Ester Aminolysis Catalyzed by Nucleosides in a Nonpolar Medium

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Received July 12, 1996

Since the discovery of catalytic RNA, a number of examples have emerged illustrating the ever increasing diversity of RNA catalysis.[1] This diversity ranges from phosphoryl transfer reactions as seen in group I and group II introns, RNase P, hammerheads, and snRNPs to evidence of amide bond catalysis by rRNA during protein synthesis.[2] Moreover, the advent of polymerase chain reaction technology has led to the evolution and selection of both ribo- and deoxyribozymes with impressive catalytic abilities that include aminoacyl esterase,[3] amidase,[4] DNA[5] and RNA ligase,[6] and biphenyl isomerase[7] activities. Virtually all of the examples of RNA or DNA catalysis to date have dealt with relatively complicated three-dimensional nucleic acid structures, and thus, the various components contributing to the catalysis have been difficult to sort. Moreover, the focus of the catalysis has been attributed to the RNA in terms of template effects, effective molarities, binding of substrates and metal ions, and orbital steering of functional groups for optimal reactivity. In this communication, we report that the functional groups of certain nucleoside bases of RNA are capable of catalyzing amide bond formation and do so without the aid of metal ions. This bond is the key bond formed during protein synthesis, and its formation is catalyzed by the ribosome.

The role of the functional groups that comprise each nucleotide base offers a multitude of donor−acceptor possibilities when arranged in 3-dimensional space. Unlike their protein counterparts, these functional groups lack the chemical diversity found in the side chains of the 20 different amino acids. Despite this apparent shortcoming, there are unique chemical features of the nucleic acid bases that are not found in the amino acid side chain functionality. Such features range from the varying degree of bifunctionality seen in the relatively acidic imide moiety of uracil and Ψ to the weakly basic amidine group in cytosine.

In order to investigate the inherent catalytic abilities of the functional groups contained within the nucleic acid bases, each nucleoside was examined individually for its ability to catalyze amide bond formation (Figure 1). The experiment was designed to examine the relative contributions of each base toward stabilizing the transition state of the aminolysis reaction by hydrogen bonding. It was hypothesized that under appropriate conditions, individual base functionalities could operate as *bifunctional* catalysts in much the same way as two separate histidine and aspartate residues act in concert within the active site of a protein enzyme.[8] To test this notion, the effect of each protected ribose nucleoside A, C, G, U, and Ψ was examined for its ability to catalyze amide bond formation in a nonpolar medium, such as deuterochloroform (Figure 2). A nonpolar medium was chosen for several reasons; most importantly, it would enable an optimal enthalpic contribution to catalysis due to any hydrogen bonding stabilization of the presumed ionic transition state. In addition, many active sites in enzymes are hydrophobic in nature, and these conditions may very well model the hydrophobic environs in proteins,[8] nucleic acids,[9] and protein-nucleic acid complexes.

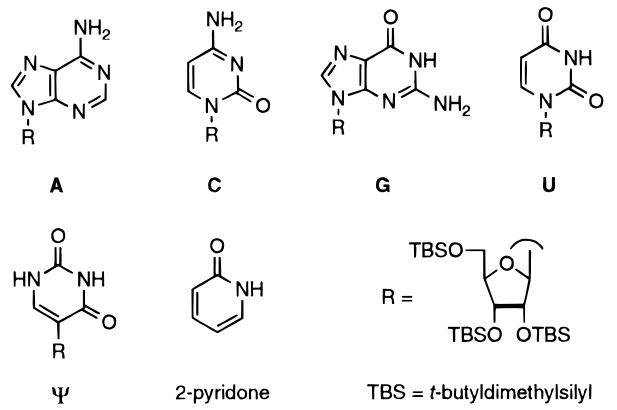

Figure 1. *tert*-Butyldimethylsilyl protected nucleosides A, C, G, U, and Ψ and 2-pyridone.

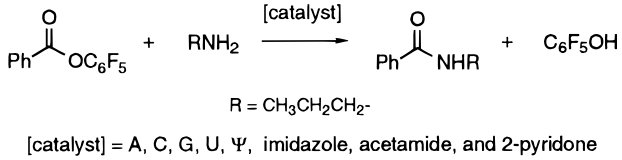

Figure 2. Aminolysis reaction of pentafluorophenyl benzoate by propylamine catalyzed by various catalysts.

Figure 3 shows the effects of the various bases as catalysts for the aminolysis reaction of pentafluorophenyl benzoate by propylamine at 23 °C in deuterochloroform. These results are summarized in Table 1. The bases A and U showed less than 10% catalysis over the uncatalyzed rate, which is consistent with recent reports of similar systems.[10] G and Ψ showed rate enhancements of 24% and 29%, respectively, while C produced a relatively high rate increase of 210%. For comparison, 2-pyridone, a well-known bifunctional catalyst,[11] showed a rate increase of 110% under the same conditions. Imidazole, which constitutes the heterocyclic base in histidine, is critical to a number of enzymatic proton transfer processes and showed a rate increase of less than 10%. These results indicate that the functional groups contained in C, G, and Ψ are not only capable of catalyzing amide bond formation but do so more effectively than imidazole. The relatively high value for C, as compared to the bifunctional catalyst 2-pyridone, suggests that C might be operating in a similar manner

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S0022-3263(96)01331-X CCC: $12.00

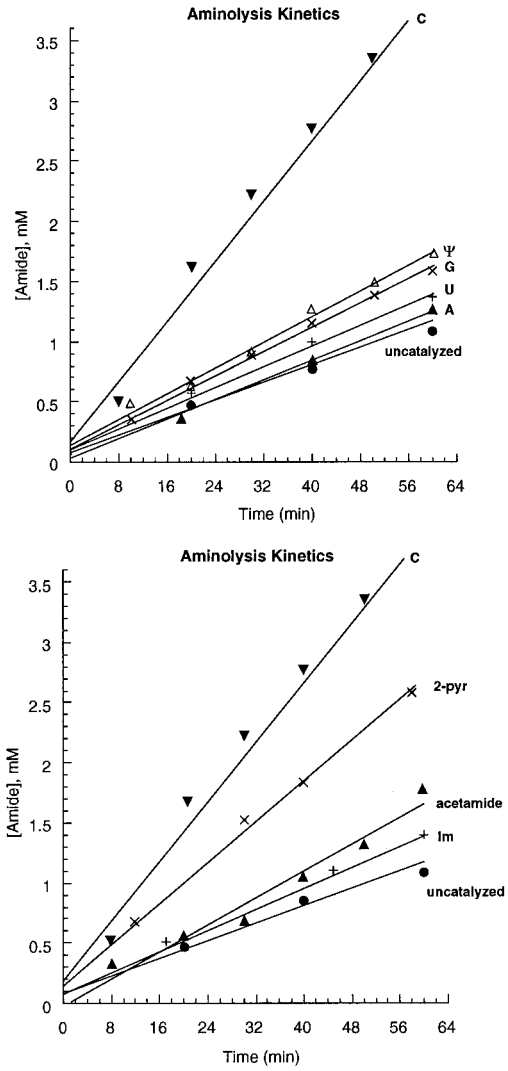

Figure 3. Time course of ester aminolysis of pentafluorophenyl benzoate by propylamine in $CDCl_3$ at 23 ± 1 °C. The kinetics measurements were performed with 14.6 mM amine, 14.0 mM ester, 14.0 mM catalyst, and 55 mM triethylamine. The progress of the reaction was monitored by 400 MHz ^1H NMR by integrating the appearance of the β-hydrogens of the amide product (1.64 ppm) while simultaneously integrating the disappearance of the amine β-hydrogens (1.45 ppm) of *n*-propylamine. The values are the average of three different runs and are reproducible within ±5%. Initial rates were determined over the first 60 min of the reaction by plotting product formation vs time. The rate for the uncatalyzed reaction is 2.0×10^{-5} M min^{-1}. Abbreviations are as follows: Im = imidazole, 2-pyr = 2-pyridone.

Table 1. Summary of Catalysts

catalyst	rate ($\times 10^5$ M/min)	rate increase (%)
imidazole	2.2	<10
A	2.2	<10
U	2.2	<10
G	2.5	25
Ψ	2.6	29
acetamide	2.8	38
2-pyridone	4.2	110
C	6.2	210

(Figure 4).[12] In addition, the *N*,*N*-dimethylcytidine analog was prepared and tested. Less than a 10% increase

(12) A full kinetic description of this system will be reported elsewhere.

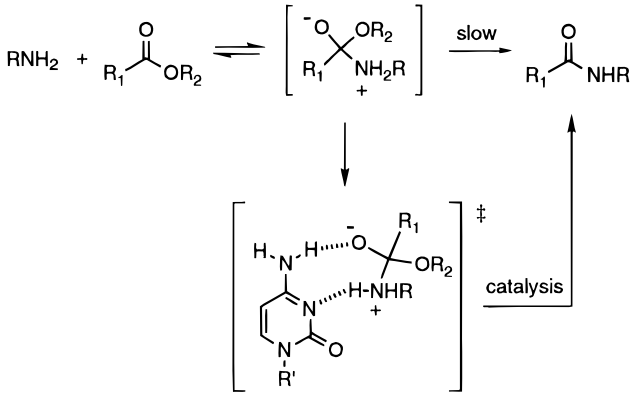

Figure 4. Generalized mechanism of ester aminolysis. The rate determining step is breakdown of the tetrahedral intermediate. Stabilization of the ionic transition state by C acting as a bifunctional catalyst facilitates the breakdown of the intermediate to products.

in the rate over the uncatalyzed reaction was observed (data not shown). This result supports a major role for the $-NH_2$ group in the catalysis. It is known that soluble analogs of G, C, and 2-pyridone self-associate in pure solvents such as benzene[11] and chloroform,[13] with association constants as high as 10^3 M^{-1}. Since the associated species is believed to be, for the most part, catalytically inert, it will be interesting to determine the catalytic rate constant for each catalyst since self-association may be the underlying cause for the modest rate increase observed for G and Ψ. Interestingly, the modified base, Ψ, which also possesses potential chemical bifunctionality, exhibits catalysis, whereas U does so only minimally, thus suggesting a possible catalytic role for Ψ in rRNA.[14] Overall, the magnitude of the catalysis for this *intermolecular* system is rather modest, but one would not expect to see a large rate increase due to the substantial negative entropy that is associated with forming the termolecular complex of the transition state.

Early studies in the aminolysis of activated esters showed that simple amides, such as N,N-dimethylacetamide, could catalyze the aminolysis of esters.[15] Recent studies further support these findings.[10] The seminal work by Menger[16] showed that in a nonpolar medium, the rate-limiting step is the breakdown of the tetrahedral intermediate to give the amide product and alcohol. This step could be accelerated by the addition of various dipolar oxygen functionalities, amine bases, and tautomeric catalysts, such as 2-pyridone.[15] Stabilization of the ionic transition state by these hydrogen bond acceptors facilitates the breakdown of the tetrahedral intermediate in the forward direction. The transfer of positive charge to the catalyst promotes cleavage of the carbon−oxygen bond toward the products while retarding cleavage of the carbon−nitrogen bond back toward the reactants.

The findings presented here establish that the intrinsic chemical functionalities of the nucleoside bases can stabilize a transition state that is associated with a chemical reaction resulting in catalysis. In the present case, nucleosides manifest their catalysis as general bases or bifunctional catalysts during amide bond formation.

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To our knowledge, this is the first example that demonstrates the direct participation of a nucleic acid base functionality in catalysis without the participation of a metal ion. These results also provide chemical evidence that RNA solely has the potential to catalyze amide bond formation which may have relevance to peptide bond formation as catalyzed by the ribosome.[2] Furthermore, the data presented here support the proposal that nucleic acids may have played an important role in life's early evolution by catalyzing amide bond formation[17,18] and that early nucleic acids may, in fact, have recruited proteins for desolvation purposes in generating the hydrophobic cores necessary for nucleic acid mediated catalysis. Investigations to address the scope of the reactions that are potentially catalyzed by the functional groups of nucleic acids are currently in progress.

Acknowledgment. Financial support from the National Science Foundation (NSF Young Investigator Award to D.A.H.) and the American Chemical Society Petroleum Research Fund is gratefully acknowledged.

JO961331Q

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