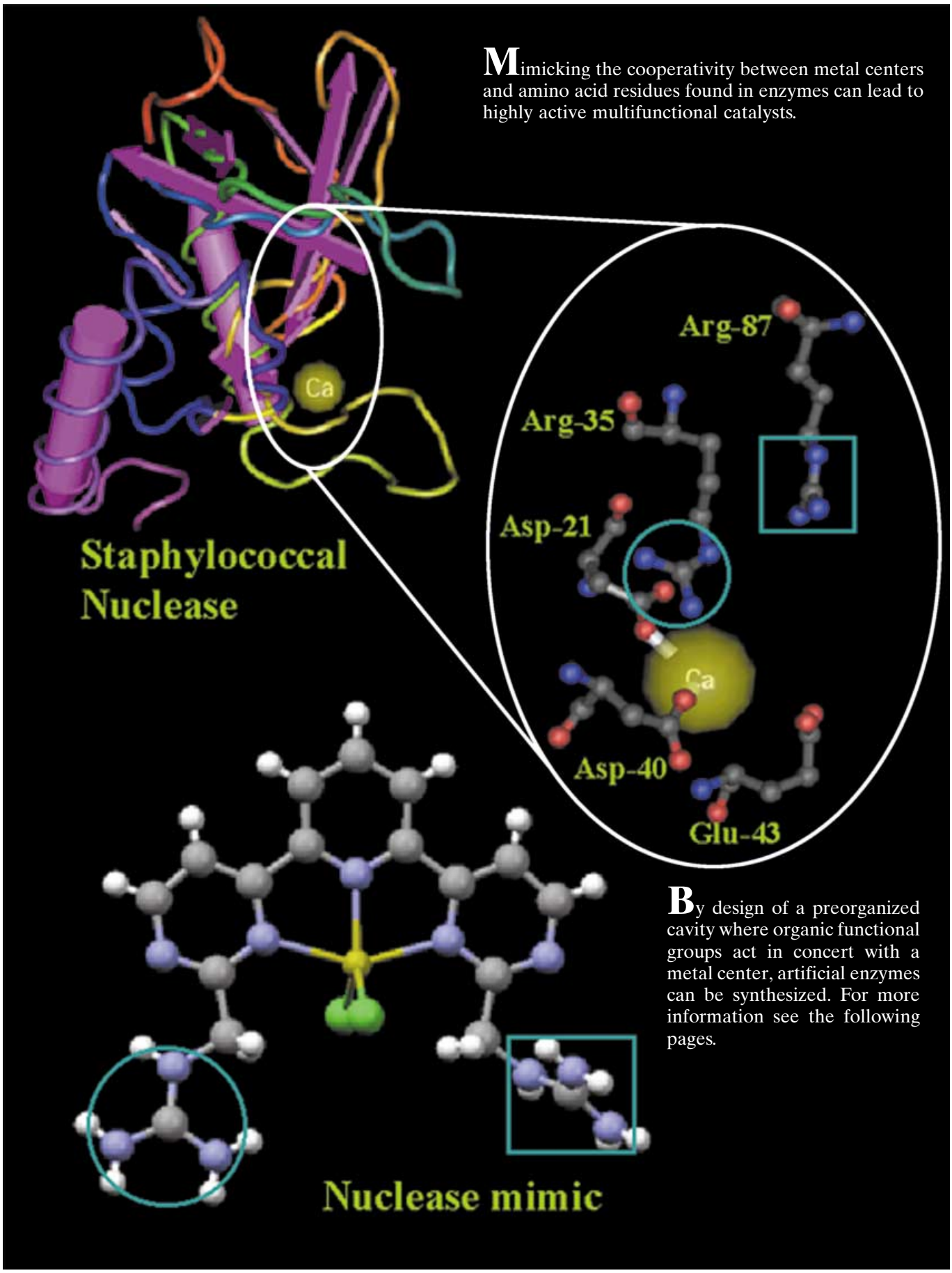

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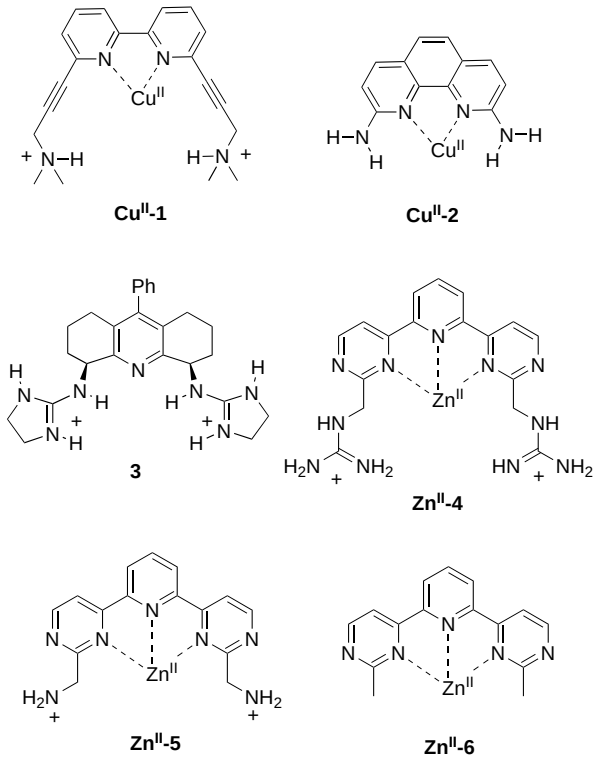
Remarkable Cooperativity between a Zn^{II} Ion and Guanidinium/Ammonium Groups in the Hydrolysis of RNA**

Hassan Aït-Haddou, Jun Sumaoka, Sheryl L. Wiskur, J. Frantz Folmer-Andersen, and Eric V. Anslyn*

In nature, the hydrolysis of the phosphodiesters in DNA and RNA is often performed with enzymes that exploit cooperation between metal centers and amino acid residues, which act as general acid/base catalysts.[1] The positively charged residues of lysine, arginine, and histidine are thought to stabilize phosphorane-like transition states by electrostatic interactions, hydrogen bonding, and/or proton transfer.[2] In the case of the metalloenzyme staphylococcal nuclease (SNase), site-directed mutagenesis studies have revealed the extent of cooperativity between a Ca^{II} metal center and guanidinium residues.[3] For example, the native enzyme containing guanidinium groups has a k_{cat} approximately 35000-fold higher than a mutant lacking the guanidinium groups. Furthermore, it was found that replacement of the Ca^{II} center with an Mn^{II} center, which maintains the tertiary structure but is catalytically inactive, dropped the reactivity approximately 36000-fold. Hence, the guanidinium and Ca^{II} units enhance each other's reactivity by factors of nearly 3×10^4, which can be considered a measurement of their cooperativity.

The hydrolysis of RNA is a two-step reaction. The first step is a transesterification of the phosphodiester by the 2′-OH group, to create a 2′,3′-cyclic phosphodiester, and the second step is nucleophilic opening that completes the hydrolysis.[2] Artificial enzymes that could efficiently catalyze this sequence would be very useful in gene therapy.[4] Owing to this potential, there is considerable interest in creating catalysts that efficiently hydrolyze RNA.[5] Taking their lead from enzymes, numerous catalysts containing metal centers or general acids/bases have been explored.[6] Although catalytic systems have been created that are very active using either metal centers or acids and bases alone, few demonstrate the high cooperativity between the two moieties that are hallmarks of enzymatic catalysis. In fact, there are few artificial enzymes with which the cooperativity between a metal ion and a charged auxiliary have been explored,[7] relative to the number of systems with which one of these effects was analyzed independently.

One potential way to achieve multifunctional catalysis is to create a rigid cavity where multiple functional groups converge upon and form contacts with the bound substrate. This strategy may promote cooperativity between the functional groups. We do not presume to be sophisticated enough to create a cavity that complements the shape of the transition state over the substrate. We simply want the catalytic groups to reside within a cleft or groove so that they form contacts with the substrate. The precedent for this design concept, and the catalysts reported herein, resides primarily in three studies: to study the cooperativity between a metal ion and ammonium groups in phosphate ester hydrolysis, Krämer and Kávári designed the complex **Cu^{II}-1** (Scheme 1).[8] This complex was found to be 2900 times more reactive in promoting the hydrolysis of an activated phosphodiester, bis(*p*-nitrophenyl) phosphate, than a control structure missing the ammonium ions. This catalyst has a preorganized cleft, such that binding of the substrate to the metal center ensures close proximity of the ammonium groups. Similarly, the complex **Cu^{II}-2** (Scheme 1), reported by Chin and co-workers, has two amino groups proximal to the metal, which leads to a 2×10^4 enhancement of the rate of the hydrolysis of 2′,3′-cyclic monophosphate (2′,3′-cAMP) compared to a catalyst lacking the amino groups.[9] Our current design is also influenced by solely guanidinium-based catalysts. The rigid bisguanidinium receptor **3** (Scheme 1), where each guanidinium unit interacts with the substrate, has been found to enhance the rate of hydrolysis of RNA by a factor of 20 under neutral conditions.[10]



Scheme 1.

Based upon these three studies, it seemed reasonable that a rigid bisguanidinium cleft that also contained an appropriately positioned metal center could act as an efficient RNA hydrolysis catalyst. Consequently, **Zn^{II}-4** (Scheme 1) was designed.[11] The compound **Zn^{II}-4** incorporates guanidinium "arms" capable of hydrogen-bonding, designed to make direct

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[**] We gratefully acknowledge the National Science Foundation for the support of this work.

  0044-8249/02/4121-4014 $ 20.00+.50/0

contacts to a phosphodiester that is bound to the Zn^{II} center. Herein, we report the reactivity of **Zn^{II}-4** and **Zn^{II}-5** (Scheme 1) in the hydrolysis of the RNA dimer adenylyl (3′→5′)phosphoadenine (ApA), and compare it to the reactivity of **Zn^{II}-6** (Scheme 1), to ligand **4** alone, as well as to background hydrolysis. We observed high cooperativity between the guanidinium groups and the metal center.

The hydrolysis of ApA to adenosine 3′-monophosphate, adenosine 2′-monophosphate, adenosine 2′,3′-cAMP, and adenosine, was monitored by reverse-phase high-performance liquid chromatography (HPLC). The k_{obs} for hydrolysis increases linearly with **Zn^{II}-4** concentration at pH 7.4 (Figure 1 A), which indicates a first-order dependence on **Zn^{II}-4**. It is important to note that in all cases, the concentration of catalyst is above that of the substrate, and therefore there is no turnover. The k_{obs} values increase with pH until an optimum rate is reached around pH 7.5 (Figure 1 B). This observation is in excellent agreement with the pK_a value of a water molecule coordinated to the zinc(II) center in **Zn^{II}-4**, which was determined to be 7.3 by potentiometric titration. This result suggests that the Zn-hydroxide form of complex **Zn^{II}-4** is the active catalyst in ApA hydrolysis. The low pK_a value for the metal-bound water is because of the close proximity of the guanidinium arms. Similarly, Krämer et al. showed that the pK_a of the water molecule coordinated to the copper(II) center in **Cu^{II}-1** is lowered by the proximal ammonium groups.[8] As shown in Figure 1 B, the reactivity of **Zn^{II}-4** drops at higher pH values. This effect is the result of two factors: the slight precipitation of the catalyst and the deprotonation of one of the two guanidinium groups, the pK_a value (10–11) of which is lower than normal for guanidinium groups (around 13).

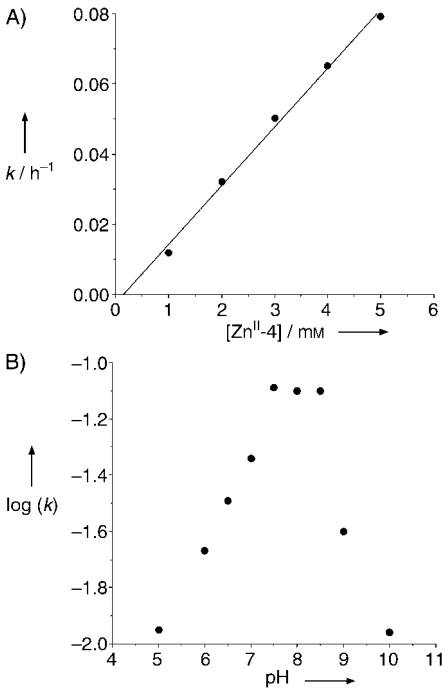

Figure 1. A) Pseudo first-order-rate constants (h^{-1}) for the hydrolysis of ApA promoted by **Zn^{II}-4**. B) Log-pseudo-first-order rate constants for hydrolysis of ApA as a function of pH value.

The pseudo-first-order rate constants for **Zn^{II}-4**, **Zn^{II}-5**, and **Zn^{II}-6** (5 mM) promoted hydrolysis of ApA (0.05 mM) at pH 7.4 (in water buffered with 10 mM of *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid (HEPES), at 37 °C) are $8.0\times10^{-2}\ h^{-1}$, $9.0\times10^{-3}\ h^{-1}$, and $2.4\times10^{-5}\ h^{-1}$, respectively. The reactions were followed to near 20% completion. Although these rate constants are very high, **Zn^{II}-4** is not as active as the most active catalysts previously reported. For example, a comparison of the rate constant for **Zn^{II}-4** to that of a very active dinuclear copper(II) complex reported by Chin and Young[6g] shows that **Zn^{II}-4** is approximately 25 times less active. The key factor is this study is the degree of cooperativity achieved with **Zn^{II}-4**.

Mechanistically, the hydrolysis of ApA proceeds by an intramolecular attack by the 2′-OH group of the ribose. This step was confirmed by the fact that the 2′-deoxyadenylyl(3′→5′)-2′-deoxyadenosine is not hydrolyzed to any measurable extent by **Zn^{II}-4**. However, the intermediate 2′,3′-cAMP does not accumulate, but is rapidly hydrolyzed in situ. The rate constant of the hydrolysis of 2′,3′-cAMP by **Zn^{II}-4** is fivefold larger than that of ApA. Therefore, the products of the hydrolysis reaction are adenine, and an equal mixture of 2′- and 3′-adenine monophosphate. The cooperativity is evidenced by the fact that there is no significant hydrolysis after 24 days using **4** alone, without the zinc center. Hence, **Zn^{II}-4** is about nine times more effective than **Zn^{II}-5**, over 3300 times more effective than **Zn^{II}-6**, and over 10^5 times more effective than **4** alone for enhancing the hydrolysis of ApA. To estimate the enhancement over the background hydrolysis by pure water at pH 7.4, a comparison to the buffer hydrolysis of cytidylyl(3′→5′)cytidine (CpC) at 60 °C was made.[12] The half-life of this second-order reaction was estimated to be about 10000 days, which gives an extrapolated enhancement for **Zn^{II}-4** of near 10^6 at 37 °C.

The rate enhancement of **Zn^{II}-4** over **4** is greater than that observed for native SNase containing the Ca^{II} center over SNase containing a nonproductive metal center. Clearly the enhancement obtained by adding metals to the guanidinium groups is very large in both the natural and synthetic systems. Yet, there is also a large enhancement as a result of adding a guanidinium to the metal centers in each case. The 3300-fold rate enhancement imparted by **Zn^{II}-4** compared to **Zn^{II}-6** is only one order of magnitude less than the enhancement attributed to the guanidinium groups for SNase, which demonstrates a very significant cooperativity between the Zn^{II} center and the guanidinium groups in **Zn^{II}-4**. The difference in reactivity cannot be entirely related to the pK_a value of the water coordinated to the Zn^{II} center in each complex, although undoubtedly the pK_a value of the Zn-bound water in **Zn^{II}-6** would be a unit or two higher than in **Zn^{II}-4** and **Zn^{II}-5**. Instead, some combination of the hydrogen bonding, electrostatic effects, and proton-donating ability of the guanidinium- and ammonium-containing arms must be the key to the enhanced reactivity. As these catalytic groups reside within a cleft containing the zinc ion, they form contacts with a bound substrate and play a role in the stabilization of the transition state. At this point we cannot rule out higher order complexes of **Zn^{II}-4** with itself, or with **4**, or with Zn^{II} as the catalytically active species.

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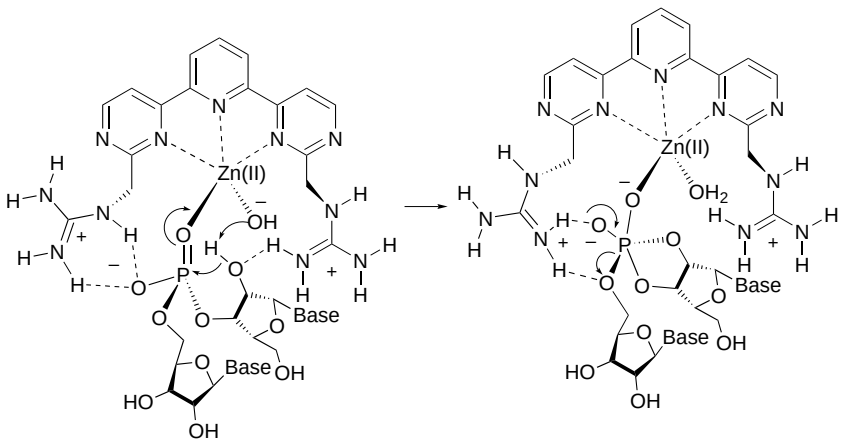

Scheme 2. Proposed mechanism for nucleophilic attack and leaving-group departure facilitated by **Zn^{II}-4**.

A possible mechanism (Scheme 2) for the hydrolysis of ApA by **Zn^{II}-4** includes double activation of the phosphate by coordination to the zinc center and to one of the guanidinium fragments, followed by Zn^{II}-OH general-base-promoted delivery of the 2′-OH group. Hence the guanidinium and ammonium arms are postulated to play several roles:[13] 1) to assist the binding of the phosphate group to the zinc ion, 2) to act as additional Lewis acids which polarize the phosphate group, 3) to lower the pK_a of a zinc-bound water molecule, and 4) potentially to protonate the leaving group.

In summary, a general strategy used by natural metallonucleases is to achieve the hydrolysis of phosphodiesters by cooperation between metal centers and amino acid residues. By creating a cleft that ensures guanidinium or ammonium groups and a zinc ion are proximal to one another, and preorganized to form contacts with a bound phosphodiester, we discovered cooperativity between the catalytic groups that is comparable to the enzyme SNase. The creation of other well-defined clefts wherein multiple productive contacts are made between catalyst and substrate may be anticipated to generate other efficient artificial enzymes.

Received: May 14, 2002 [Z19298]

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Fabrication of Ultrafine Conducting Polymer and Graphite Nanoparticles**

Jyongsik Jang,* Joon H. Oh, and Galen D. Stucky

The ability to selectively tune defects, electronic states, and surface chemistry has motivated the development of a variety of methods to fabricate metallic,[1] inorganic,[2] and polymeric nanoparticles.[3] While metallic and inorganic semiconductor nanoparticles with dimensions of around 1 nm are routinely made, polymer nanoparticles with dimensions less than 5 nm have not been reported. Here we report the selective fabrication of amorphous polypyrrole (PPy) nanoparticles as small as 2 nm in diameter, using microemulsion polymer-

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[**] This work was supported in part by the Brain-Korea 21 Program of the Korea Ministry of Education, by the Hyperstructured Organic Materials Research Center of Seoul National University, by the U.S. National Science Foundation (NSF), and by the Materials Research Laboratory Program of the NSF.

  0044-8249/02/4121-4016 $ 20.00+.50/0