Precomplexation and Activation of Carboxylate and Phosphate Esters

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This is the final report for contract N00014-88-K-0309. It summarizes our previously submitted Technical Reports #1-9.
Statement of objectives

We are looking for reaction types that simultaneously: (1) provide for the reaction of acyl and phosphoryl groups under non-forcing conditions; (2) suggest ways for elaboration into catalytic cycles with turnover behavior; and, (3) survive translation onto binding moieties. To date, we have focused on artificial metalloenzymes derived from Co(III) and Cu(II) coordination complexes with cyclodextrins, preassociating α-nucleophiles, and binuclear metal ion complexes.

Statement of accomplishments

PREASSOCIATING α-NUCLEOPHILES

Cyclodextrins have been prepared bearing imidazole as a group with reactivity at pH 7; monodant coordination complexes have likewise been employed. However, as potential pendant groups, α-nucleophiles such as hydrazine or hydroxylamine offer unique properties. (1) In solution, α-nucleophiles show enhanced reactivity towards acyl transfer as compared to isosteric alcohols or amines. (2) Despite their greater reactivity towards acyl compounds, hydroxylamine (pKₐ 5.97) and hydrazine (pKₐ 8.0) are less basic than isosteric amines (pKₐ 9-10), and thus exist in a reactive form near neutral pH. (3) Both hydroxylamine and hydrazine transacylate alkyl esters and amides. (4) Because they are physically small, pendant α-nucleophiles would necessarily reside proximal to the CD binding cavity. We now report on the syntheses, characterizations, and reactivities of βCDNHNH₂ and βCDNHOH.

Reaction of βCD-1-O-tosylate (1) in anhydrous hydrazine (2) at RT for 4 h, followed by precipitation from EtOH, gave the crude product (3). Physically entrained NH₂NH₂ was removed by reprecipitation from EtOH (5x), which gave 3 in 60% yield. In an analogous manner, reaction of 1 with a 6% aqueous solution of hydroxylamine (4) at 90°C for 3 h, followed by multiple reprecipitation from EtOH, gave 5 in 86% yield. While either the N- or the O-alkylation product might have been formed, catalytic hydrogenation, which yielded βCDNH₂ and not βCD itself, confirmed the former. Notably for an unsymmetrically substituted CD derivative, 5 yields colorless plates (dec 207-210°C) from water.

Both βCDNHNH₂ and βCDNHOH are acylated rapidly by p-nitrophenylacetate (pNPA) with saturation behavior. The reaction of pNPA (0.05 mM) fully complexed to 5 at pH 7.0 and 25°C is faster than that with equimolar CH₃NHOH (k₂=1.0 M⁻¹ s⁻¹), demonstrating an effective
RNHOH concentration of 37 mM. βCDNHOH is acylated as efficiently at pH 7.0 as at pH 9.5; furthermore, the rate of acyl transfer is 1500-times faster than that afforded using equimolar βCD, which is not reactive under neutral conditions (pub. 7).

CATALYSIS VIA REVERSIBLE COVALENT BOND FORMATION

Concurrently, we have been investigating the potential of a different mode of reversible complexation: the Michael reaction. Without added metals, the hydrolysis of ester 9 (1.8 mM) to acid 12 proceeded at pH 7.50 and 23°C with $k_{obs}$ = $1.0 \times 10^{-6}$ s$^{-1}$ ($t_{1/2}$ = 670,000 s). Addition of divalent metal ions accelerated the rate of the hydrolysis by varying extents. The addition of 1 eq of Co(II) or Ni(II) accelerated the hydrolysis, yielding $k_{obs}$ = $3.9 \times 10^{-6}$ s$^{-1}$ ($t_{1/2}$ = 170,000 s) and 1.2 x $10^{-5}$ s$^{-1}$ ($t_{1/2}$ = 57,000 s), respectively. The use of copper ion led to the largest accelerations; addition of 1 eq of Cu(II) gave $k_{obs}$ = $6.2 \times 10^{-5}$ s$^{-1}$ ($t_{1/2}$ = 110 s), and addition of 5 eq of Cu(II) gave $k_{obs}$ = $1.6 \times 10^{-2}$ s$^{-1}$ ($t_{1/2}$ = 44 s). Therefore, the hydrolysis rate for the fully complexed ester is 16,000-times faster than the same reaction without added metal. Significantly, the reaction was catalytic with respect to copper ion; 0.01 eq of Cu(II) gave $k_{obs}$ = $6.2 \times 10^{-6}$ s$^{-1}$ ($t_{1/2}$ = 110,000 s), and the reaction proceeded to >90% completion while following first order
CZARNIK, Anthony W.

kinetics. As we did not observe product inhibition, it must be inferred that the complexation of Cu(II) to acid 12 is readily reversible and largely incomplete at the lower Cu(II) concentrations (pub. 2).

Even more encouragingly, the same scheme demonstrates metal-catalyzed hydrolysis of an unactivated amide. Without added metals the hydrolysis of amide 16 to acid 19 is too slow at pH 7.50 to measure readily, but no (≤2%) reaction had occurred after 650 h at 50°. As one basis for comparison, Still has measured the rate of peptide hydrolysis at 25° and pH 6-8 as $k_{\text{obs}} = 3 \times 10^{-9}$ s$^{-1}$ (t1/2 = ca. 7 years). With 1 equivalent of Cu(ClO$_4$)$_2$ at pH 7.0 and 23°, the hydrolysis of 16 occurs to at least 97% completion with $k_{\text{obs}} = 2.5 \times 10^{-6}$ s$^{-1}$. The reaction with 0.5 eq of Cu(II) at pH 7.5 and 50° proceeded to >97% completion and showed biphasic kinetics: $k_{\text{obs}} = 8.7 \times 10^{-5}$ s$^{-1}$ (first half) and $4.5 \times 10^{-6}$ s$^{-1}$ (second half). Reference 20 did not hydrolyze under metal catalyzed conditions. While such observations of metal ion promotion of amide hydrolysis have been made before, the reaction we observe is catalytic with respect to copper ion. The use of 8 mM 16 and 2 mM Cu(II) (0.25 equivalents) at pH 7.5 and 50° resulted in 80% conversion to acid 19 after 10 days. Likewise, the use of 1.2 mM Cu(II) (0.15 equivalents) at pH 7.0 and 50° resulted in 74% conversion to acid 19 after 15 days. These reactions, while not of enzyme-like speed, represent the first examples of metal catalyzed amide hydrolysis in a synthetic system (pub. 5).

METALLOENZYME MIMICS

We have prepared isomeric cyclodextrin/Co(III)-azamacrocycle conjugates 25 and 29. The primary side derivative (25) increases the rate of p-nitrophenylacetate hydrolysis by a factor of 900-fold; equally significant is the fact that the acceleration is observed at pH 7, at which cyclodextrin itself shows no activity (pub. 1,8).
We have also prepared binuclear complex 35, which accelerates the hydrolysis of the bis(PNP)phosphodiester to a greater extent than two equivalents of the monomeric cyclen-Co(III) complex (pub. 4).
(3) List of publications to date resulting from this work


