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The syn-Oriented 2-OH Provides a Favorable Proton Transfer Geometry in 1,2-Diol Monoester Aminolysis: Implications for the Ribosome Mechanism

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1,2-Diol monoester aminolysis, the iteration of which on the living cell ribosome results in protein biosynthesis, is a fundamental chemical reaction.[1] A neighboring 2-OH has a small effect on ester reactivity in aqueous solutions,[2] but this effect increases strongly in non-hydrogen bonding solvents.[2c] The importance of the 2′-OH of the invariant peptidyl tRNA 3′-terminal adenosine now seems well established, but the nature of its catalysis is less clear.[3] The simplest model reaction of the 1,2-diol monoester aminolysis is the ammonolysis of 1-*O*-formyl 1,2-ethanediol **1** (Scheme 1). Previous computational studies[4] of the transition state structures and energetics of the aminolysis of its 2-deoxy derivatives revealed two reaction pathways or mechanisms viable in the gas phase/aprotic medium. The first is a concerted pathway **A** involving direct nucleophilic substitution coupled with proton transfer from the nucleophile (NH_3) to the leaving alkoxy group (Scheme 1). The second mechanism is a stepwise addition/elimination pathway **B** in which the addition and elimination steps are coupled with proton transfer to maintain neutrality in the tetrahedral intermediate **I** formed. Inclusion of one water[4b] or a second amine[4c] molecule to assist proton transfer substantially lowers the activation energies for both mechanisms, which is consistent with the well-known acceleration of alkyl ester aminolysis by a water, alcohol, or amine molecule.[5] In the present computational study, we wanted to find out whether the syn-oriented 2-OH in 1-*O*-formyl 1,2-ethanediol **1a** can act in a manner similar to that of an external water, alcohol, or amine molecule in the aminolysis of its 2-deoxy analogue. To eliminate any interference of the through-bond effect of 2-OH, the aminolysis of the conformer with an anti-oriented 2-OH **1b** was studied as a reference reaction instead of the aminolysis of the 2-deoxy derivative, 1-*O*-formyl ethanol.

The calculations were performed at the B3LYPlevel with 6-31++G(d,p) basis set.[6] Gibbs free energy profiles generated for the concerted (**Ac$_4$** and **Ac$_6$**) and stepwise (**Bs$_4$** and **Bs$_6$**) pathways for the ammonolysis of both anti (**1b**) and syn (**1a**) conformers of 1-*O*-formyl 1,2-ethanediol are shown in Figure 1. Characteristically, the energy barriers for the concerted **Ac$_4$** and stepwise mechanism **Bs$_4$** of the aminolysis of the conformer with anti-oriented 2-OH, **1b**, are similar, as it has been previously calculated for the aminolysis of 2-deoxy esters.[4b,c] This suggests that the anti-oriented 2-OH does not affect the mechanism of alkyl ester aminolysis.

The syn-oriented 2-OH, however, removes the similarity in the energy barriers of the concerted and stepwise pathways. It leaves unchanged the activation energy of the concerted mechanism **Ac$_4$** but lowers dramatically (by ca. 18 kcal/mol) the energy of the rate-limiting first step of the stepwise mechanism **Bs$_4$**, while the decrease for the second step is modest (by ca. 5 kcal/mol) (Figure 1). Thus, the syn-2-OH induces a change in the rate-limiting step, providing a lower energy stepwise pathway **Bs$_6$**, with the second step

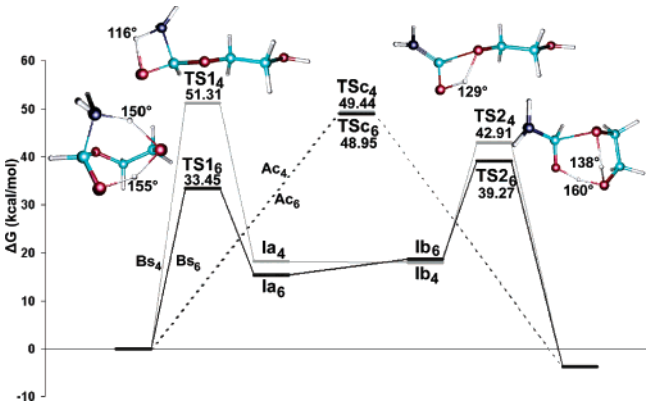
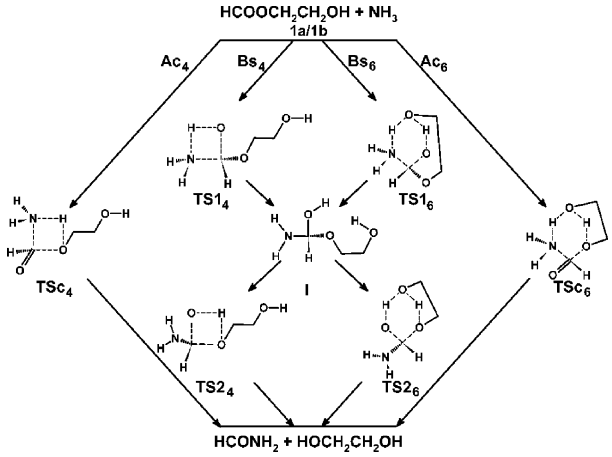


Figure 1. Energy profiles and transition state structures for concerted (dotted line) and stepwise (solid line) mechanisms of the aminolysis of conformers of 1-*O*-formyl 1,2-ethanediol with an anti- (light line) and syn (heavy line)-oriented 2-OH.

Scheme 1. Proposed Mechanisms of Aminolysis of 1-*O*-Formyl 1,2-Ethanediol with anti- (**Ac$_4$** and **Bs$_4$**) and syn (**Ac$_6$** and **Bs$_6$**)-Oriented 2-OH



becoming now rate-limiting. A similar catalytic effect has been predicted for water-[4b] and amine[4c]-assisted aminolysis of 2-deoxy esters. Therefore, the catalytic role of the added water and amine molecules in 2-deoxy ester aminolysis is taken over by the syn-oriented 2-OH in 1,2-diol monoester aminolysis. The decrease in the activation energy of the overall reaction is ca. 12 kcal/mol corresponding to an almost billion-fold (0.67×10^9) rate acceleration.

Understanding the mechanism of this tremendous catalytic effect of syn-oriented 2-OH is possible provided that we know the response of the rate-limiting transition state structure **TS1$_4$** to the presence of syn-oriented 2-OH. The optimized **TS1$_4$** structure is shown in Figure 1 and Scheme 1. It is a four-membered ring in

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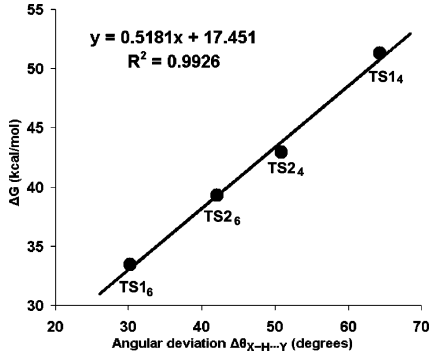

Figure 2. Transition state energy as a function of the angular deviation $\Delta\theta_{X-H\cdots Y}$ for the H-bond of the rate-limiting transition state proton transfer.

which the proton transfer has hardly begun, while C−N bond formation and ester carbonyl bond cleavage are almost complete, that is, it has a zwitterionic-like character. After the reaction passes this transition state structure, the proton transfer occurs for the next 30 kcal/mol down the reaction path. Therefore, the barrier for this step is the creation of a geometrically and electrostatically favorable transition state structure for proton transfer.

The created proton transfer geometry in **TS1$_4$**, however, is highly unfavorable since the distance between the donor (NH_3 nitrogen) and acceptor (C═O_c oxygen) atoms is stretched and the hydrogen bond is bent. It is known[7] that proton transfer barriers rise quickly as the angular deviation from the requisite 180° for a linear hydrogen bond $\Delta\theta_{X-H\cdots Y} = 180 - \theta_{X-H\cdots Y}$ gets beyond 40°. This is found in the tetragonal transition state **TS1$_4$** ($\Delta\theta_{N-H\cdots Oc} = 64.3°$) accounting for the high activation energy of the anti conformer aminolysis. When 2-OH is syn-oriented, however, it can be inserted in the four-membered ring, expanding it to a six-membered one, and in this hexagonal transition state **TS1$_6$** (pathway **Bs$_6$**), it bridges the N to O_c proton transfer by accepting a hydrogen bond from NH_3 and donating a hydrogen bond to the carbonyl oxygen. The angular distortions for these two transition state hydrogen bonds are well below 40°: $\Delta\theta_{N-H\cdots O2} = 30.8°$ and $\Delta\theta_{O2-H\cdots Oc} = 25.2°$. Therefore, the presence of syn-oriented 2-OH provides a more favorable, that is, more linear, proton transfer geometry for the syn addition of ammonia N−H to the ester C═O_c. The single unfavorable proton transfer in **TS1$_4$** is substituted by two favorable proton transfers in **TS1$_6$**. This efficient double proton transfer promotes such a dramatic lowering of the activation energy of the first step that it is not rate-limiting anymore along the addition/elimination pathway **Bs$_6$**.

The transition states **TS1$_4$** and **TS1$_6$** evolve to the neutral tetrahedral intermediates **Ia$_4$** and **Ia$_6$**, respectively (Figure 1). The next step, the syn elimination of 1,2-ethanediol, occurs after the low-energy conformation **Ia$_6$** isomerizes to the more reactive conformation **Ib$_6$**. The syn elimination mechanism requires specific geometric arrangements and particularly, in this case, syn-coplanar conformations of the transition state **TS2$_6$**. The proton transfer geometry for this step evidently is not so favorable since the reduction of its activation barrier by the presence of syn-2-OH is modest (ΔG = ca. 5 vs 18 kcal/mol for the first step). Actually, the angular deviation from linearity of hydrogen bonding and particularly that for the second (O2 to O3) proton transfer ($\Delta\theta_{O2-H\cdots O3} = 42.1°$) is larger than that calculated for both transition state proton transfers of the first step **TS1$_6$**. As a result, this step becomes rate-limiting in the overall aminolysis reaction. Finally, a linear plot of the transition state energy versus the angular deviation from linearity of the hydrogen bond for the rate-limiting transition state proton transfer was obtained (Figure 2), suggesting that the latter controls the aminolysis rate.

It is noteworthy that we did not localize the hexagonal transition state **TS1$_6$** on the potential energy surface in the ammonolysis of 1-*O*-acetyl 1,2-benzenediol (acetyl catechol).[8] In contrast to a free rotation around the single C1−C2 bond connecting the vicinal hydroxyls in 1,2-ethanediol, the rotation around this bond in catechol is restricted by the partially double bond C1═C2. This conformational constraint prevents **TS1$_6$** formation, and the small catalytic effect (2 kcal/mol) of the un-ionized *o*-OH is attributed to its hydrogen bonding to the carbonyl oxygen[8] only.

The only lethal modifications in the ribosome−peptidyl tRNA complex are the substitutions of the peptidyl tRNA A76 2′-OH by H or F[3b] and of its proton by a methyl group.[3c] On the basis of these findings, we ascribed[3c] a proton shuttling role to A76 2′-OH, which has been recently supported by computational modeling[3d] and crystal structure analysis.[3e] Here we report that the syn-oriented 2-OH provides a more favorable proton transfer geometry resulting in an almost billion-fold rate acceleration. Since the A76 2′-OH is syn-oriented to the peptidyl group in peptidyl tRNA, these findings provide a structural basis for the explanation of its efficiency in proton shuttling as a possible catalytic strategy used by the ribosome. As a matter of fact, in this double proton transfer mechanism, the developing carbonyl oxyanion acts as a general base which deprotonates the attacking α-NH_2 by the intermediacy of the syn-oriented A76 2′-OH[3c] (transition state **TS1$_6$**). The predicted necessity for a geometrically and electrostatically favorable situation for the rate-limiting transition state proton transfer assigns a crucial role to the ribosome peptidyl transfer center in the precise positioning of peptidyl and aminoacyl tRNAs.

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Supporting Information Available: Computational details, structure of all transition states and intermediates, including bond lengths and proton transfer angles. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Wilson, D. N.; Nierhaus, K. N. *Angew. Chem. Int. Ed.* **2003**, *42*, 3464−3486.
(2) (a) Sprinzl, M.; Cramer, F. *Nat. New Biol. (London)* **1973**, *245*, 3−5. (b) Sharma, P. K.; Xiang, Y.; Kato, M.; Warshel, A. *Biochemistry* **2005**, *44*, 11307−11314. (c) Changalov, M. M.; Bayryamov, S. G.; Mladjova, A. P.; Yomtova, V. M.; Petkov, D. D. Unpublished results.
(3) (a) Das, G. K.; Bhattacharyya, D.; Burma, D. P. *J. Theor. Biol.* **1999**, *200*, 193−205. (b) Weinger, J. S.; Parnell, K. M.; Dorner, S.; Green, R.; Strobel, S. A. *Nat. Struct. Biol.* **2004**, *11*, 1101−1106. (c) Changalov, M. M.; Ivanova, G. D.; Rangelov, M. A.; Acharia, P.; Acharya, S.; Minakawa, N.; Foldesi, A.; Stoineva, I. B.; Yomtova, V. M.; Roussev, C. D.; Matsuda, A.; Chattopadhyaya, J.; Petkov, D. D. *ChemBioChem* **2005**, *6*, 972−977. (d) Trobro, S.; Aqvist, J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12395−12400. (e) Schmeing, T. M.; Huang, K. S.; Kitchen, D. E.; Strobel, S. A.; Steitz, T. A. *Mol. Cell* **2005**, *20*, 437−448. (f) Youngman, E. M.; Brunelle, J. L.; Kochaniak, A. B.; Green, R. *Cell* **2004**, *117*, 589−599. (g) Sievers, A.; Beringer, M. V.; Rodnina, R.; Wolfenden, R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7897−7901.
(4) (a) Zipse, H.; Wang, L.; Houk, K. N. *Liebigs Ann.* **1996**, 1511−1522. (b) Yang, W.; Drueckhammer, D. G. *Org. Lett.* **2000**, *2*, 4133−4136. (c) Ilieva, S.; Galabov, B.; Musaev, D. G.; Morokuma, K.; Schaefer, H. F., III. *J. Org. Chem.* **2003**, *68*, 1496−1502.
(5) (a) Gordon, M.; Miller, J. G.; Day, A. R. *J. Am. Chem. Soc.* **1949**, *71*, 1245−1250. (b) Menger, F. M.; Smith, J. H. *J. Am. Chem. Soc.* **1972**, *94*, 3824−3829.
(6) Computational details are found in the Supporting Information.
(7) Duan, X.; Scheiner, S. *J. Am. Chem. Soc.* **1992**, *114*, 5849−5856.
(8) Rangelov, M. A.; Vayssilov, G. N.; Yomtova, V. M.; Petkov, D. D. *Org. Biomol. Chem.* **2005**, *3*, 734−744.

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