# A Protease Mimic with Turnover Capabilities

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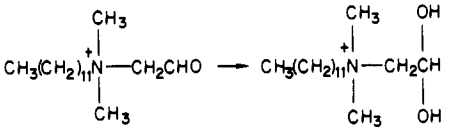
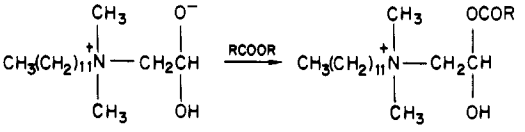
In an effort to mimic the action of serine proteases, Breslow and Cram synthesized models based on cyclodextrin and chorand derivatives, respectively.[1,2] Disagreement arose as to the relative effectiveness of their enzyme models. From one point of view, however, *neither* is satisfactory. Although the Breslow and Cram compounds are rapidly acylated by ester substrates, the resulting products permanently retain their acyl groups. The models are, therefore, stoichiometric acyl-transfer reagents, not catalysts. If actual catalysis is to be observed, acylated material must deacylate in a second and even faster step. Examples of such behavior are rare, the most notable exception being an iodoso-containing system studied by Moss.[3] In the present communication, we describe a new protease model which (1) noncovalently binds ester substrates, (2) accepts their acyl groups, and (3) deacylates to regenerate the original catalytic entity. Thus, true catalysis is achieved.

The protease model **1** possesses an aldehyde group and a long hydrocarbon chain linked to a quaternary nitrogen.[4,5] Several features of the compound seemed attractive. (1) Being amphiphilic, **1** forms micelles which can associate with esters and other substrates.[6] (2) Electronegative groups are known to promote aldehyde hydration (e.g. $Cl_3CCHO$ is totally hydrated in water). Thus, we could reasonably expect the formation of hydrate **2** with our micellar catalyst. (3) A hydroxyl group of the hydrate should



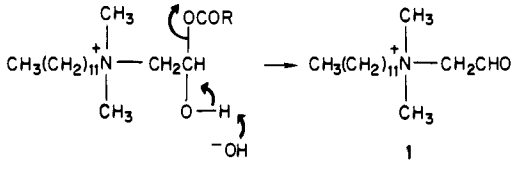
**1** **2**

be several p$K_a$ units more acidic than a simple primary alcohol; this permits kinetically significant levels of anion **3** to exist under mildly basic conditions. (4) Anion **3** can attack the carbonyl of a bound ester, thereby acylating the catalyst to give **4**. (5) Owing



**3** **4**

to the second hydroxyl (and this is critical), a facile pathway is available for the deacylation of the catalyst and regeneration of **1**. Since anionic counterions tend to collect at the surface of



**4**

cationic micelles,[7] hydroxide ion likely accelerates the deacylation.

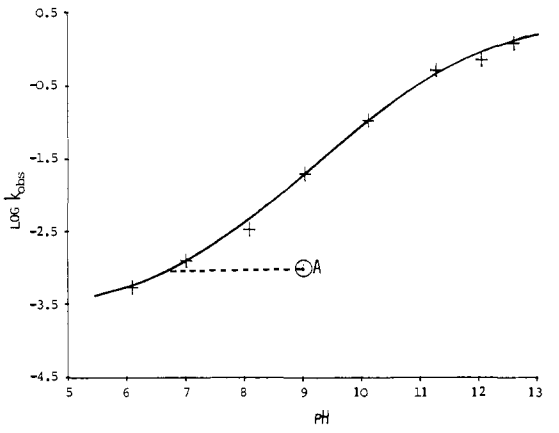



**Figure 1.** Dependence of log $k_{obsd}$ on the pH for the hydrolysis of *p*-nitrophenyl diphenyl phosphate catalyzed by micellar **2**. The profile, distorted by micellization, shows an approximate p$K_a$ of 10.9. Point A represents the rate at pH 9.0 for the functionalized surfactant described in ref 9.

We show below that deacylation does in fact proceed rapidly relative to acylation, so that recycling or "turnover" is operative.

According to surface tension measurements, the concentration at which our catalyst abruptly forms micelles (i.e., its CMC) equals $8 \times 10^{-4}$ M. $^{13}C$ NMR data prove that the catalyst exists totally as the hydrate **2**. Thus, no downfield peaks corresponding to the carbonyl and its enol are evident; a peak at 87 ppm is assigned to the hydrate carbon. In contrast, a ketonic analogue of **1** (having $CH_2COCH_3$ instead of $CH_2CHO$) displays neither hydrate formation nor catalytic activity.

The efficiency of our catalyst was examined using *p*-nitrophenyl diphenyl phosphate **5** as the substrate. This water-insoluble phosphate ester binds totally to the micelles under typical kinetic conditions: [surfactant] = $2 \times 10^{-3}$ to $2 \times 10^{-2}$ M; [**5**] = $4 \times 10^{-5}$ M; pH 9.00; 25.0 °C. Observed rate constants for the hydrolysis of **5** (determined spectrophotometrically at 400 nm) increase from $1.1 \times 10^{-5}$ $s^{-1}$ without any catalyst to a maximum of $2.0 \times 10^{-2}$ $s^{-1}$ at $8.0 \times 10^{-3}$ M catalyst. (A Berezin–Martinek[8] effect diminshes the maximum rate 15% at $2 \times 10^{-2}$ M catalyst.) The catalytic efficiency of hydrate **2** can be assessed from three comparisons: (1) The rate with $8 \times 10^{-3}$ M catalyst is 1800 times faster than that in pure pH 9.00 buffer. (2) The maximum hydrolysis rate is 210-fold larger with **2** than with a nonfunctionalized surfactant, dodecyltrimethylammonium bromide (DTAB). Adding 10 mol % octanal to the latter does not change this ratio. (3) Micellar **2** ($8.0 \times 10^{-3}$ M) catalyzes the hydrolysis of **5** about 21 times more effectively than does the Bunton surfactant[9] $C_{12}H_{25}N^+(CH_3)_2CH_2CH_2OH$ ($1.3 \times 10^{-2}$ M) under identical conditions. Thus, hydrate **2** manifests one of the larger known micellar catalyses.

Hydrolysis of **5** by micellized **2** possesses a sigmoidal pH–rate profile (Figure 1). The plot indicates that, prior to attacking the substrate, a hydrate hydroxyl ionizes with an approximate p$K_a$ of 10.9. By comparison, the Bunton surfactant[9] has a p$K_a$ of 12.4. Point A in Figure 1 corresponds to the hydrolysis rate at pH 9.00 catalyzed by the Bunton surfactant. The horizontal dotted line emanating from point A emphasizes the fact that surfactant **2** operates at pH 7 as effectively as does the monohydroxyl analogue at pH 9. We ascribe this improvement to the 1.5 p$K_a$ unit greater acidity of the hydrate over the alcohol; this permits a greater concentration of anionic nucleophile under mildly basic conditions.

Turnover by the surfactant catalyst **2** was demonstrated with the aid of a more water-soluble substrate, sodium 3-nitro-4-acetoxybenzenesulfonate (**6**). All experiments were carried out at pH 9.00 and 25.0 °C with $4.0 \times 10^{-3}$ M catalyst and $5.0 \times 10^{-3}$ M DTAB (the latter being necessary to preclude precipitate

(1) Breslow, R.; Trainor, G.; Ueno, A. *J. Am. Chem. Soc.* **1983**, *105*, 2739.
(2) Cram, D. J.; Katz, H. E. *J. Am. Chem. Soc.* **1983**, *105*, 135.
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(4) The design of this catalyst was motivated by work of Bender and Silver over 20 years ago: Bender, M. L.; Silver, M. S. *J. Am. Chem. Soc.* **1962**, *84*, 4589.
(5) Several obvious approaches to the synthesis of **1** failed for various reasons. Synthetic details of a method that finally succeeded are available on request.
(6) Menger, F. M.; Portnoy, C. E. *J. Am. Chem. Soc.* **1967**, *89*, 4698.
(7) Al-Lohedan, H.; Bunton, C. A. *J. Org. Chem.* **1982**, *47*, 1160.
(8) Yatsimirski, A. K.; Martinek, K.; Berezin, I. V. *Tetrahedron*, **1971**, *27*, 2855.
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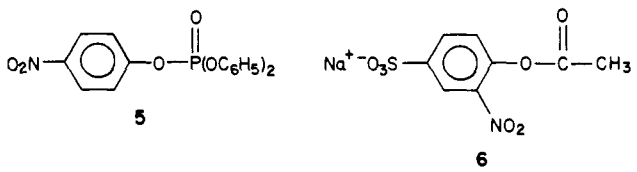

5



6

formation). Varying the ratio of **2** to **6** from 10/1 to 1/1 to 1/2 to 1/5 led to two important observations: (1) Substrate is completely hydrolyzed even when present in excess over the catalyst. (2) The second-order rate constant $k_{OH}$ is identical for all four runs (600 $M^{-1}$ $s^{-1}$). These results can only be explained by a rate-determining acylation of the surfactant followed by rapid deacylation and regeneration of the original catalytic species.

Since the $CH_2CHO$ unit can be attached easily to any molecule bearing an amino group (e.g., an aminocyclodextrin), the prospects seem good for creating additional biomimetic catalysts.

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## Chiral Discrimination in the Covalent Binding of Bis(phenanthroline)dichlororuthenium(II) to B-DNA

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Recently there has been increased attention focused on the binding of metal complexes to nucleic acids and nucleic acid constituents.[1] This interest stems in large part from the successful application of cis-dichlorodiammineplatinum(II) (*cis*-DDP) as an antitumor drug.[2] Our laboratory has utilized chiral transition-metal complexes in designing specific probes for nucleic acid structure. The tris(phenanthroline) complexes of zinc(II)[3] and ruthenium(II)[4] display enantiomeric selectivity in binding to DNA by intercalation. Because of their high specificity in noncovalent binding to right- or left-handed DNAs, enantiomers of tris(4,7-diphenylphenanthroline)ruthenium(II) and -cobalt(III) provide respectively spectroscopic probes[5] and cleaving agents[6] that are DNA conformation-specific. We became interested in developing a covalent analogue of this series in order to incorporate some stereospecificity into new drug design. We report here that bis(1,10-phenanthroline)dichlororuthenium(II) ($(phen)_2RuCl_2$) binds covalently to the DNA duplex and exhibits striking enantiomeric selectivity different from that seen on intercalation.

In buffer containing 10% ethanol, 50 mM $NaNO_3$, 5 mM Tris at pH 7.1, *rac*-$Ru(phen)_2Cl_2$[7] (50 $\mu$M) was incubated either at ambient temperatures or 37 °C for variable amounts of time with calf thymus DNA[8] (500 $\mu$M nucleotide). Following incubation, NaCl and 95% ethanol were added to quench the reaction and precipitate the DNA, with unbound ruthenium remaining in solution. After centrifugation, the supernatant was assayed spectrometrically, compared to controls lacking ruthenium or DNA, and levels of bound and free metal complex were determined. This experiment measures only covalent binding to the DNA. We repeated the procedure using the coordinatively saturated tris(phenanthroline)ruthenium cation, $Ru(phen)_3^{2+}$, which binds by intercalation;[4] under these assay conditions no binding to DNA was observed.[9] A plot of the extent of coordination to DNA by the $(phen)_2Ru^{2+}$ cation as a function of time is shown in Figure 1. A maximum binding ratio of 0.045, or one $(phen)_2Ru^{2+}$ moiety for every 11 base pairs, is obtained at about 3.5 h. This dependence on time is consistent with kinetics of ligand substitution in ruthenium(II) complexes.[10]

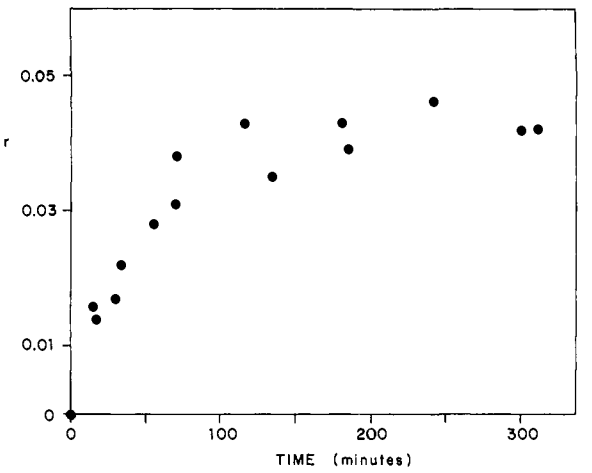


Figure 1. Plot of $(phen)_2RuCl_2$ binding to calf thymus DNA as a function of time; $r$ is the ratio of bound ruthenium to nucleotide concentrations.

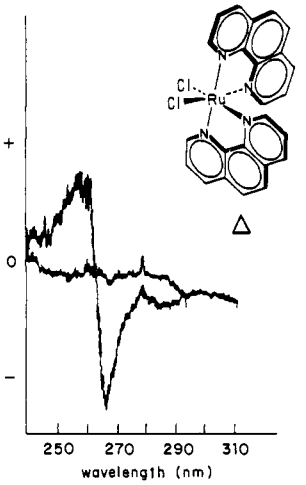


Figure 2. Circular dichromism of the supernatant after ethanol precipitation of the ruthenium complex bound to B-DNA. Binding to B-DNA is stereoselective and leads to enrichment of the supernatant in the unbound Δ isomer (inset).

(1) (a) Lippard, S. J.; Ed. "Platinum, Gold and Other Metal Chemotherapeutic Agents"; American Chemical Society: Washington DC, 1983; ACS Symp. Ser. No. 209. (b) Barton, J. K.; Lippard, S. J. *Met Ions Biol.* **1980**, *1*, 31. (c) Marzilli, L. G.; Kistenmacher, T. J.; Eichhorn, G. L. *Met. Ions Biol.* **1980**, *1*, 179. (d) Martin, R. B.; Mariam, Y. H. *Met. Ions Biol. Syst.* **1979**, *8*, 57. (e) Marzilli, L. G. *Prog. Inorg. Chem.* **1977**, *23*, 255. (f) Hodgson, D. J. *Prog. Inorg. Chem.* **1977**, *23*, 211.

(2) (a) Rosenberg, B.; Van Camp, L.; Trosko, J. E.; Mansour, V. H. *Nature (London)* **1969**, *222*, 385. (b) Rosenberg, B.; Van Camp, L. *Cancer Res.* **1970**, *30*, 1979. (c) For a review, see: Hill, J. M.; Loeb, E.; MacLellan, A.; Hill, N. V.; Khan, A.; King, J. J. *Cancer Treat. Rep.*, **1975**, *59*, 647. (d) Lippard, S. J. *Acc. Chem. Res.* **1978**, *11*, 211. (e) Hacker, M. P., Doyle, E. B., Krakoff, I. H., Eds. "Platinum Coordination Complexes in Cancer Chemotherapy"; Martinus Nijhoff: Boston, 1984.

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(7) The preparation of this compound was analogous to the synthesis of *cis*-bis(2,2′-bipyridine)ruthenium(II) dichloride by: Sullivan, B. P.; Salmon, D. J.; Meyer, T. J. *Inorg. Chem.* **1978**, *17*, 3334. Spectroscopic data in EtOH were as follows: $\epsilon$ 1.08 × $10^4$ $M^{-1}$ $cm^{-1}$ at 496 nm, $\epsilon$ 7.25 × $10^4$ $M^{-1}$ $cm^{-1}$ at 267 nm. In aqueous solution the complex may be considered a mixture of hydrolyzed species.

(8) Supplied by Sigma and purified by phenol extraction as in: Barton, J. K.; Lippard, S. J. *Biochemistry* **1979**, *12*, 2661.

(9) Subsequent precipitation of the resuspended $(phen)_2Ru^{2+}$–DNA pellet released no free ruthenium. This observation is also consistent with covalent binding.

(10) Isied, S. S.; Taube, H. *Inorg. Chem.* **1976**, *15*, 3070.

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