Catalysts, Anticatalysts, and Receptors for Unactivated Phosphate Diesters in Water

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A set of substituted bisguanidines have been prepared and examined for their ability to bind and catalyze the hydrolysis of uridylyl-3′,5′-uridine (UpU), an unactivated RNA substrate in water. The unexpected result is that this set includes both catalysts (binding the transition state better than the ground state) and anticatalysts (binding the ground state better than the transition state), each with respectable rate enhancements and/or affinities, despite the fact that these molecules all have very similar structures. These results therefore show the level of sophistication that must be achieved in the conformational theory of small molecules if we hope to truly "design" supramolecular structures that bind preferentially to a transition state over the ground state.

A half century ago, Linus Pauling pointed out that if the formalism of transition state theory is accepted, a molecule is a catalyst if it binds a transition state more tightly than it binds the corresponding ground state.[1] This insight has challenged chemists to use their synthetic talents to make molecular cavities that bind small molecules and transition states differentially, and therefore catalyze reactions as natural enzymes do.[2] While an uncounted number of efforts in this direction have been made, relatively little work has addressed the general consequence of Pauling's insight, that there is no qualitative distinction between a receptor that binds a transition state tighter than a ground state (a catalyst) and one that binds a ground state tighter than a transition state (an "anticatalyst"). In the design of receptors, one might expect both to be generated with approximately equal frequency, if the geometric perturbations that distinguish ground and transition states are small relative to the precision with which organic molecules can be designed and their conformations in solution predicted.

As part of our work studying the origin and evolution of the enzyme ribonuclease (RNase),[3] we needed a chemical perspective on the transesterification of unactivated phosphate esters (the natural substrates for RNase) to cyclic phosphates (the natural products) in water (the natural solvent). Anslyn,[4] Hamilton,[5] Goebel,[6] and their co-workers designed bisguanidinium structures (e.g., **1a**) to provide receptors complementary to the pentacoordinated phosphate at the transition state of the reaction (Figure 1). For example, the simple bisguanidinium species **1a** and **2** are catalysts (rate enhancements 360- and 4800-fold greater than that for guanidinium itself) for the alcoholysis by 2-phenylethanol of catechol cyclic phosphate as an activated substrate in dimethylformamide as solvent at 30 °C.[7,8]

To enhance the biological relevance of these experiments, we reexamined catalysis by **1a** and **2** in buffered water (triethanolamine, pH 7.5, 80 °C)[9] using the unactivated natural uridylyl-3′→5′-uridine (UpU) as substrate (Table 1). The rate of transesterification of UpU to give 2′,3′-cUMP and uridine was followed by monitoring the appearance of uridine by reversed phase HPLC.[10] The amount of uridine was quantified by calibration with standards of known concentrations. Each experimental data point was obtained in triplicate.

Both **1a** and **2** were found to be catalysts for physiological substrates under physiological conditions. Because the rate constant with guanidinium itself under these conditions was not measurably different from zero ($<8 \times 10^{-8}$ M^{-1} min^{-1}), only a lower limit on the rate enhancement (a factor of >10000, for **1a**) could be

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(7) The bisguanidinium derivatives were synthesized by treating *m*-bis(aminomethyl)benzene with thiophosgene following the procedure of Gross et al.[7a] to yield the bis(*iso*thiocyanate), which was then treated with the appropriate amine in CH_3CN or DMF to yield the corresponding thiourea, which was treated with methyl iodide to yield the *iso*thiomethylurea, which was then converted to the bisguanidinium species by treatment with ammonia or an appropriate amine. All new compounds yielded satisfactory analytical data.[8]

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(10) Supelco LC 18-Sm 250 × 5 mm column, particle size 5 mM; elution normally with NH_4OAc 60 mM, pH 4.2, from 0 to 10% CH_3CN; for **1g**, **1h**, and **1i**, an NH_4PO_4 buffer, 5 mM, pH 7.0, 0 to 20% MeOH was used.

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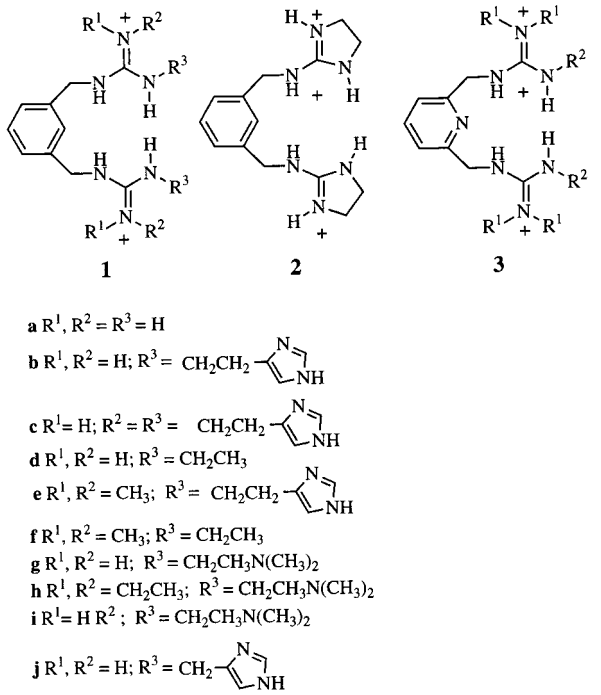

Figure 1. Structures of bisguanidinium receptors examined.

Table 1. Binding of Bisguanidinium Derivatives to Uridylyl-3′,5′-uridine (UpU) and the Transition State for the Cleavage Reaction[a]

compd	catalyst or anticatalyst[a]	ground state K_{diss}[b] (mM)	k_{cat}[c] ($\times 10^6$ min^{-1})	transition state K_{diss}[d] (mM)
1a	catalyst	16 ± 3	72 ± 3	11.1
1b	anticatalyst	24 ± 2	1 ± 1	>1200
1c	anticatalyst	18 ± 4	1 ± 1	>900
1e	anticatalyst	9 ± 3 16 ± 5†	1 ± 1	>450
1f	anticatalyst	45 ± 10	15 ± 3	150
1g	catalyst	35 ± 8 31 ± 6†	175 ± 10	9
1h	neither	13 ± 4†	50 ± 5	13
2	catalyst	18 ± 3	70 ± 3	13
3	catalyst	24 ± 3	100 ± 7	12

[a] A compound is a catalyst if $k_{cat} > k_{uncat}$ and an anticatalyst if $k_{cat} < k_{uncat}$. [b] All values are determined by kinetics unless otherwise marked and are measured in water with triethanolamine buffer (100 mM, pH = 8.6 at 25 °C, giving a pH = 7.5 at 80 °C using the temperature dependence in ref 9) and KCl (added to ionic strength of 0.5 M) at 80 °C at pH 7.5 at [UpU] = 1 mM and [catalyst] from 1 to 40 mM. Values marked by † were determined by equilibrium measurements using NMR at 80 °C under the same conditions, but with [UpU] = 2.4 mM and [catalyst] from 2.4 to 57.6 mM. Values in parentheses are estimates based on fewer data points. [c] All values in water with triethanolamine buffer (100 mM, pH=8.6 at 25 °C, giving a pH = 7.5 at 80 °C using the temperature dependence in ref 9) and KCl (added to ionic strength of 0.5 M) at 80 °C at pH 7.5 at [UpU] = 1 mM and [catalyst] from 1 to 40 mM. Products were resolved by HPLC and quantitated by coinjection of an internal standard. [d] Estimated from k_{cat} and K_{diss} for the ground state. RT ln $k_{cat}k_{uncat}$ is a measure of how effective the catalyst is; it is a $\Delta\Delta G^{\#}$. Given K_{diss} for the ground state, $K_{diss}K_{diss}^{\#} = k_{cat}k_{uncat}$. Values marked with ">" are for anticatalysts with k_{cat} close to zero; division by a small number creates inexact values.

obtained. The catalytic power was 3-fold higher if the phenyl ring was replaced by a pyridine ring (**3**).

We then asked whether this catalytic power could be rationally improved. The structure of the active site of the enzyme RNase A[11] suggested that this might be possible by appending imidazoles that abstract and/or donate protons to the attacking and departing oxygen

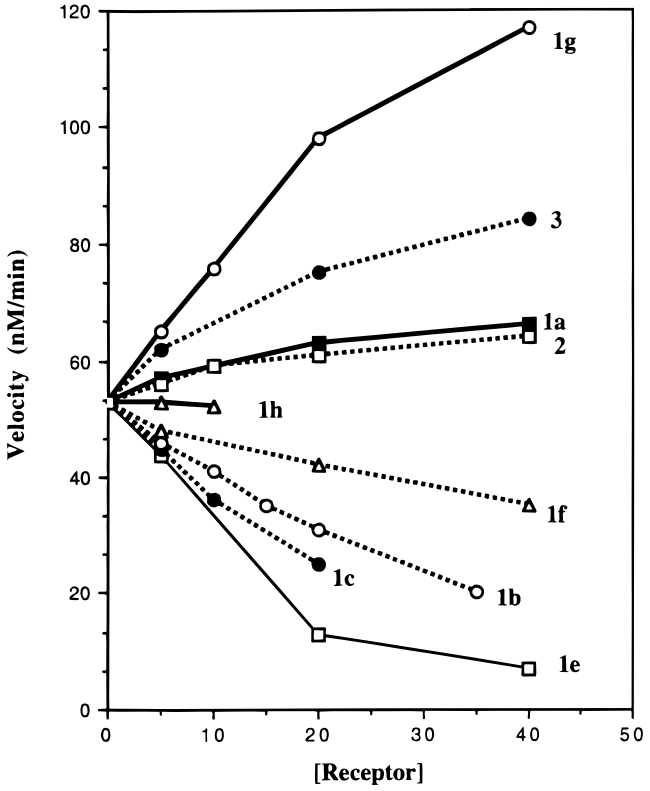

Figure 2. Velocity of cleavage of uridylyl-3′→5′-uridine in buffered water (triethanolamine, pH 7.5, 80 °C) as a function of the concentration of the receptor, for nine different receptors: **1a** —■—; **1b** ···○···; **1c** ···□···; **1e** —△—; **1f** ···△···; **1g** —○—; **1h** —△—; **2** ···□···; **3** ···●···.

atoms. Molecular models suggested that imidazoles attached via ethylene linkages (e.g., compound **1b**) might be positioned appropriately to do this (compare the analogous species **1j** prepared while this work was in progress by Hamilton and co-workers, which was a catalyst in acetonitrile with an activated substrate, displaying a $k_{cat}/k_{uncat} = 85$).[5b] Surprisingly, when **1b** bearing two imidazoylethyl appendages was added to a solution of UpU in buffer, the rate of cleavage of UpU *decreased*, not increased, with increasing concentrations of "catalyst" (Figure 2). Analysis of the rate of the reaction as a function of the concentration of **1b** suggested that UpU binds to **1b** with a disassociation constant of 24 ± 2 mM and that the rate constant for the cleavage of UpU in the complex is not measurably different from zero. Thus, **1b** is an "anticatalyst" for the reaction; it binds to the ground state for the reaction more tightly than the transition state. Further studies showed that this anticatalysis was achieved by "differential binding", analogous to that discussed for natural enzymes two decades ago by Albery and Knowles.[12] **1b** binds UpU more weakly than **1a** (K_{diss} = 16 mM for **1a**, 24 mM for **1b**, Table 1) but binds even more weakly to the transition state for the cleavage reaction (K_{diss} = 11 mM for **1a**, >1200 mM for **1b**).

The identification of an anticatalyst closely related in structure to a catalyst encouraged us to make further structural modifications of the molecule to examine the

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phenomenon. These focused on modifications of the imidazoles to alter their hydrogen bonding potential. Compound **1b** was first found to be a catalyst rather than an anticatalyst at pH 5.5, where the imidazoles are largely protonated. Similarly, the bis(ethyl) analogue **1d** lacking the imidazole groups displayed the catalytic activity of **1a**, not the anticatalytic behavior of **1b**. Last, compound **1c**, with four appended imidazoles, was also an anticatalyst at pH 7.5 and a better receptor for UpU as well ($K_{diss} \approx 18$ mM, Table 1). Methyl groups were also placed on the guanidinium group to yield **1e**, blocking the ability of imidazole to accept a hydrogen bond from the guandinium group, an interaction that might distort its conformation to make it less complementary to the transition state. Rather than having restored catalysis, **1e** proved to be a more effective anticatalyst than **1b** because of tighter binding of the ground state ($K_{diss} \approx 9$ mM, determined kinetically, Table 1). A comparable thermodynamic value for K_{diss} (16 ± 5 mM) was measured by NMR, exploiting the fact that the P-31 chemical shift of UpU is perturbed upon binding to bisguanidinium salts. This suggested that hydrogen bonding between the imidazole and guanidinium groups does not produce the perturbation in structure leading to the anticatalytic behavior of **1b**.

The existence of a number of closely related structures having interactions with UpU that range from those distinctly catalytic to those distinctly anticatalytic led us to examine most closely the nature of the interaction. The affinity of **1e** for UpU (having a 2′-OH group) was approximately 3-fold higher than its affinity for TpT (lacking the 2′-OH group). This suggested that a functionality of **1e** interacts with this OH. As the 2′-OH group almost certainly acts as a nucleophile in the reaction, this interaction might also have an anticatalytic consequence. Consistent with this model was the fact that k_{cat} is not measurably different from zero for any compound containing an appended imidazole, regardless of its affinity of the receptor for the ground state.

Intriguingly, alkyl substitution on the nonbridging nitrogens of the guanidinium groups itself affects differentially the binding of ground and transition states of the bisguanidinium receptor. Thus, **1f**, having alkyl substitutions but no imidazoles, binds the ground state 3-fold less tightly than **1a** but the transition state 14-fold less tightly. Thus, **1f** is also an anticatalyst. Most plausibly, the methyl groups distort the geometry of the bisguanidinium receptor sufficiently to generate a receptor more complementary to the ground state and less complementary to the transition state. An alternative model, where the alkyl substitutions desolvate the complex, is not consistent with the improved binding of UpU by **1e** and **1h**.

As these results suggested that the precise placement of the base in the side chain might be decisive in converting a catalyst to an anticatalyst, the bis(dimethylaminoethyl)bisguanidinium species **1g**[5b] was prepared and examined in water with UpU as substrate. The first-order rate constant for **1g** is 2.5-fold higher than that for **1a** in water at pH 7.5 (compare the 21-fold higher rate constant with those of activated substrates in acetonitrile)[5b] and was the best catalyst observed for this unactivated substrate. The improved catalysis displayed by **1g** was generated by differential binding.[12] The ground state is bound by **1g** 2-fold less tightly than by the parent **1a**, while the transition state is bound 20% better by **1g**

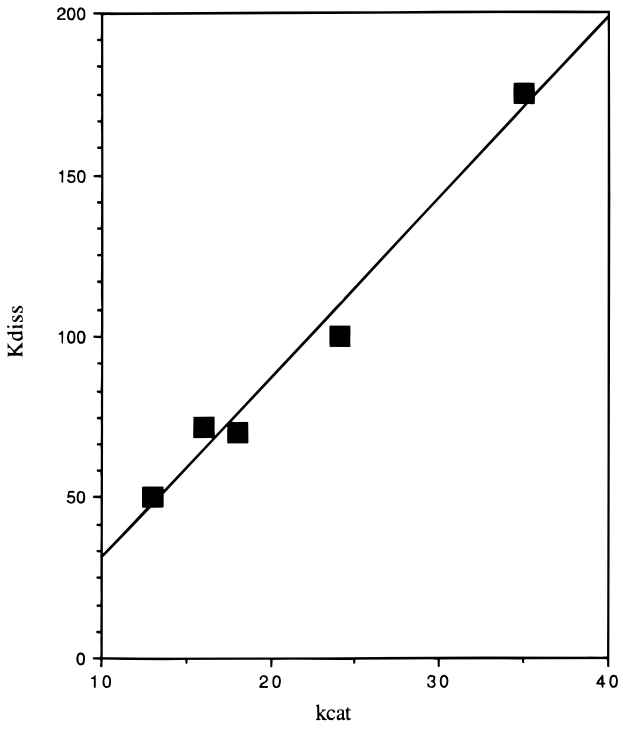

Figure 3. Plot showing a correlation between the disassociation constant of substrate uridylyl-3′→5′-uridine (measuring binding to the ground state) from the bisguanidinium receptor (K_{diss}) and the rate of transformation of receptor-bound substrate to give products (k_{cat}) (measuring differential binding of the ground and transition states).

than by **1a**. Remarkably, the catalytic power of **1g** was also diminished by alkylation on the nonbridging nitrogen in the guanidinium group to give **1h**, which was neither a catalyst nor an anticatalyst. The rate of cleavage of UpU bound to **1h** was, within experimental error, identical to the rate of cleavage of UpU free in solution. A thermodynamic K_{diss} of 13 mM between UpU and **1h** was directly determined by P-31 NMR at 80 °C (Table 1). These results suggest a remarkable independence of two structural features acting on the ground and transition states, the first the functionality appended to the guanidinium groups, the second the (very) detailed geometry of the bisguanidinium ligands surrounding the phosphate. A linear correlation (Figure 3) exists between k_{cat} and K_{diss}, conforming to a correlation proposed two decades ago by Fersht[13] that faster enzymes are those that bind their substrates more weakly.

The field of receptor and catalyst design has a "sociology" that rewards "success" and punishes "failure" as judged by the performance of a compound relative to a technological standard. The standards are different for ligands and catalysts, tightness of affinity used to judge the former, and rate enhancement used to judge the latter. We suggest here that this value system is not appropriate for our present level of understanding of the interaction between solvents and solutes and of the structure of ground and transition states. These bisguanidinium receptors differ very subtly in their geometry. These differences are certainly small relative to the resolution of the molecular models used to design the

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molecules.[14] Nevertheless, their reactivity presents a continuum between sociologically acceptable rate enhancements (>10000 in the best example from the work presented in this manuscript) to sociologically acceptable ligand−receptor interactions (K_d of 5 mM in water, for the best example from this work) accompanied by anticatalysis. The results therefore carry the message that the level of sophistication in contemporary conformational theory in small molecules is below that required to truly design supramolecular structures that bind preferentially to a transition state over the ground state. This message may be more important than the statement that any particular molecule is effective as a catalyst, while another is effective as a receptor, as it directs our focus toward the experiments that must be done if the development of either is to be possible.[15]

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Supporting Information Available: Detailed experimental procedures for preparing compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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