of the solvation entropy $\Delta S_{sol}$ than of the solute configurational entropy $\Delta S_{prox}$ considered here. The authors, however, do not mention the ratio of the corresponding enthalpic contributions, $\Delta H_{sol}$ and $\Delta H_{prox}$.[6b]

The acceleration of the overall aminolysis reaction is associated with the decrease in the activation energy of the rate-limiting transition state $\Delta\Delta G^*$ (Table 1). The difference between the values for intramolecular aminolysis of 1-*O*-*δ*-aminovaleryl 1,2-ethanediol with (**2b**) and without (**2a**) participation of 2-OH is 18.2 kcal/mol, which corresponds to a more than billion-fold ($2.2 \times 10^{13}$) rate acceleration. In order to check this prediction of the theory, we prepared 2′/3′-*O*-(α-*N*-*p*-nitrobenzoyl-L-ornithinyl) 5′-*O*-pivaloyl adenosine **3b** and its 2′-deoxy analogue **3a** and studied the time course of their intramolecular aminolysis to the corresponding *δ*-lactam (α-*N*-*p*-nitrobenzoyl cyclic L-ornithine) in the polar organic solvent acetonitrile. After the addition of 1/2 equiv of $Et_3N$ to 0.1 mmol of trifluoroacetate of the adenosine ester **3b**, an initial "burst" formation of ca. 0.05 mmol of cyclic L-ornithine, followed by a much slower release, was observed (Figure 1). The biphasic nature of the time course suggests the occurrence of two consecutive reactions, the first one being that of the free base *δ*-$NH_2$ and the second that of its trifluoroacetate salt. Under the same conditions, however, only slow aminolysis by *δ*-$NH_2$ is registered in the case of the deoxy analogue **3a**. The estimated minimal rate constant ratio of the aminolysis of adenosine and deoxyadenosine ester is ca. 3600.[7] Therefore, while the lactamization of the adenosine ester **3b** is instantaneous (the reaction being over during the mixing of the reagents and its quenching in the analytical sample (10 s)), that of the deoxyadenosine ester **3a** is not over until 10 h (Figure 1). Since the only difference in the substrates is the presence or not of the 2′-OH, the observed dramatic acceleration in the case of the adenosine ester can only be attributed to the catalysis by the 2′-OH group. To the best of our knowledge, this is the first unequivocal experimental evidence for efficient chemical catalysis of adenosine ester aminolysis by its 2′-OH group in a nonribosomal reaction.

The dramatic difference in the aminolysis rate of the adenosine and deoxyadenosine esters approaches the large difference in reactivity of peptidyl tRNA and its 2′-deoxy mutant in the ribosome fragment reaction.[8] The parallel change in the reactivity of the model and ribosome reaction, however, do not necessarily imply that the ribosome provides a microenvironment resembling that of a polar aprotic solvent. However, as established by molecular dynamic simulation studies,[6] enzymes and particularly the ribosome use preorganized polar electrostatic environment to catalyze the corresponding reactions. The observed time course of our bioorganic model reactions indicates that the non-hydrogen-bonding polar organic solvent acetonitrile is the simplest model of the ribosome polar electrostatic environment of the rate-limiting transition state.

After the formation of the ribosome−substrate complex, each of the ribosome substrates becomes an integral part of the ribosome and the participation of substrate acid−base groups in the catalytic process is possible. This reminds us of the formation of a holoenzyme from an apoenzyme and coenzyme, the latter being the ribosome and peptidyl tRNA, respectively. Recent studies of ribosome−substrate crystal structures[3d] reveal that the 2′-OH of the peptidyl tRNA 3′-terminal adenosine hydrogen bonds to aminoacyl tRNA α-$NH_2$ and is the only functional group positioned to act as a general base. The deleterious effect of its removal on ribosome catalysis[9] and the dramatic change in the aminolysis rate after deletion of the adenosine 2′-OH observed here strongly support its significant catalytic role in substrate-assisted ribosome catalysis.

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**Supporting Information Available:** Computational details and data, synthesis and characterization of the substrates, and description of the kinetic experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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