Kinetic Analysis of Ester Aminolysis Catalyzed by Nucleosides in a Nonpolar Medium. Evidence of Bifunctional Catalysis

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Kinetic analysis of ester aminolysis in benzene catalyzed by 2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)-protected nucleosides and 2-pyridone is reported. The catalytic rate constant $k_{3'}$ was determined for protected nucleosides A, C, G, U, and pseudouridine (Ψ). The relatively high value associated with C and 2-pyridone is indicative of bifunctional catalysis occurring through stabilization of the aminolysis transition state. The implications of this finding on the possible role C plays in biological catalysis during protein synthesis is hypothesized.

An initial report from our laboratory demonstrated that functional groups of TBS-protected ribonucleoside bases are capable of catalyzing amide bond formation in a nonpolar medium (Figure 1).[1] This bond is the seminal bond formed during protein synthesis, and its formation is catalyzed by the ribosome.[2] A relatively large rate enhancement was observed for cytidine in comparison with the other ribonucleosides, A, G, U, and pseudouridine (Ψ) (Figure 2). Although there exists a plethora of RNA-catalyzed processes including the recent ribozyme-catalyzed aminolysis reaction,[3] the catalytic role which functional groups of nucleoside bases play remains obscure due to the complex nature of the polynucleotide structure. Herein, we report a kinetic description of ester aminolysis catalyzed by nucleosides. The data indicates C catalyzes aminolysis through a bifunctional mechanism that involves simultaneous donation and acceptance of protons in the aminolysis transition state.

Previous studies have shown[4−6] that under pseudo-first-order conditions and in the presence of nonnucleoside catalysts, aminolysis of esters in aprotic solvents proceeds according to the rate expression given in eq 1.

$$k_{1obs} = k_2[\text{amine}] + k_3[\text{amine}]^2 + k_{3'}[\text{amine}][\text{catalyst}] \quad (1)$$

The k_2 term is not detectable at room temperature[7] but has been observed at higher temperatures.[8] The k_3 term has been shown to be relatively consistent, regardless of the catalyst type or concentration, and this was found to be true in the case of nucleoside catalysis (data not shown). For the case of nucleosides, a plot of k_{1obs}/[amine] vs [amine] was linear from which k_3 and $k_{3'}$ were determined. Table 1 lists the values for $k_{3'}$ of ribonucleosides as well as the well-known bifunctional catalyst 2-pyridone[9] for comparison.

In determining an accurate value for the catalytic rate constant $k_{3'}$, the correct choice of catalyst concentration is complicated by catalyst associations. For 2-pyridone,

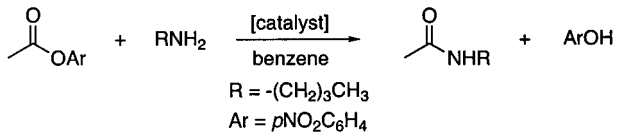

Figure 1.

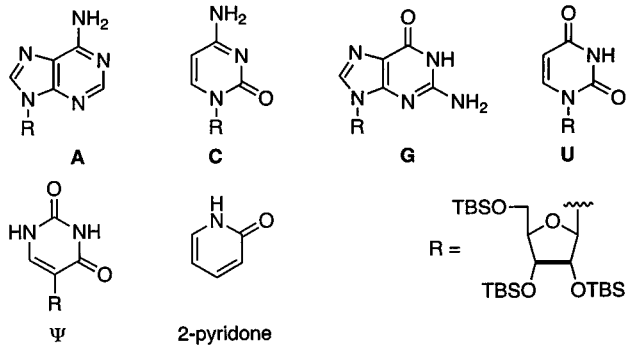

Figure 2.

Table 1. Summary of Catalysis

catalyst	[catalyst] (mM)	[amine] (M)	$10^2 k_{3'}$ (M^{-2} s^{-1})
C	0.220−1.83	0.0303−0.101	730 ± 50
2-pyridone	0.209−1.83	0.0303−0.101	610 ± 70
G	0.412−1.83	0.0303−0.101	175 ± 5
Ψ	0.915−1.83	0.0405−0.101	40.0 ± 0.4
A	0.915−1.83	0.0405−0.101	40.0 ± 2.8
U	0.915−1.83	0.0405−0.101	26.0 ± 1.6

as well as ribonucleosides, dimerization in a nonpolar medium is an unavoidable phenomenon. Since the catalytically active species is believed to manifest itself in its monomeric form,[9] obtaining true kinetic parameters requires modification of the kinetic equation to account for catalyst association. Nucleosides are known to self-dimerize weakly in nonpolar solvents,[10] but the extent of dimerization under the present conditions is unknown. Both C and 2-pyridone were assumed to self-dimerize to noncatalytically active species, and their dimerization and rate constants were calculated according to method of Watson.[8] Equation 2 shows the relationship between the dimerization constant and various rate terms. K is the dimerization constant. $k_{2obs'}$ is (k_{1obs}/[amine]) − (k_3-[amine]) and $k_{3obs'} = k_{2obs'}/T$ where T is the stoichiometric concentration of catalyst. Plots of $1/k_{3obs'}$ vs $k_{2obs'}$ for C and 2-pyridone were linear from which the catalytic rate

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$$1/k_{3obs'} = 1/k_{3'} + 2Kk_{2obs'}/(k_{3'})^2 \quad (2)$$

constant $k_{3'}$ and the dimerization constant K were calculated (Figure 3). Table 2 summarizes these results. The catalytic rate and dimerization constants for C and 2-pyridone are similar in magnitude. The values of $k_{3'}$ and K obtained for 2-pyridone under the present conditions are comparable to the previously reported values for 2-pyridone in chlorobenzene ($k_{3'} = 10 \pm 1$ M^{-2} s^{-1}, $K = 200$ M^{-1}).[8]

In an aprotic medium, breakdown of the tetrahedral intermediate has been shown to be rate determining.[11] The third-order nature of the rate expression is indicative of catalysis occurring, in part, by acceptance of the ammonium proton by catalyst in the transition state. This enables expulsion of the phenolate anion rather than the more energetic amide anion from the tetrahedral intermediate. The extent of proton transfer required for the catalysis is estimated between 10 and 30% of full proton transfer.[8] The observed rate enhancement for C is consistent with the proposal that C manifests its catalytic effect through a bifunctional mechanism (Figure 4). Stabilization of the aminolysis transition state by the amidinone functionality occurs through simultaneous donation and acceptance of protons. The other catalytically active nucleosides, however, show only modest rate enhancements, and this method proved unreliable for determining their dimerization constants. Therefore, without experimental insight into the extent of dimerization, the catalytic rate constants for A, U, G, and Ψ were determined by simply plotting k_{1obs}/[amine] vs [amine] and dividing by the stoichiometric amount of nucleoside used.[12]

As evidenced by the data, C and 2-pyridone are superior catalysts compared to the other nucleosides and are comparable in their effects. A recent study on the mutarotation of tetramethylglucose catalyzed by nucleosides, however, revealed that C was less effective in catalyzing this process than 2-pyridone.[13] This finding suggests that C may be an inherently inferior catalyst than 2-pyridone in catalyzing processes that strictly involve bifunctional/tautomeric catalysis. This is particularly true when considering that similar dimerization constants were found for C and 2-pyridone in the present study. The transition state in Figure 4 depicts C participating in three hydrogen bonds in catalyzing the aminolysis reaction. This arrangement is reminescent of the hydrogen-bonding arrangement seen in a Watson−Crick G-C base pair. Although 2-pyridone may be a fundamentally better bifunctional catalyst than C, the added stabilization by C in delivering a third hydrogen bond in the aminolysis reaction may account for the similarity seen in the rate constants for these two catalysts. The involvement of three hydrogen bonds may also account for the fact that C is a significantly better catalyst than the other nucleosides which do not possess the requisite donor−acceptor combination.

In conclusion, the nucleoside C manifests its catalytic potential via a bifunctional mechanism, while the modest rate enhancements seen by the other nucleosides indicate some form of general base catalysis. Finally, one intriguing speculation focuses on the possible role for C75 of the universally conserved CCA tail of tRNAs in peptidyl transfer reactions. While C74 of tRNA has been shown to be involved in base pairing with a G residue of ribosomal RNA, no such function has been found for C75.[14] It is known, however, that this nucleoside is required for efficient ribosome-mediated protein synthesis. Given the close proximity of C75 to the peptidyl transferase center, the possibility of C75 participating in protein synthesis through some type of bifunctional catalysis is an intriguing possibility that has not been previously examined.

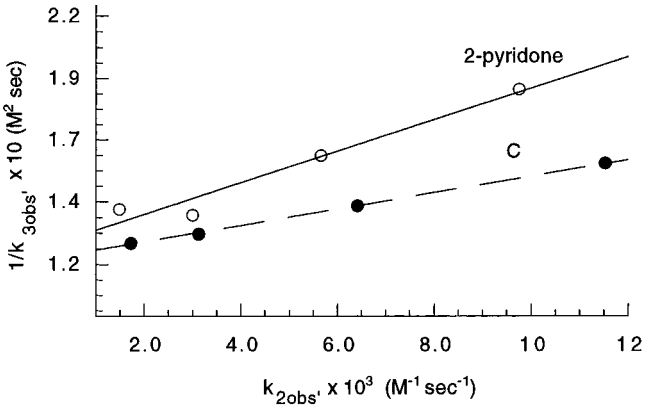

Figure 3. Determination of K and $k_{3'}$.

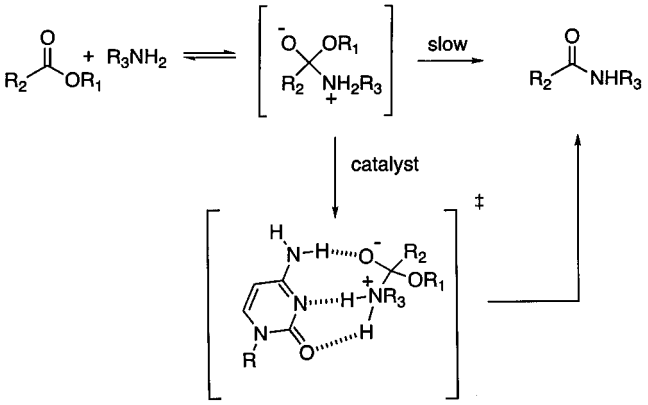

Figure 4.

Table 2. Rate and Dimerization Constants

catalyst	$10^2k_{3'}$ (M^{-2} s^{-1})	K (M^{-1})
C	830 ± 60	110
2-pyridone	790 ± 90	190

Experimental Section

Chemicals were purchased from common vendors and purified prior to use. *n*-Butylamine was distilled over KOH and stored under argon. Benzene was distilled over Na/benzophenone and stored over activated 4 Å molecular sieves under argon, 2-pyridone was sublimed twice, and *p*-nitrophenyl acetate was recrystallized from hexanes. 2′,3′,5′-*O*-Tris(*tert*-butyldimethylsilyl)nucleosides were prepared as previously described.[15]

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(12) While the catalytic effects of A, G, U, and Ψ are small, we do not interpret this result as a consequence of nucleoside self-complexation since the extent of such associations in pure solvents under the concentrations used would be small.[10] This is particularly true in the presence of 0.1 M amine. In fact, the determination of $k_{3'}$ for C without incorporating dimerization gave a value of 7.3 M^{-2} s^{-1} which is only slightly less than the value of 8.3 M^{-2} s^{-1} reported in Table 1.

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Kinetics

All reactions were conducted in benzene and were monitored by measuring the UV absorption of the released *p*-nitrophenylate anion. Reactions were carried out in a stoppered 1 mL quartz cell at a constant temperature of 25 °C. The release of *p*-nitrophenylate anion was monitored at 345 nm for the reactions containing the catalysts 2-pyridone and A, while 335 nm was used for all other measurements. The reaction was initiated by mixing an appropriate amount of *n*-butylamine to a mixture of *p*-nitrophenyl acetate and catalyst. An automatic sample changer was used for kinetic measurements, with a reference cell being used to correct for instrumental drift. Measurements were made periodically. All kinetic runs were performed under pseudo-first-order conditions under an excess of *n*-butylamine. The *n*-butylamine concentration was varied between 0.0303 and 0.101 M. The catalyst concentration ranged from 2.09×10^{-4} to 1.83×10^{-3} M, while the concentration of *p*-nitrophenylacetate was 9.15×10^{-5} M. k_{1obs} was determined from the slope of the graph $\ln[(A_\infty - A_0)/(A_t - A_0)]$. Determination of k_{1obs} was accomplished with three different concentrations of *n*-butylamine and three different concentrations of catalyst. The plot of k_{1obs}/[amine] vs [amine] was linear from which $k_{3'}$ was determined (except for 2-pyridone and C, see text).

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Supporting Information Available: Graphs of k_{1obs}/[amine] (k_2) vs [amine] for catalysts (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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