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Burst Kinetics and Turnover in an Esterase Mimic

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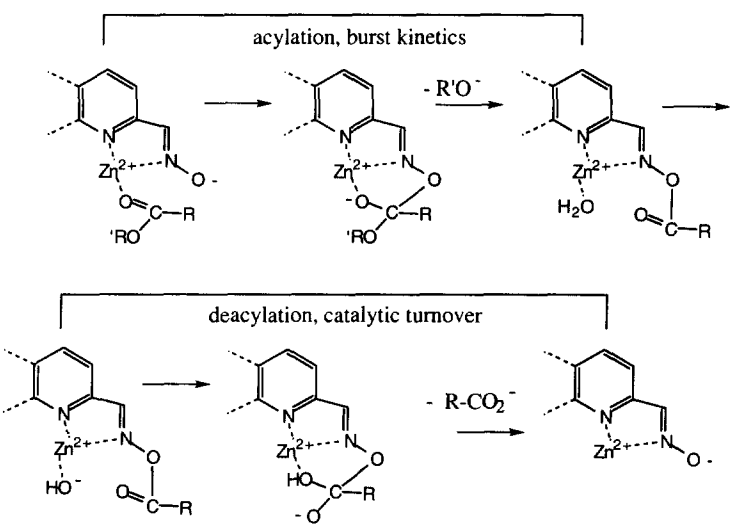
Abstract:. A catalyst combining a cyclodextrin binding group with a bound zinc cation and an oxime anion cleaves bound esters in a two step process.

Keywords: zinc, oxime, cyclodextrin

The combination of a bound Zn(II) and a coordinated pyridyl-2-carboxaldoxime can be effective in cleaving esters.[1-3] With the two nitrogens coordinated to Zn(II), the oxime hydroxyl is acidic but uncoordinated, and its anion can attack a Zn(II)-coordinated carbonyl group. The resulting O-acyl oxime is readily hydrolyzed by attack of a Zn(II)-coordinated hydroxide, both steps involving a five-membered ring.



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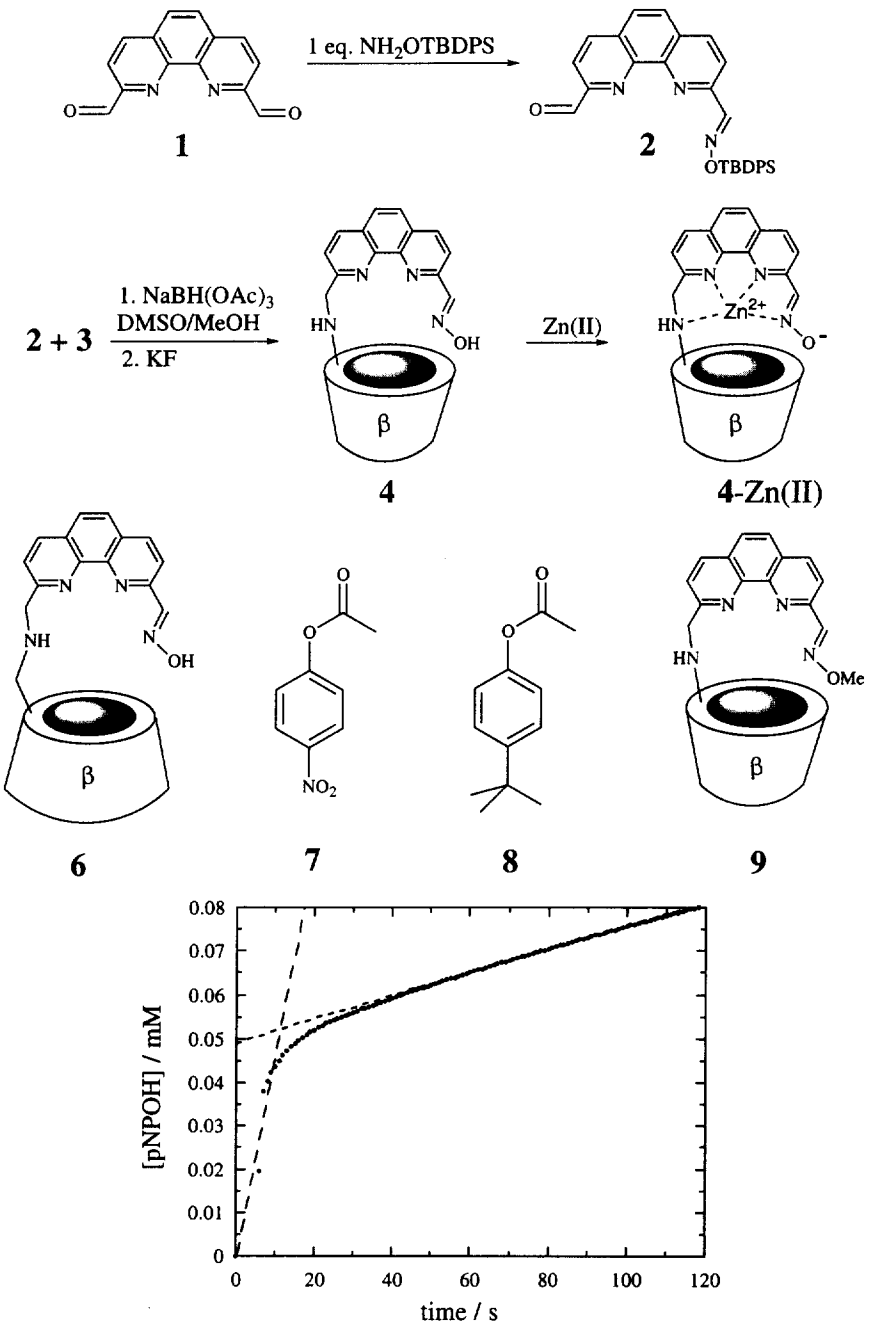
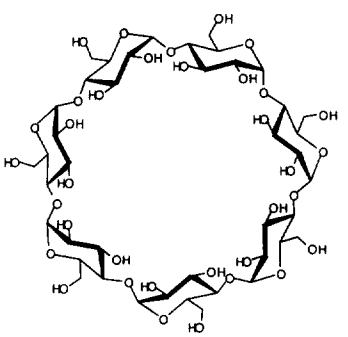


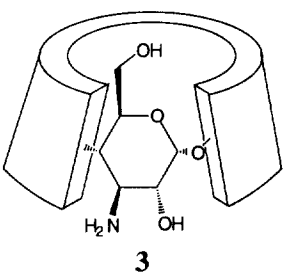
Figure 1. Formation of p-nitrophenol vs. time in the reaction of **7** with **4**-Zn(II) under the conditions described. Both the burst phase, in which the catalyst is acetylated, and the turnover phase are shown.

To date this mechanism has been used only in systems in which the oxime was part of a separate pyridinecarboxaldoxime. In the course of efforts to produce an effective esterase mimic as a single species, we have now constructed two catalysts based on cyclodextrin binding groups in which such an oxime is an intrinsic part of the molecules. With substrates that bind to the catalyst, we see ester cleavage that shows burst kinetics for catalyst-ester formation, and turnover kinetics limited by the rate of hydrolysis of the intermediate. We also see a significant preference for the catalyst with functional groups attached to the secondary face of the cyclodextrin instead of the primary face.

Phenanthroline-2,9-dicarboxaldehyde **1** was converted to its monoxime O-t-butyldiphenylsilyl derivative **2**,[4] and coupled to 3-amino-3-deoxy-β-cyclodextrin **3**[5,6] with triacetoxyborohydride to afford procatalyst **4**[7] after deprotection. Coupling of the protected monoaldehyde mono-oxime to 6-amino-6-deoxy-β-cyclodextrin **5**, then deprotection, afforded procatalyst **6**.[8] The two procatalysts, as their Zn(II) complexes, were then examined in the hydrolysis of p-nitrophenyl acetate **7**. Kinetic studies were performed in triplicate, sometimes in duplicate, with rates reproducible to ±5%.

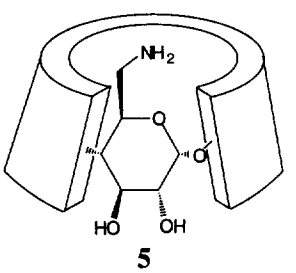


β-Cyclodextrin



3

3-aminocyclodextrin



5

6-aminocyclodextrin

A solution of **4**-Zn(II) (0.05 mM) in water with 1% methanol and 10 mM HEPES buffer (pH 7.0) was examined as a catalyst for the formation of p-nitrophenoxide ion from **7** (substrate concentration from 0.02 mM to 2.0 mM) in the burst kinetic region. Kinetic saturation was seen, from which a Lineweaver-Burk double reciprocal plot gave K_m = 0.4 mM and k_{cat} = 0.060 s^{-1}. A titration calorimetric measurement of the interaction of **4**-Zn(II) with p-nitrophenol gave a dissociation constant of 0.3 mM in water, in reasonable agreement with K_m. In the absence of the catalyst, k_{uncat} for **7** was 2.65 x 10^{-6} s^{-1}, so k_{cat}/k_{uncat} is 22,600 for the initial fast deacylation of **7**. Since k_{uncat} did not change when [buffer] was lowered from 10 mM to 5 mM and 2 mM, buffer is not participating in the reaction.

In the first rapid reaction of **4**-Zn(II) with **7** at 1.0 mM, one equivalent (0.05 mM) of p-nitrophenoxide ion is formed, but then a slower reaction ensues, with k_{cat} = 0.0075 s^{-1} (Figure 1). As in the hydrolysis of **7** by chymotrypsin,[9] this behavior

indicates the formation of an acyl-"enzyme" intermediate whose subsequent hydrolysis is slower. For this steady state turnover catalysis, k_{cat}/k_{uncat} is 2800.

Procatalyst **4** was also examined for the hydrolysis of **7** with one equivalent of Ni(II), but the reaction was 90 times slower than with Zn(II); with Cu(II), the rate was 250 times slower than for the Zn(II) complex. With the oxime methyl ether **9**, the Zn(II) complex at 0.05 mM hydrolyzed **7** at a rate only twice that of the uncatalyzed reaction, so the nucleophilic oxime anion is critical for catalysis. Substrate **8** was examined with catalyst **4**-Zn(II) under the same conditions used for substrate **7**, but at only one substrate concentration (1.0 mM) at which the catalyst should have been 90% saturated with substrate, estimated from the known binding constant for t-butylphenyl groups to β-cyclodextrin. In the burst region $k_{observed}$ was 5.3×10^{-4} s^{-1}, while k_{uncat} was 3×10^{-8} s^{-1}. The ratio $k_{observed}/k_{uncat}$ is thus 18,000, so the acceleration is comparable to that with the p-nitrophenyl substrate **7**.

We and others have made enzyme mimics with functional groups attached to either the secondary or the primary face of a cyclodextrin, and both types of catalysts have been active.[10] Thus we examined **6**-Zn(II), the isomer of **4**-Zn(II) with the catalytic group on the primary cyclodextrin face. With substrate **7** in the burst kinetic region, saturation was observed from which we derived K_m = 0.6 mM and k_{cat} = 0.0039 s^{-1}. The binding almost 50% weaker than with **4**-Zn(II), and k_{cat}/k_{uncat} is only 1500, not the 22,600 seen with **4**-Zn(II). The preference for the secondary face catalyst may reflect preferential binding geometry, and probably also the greater undesirable flexibility of the primary face catalyst.

The combination of an electrophilic metal ion and a nucleophile unquenched by the metal ion makes **4**-Zn(II) a powerful catalyst toward substrates **7** and **8**. Further work will demonstrate whether it can catalyze other reactions of interest.

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References and Notes

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4. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.3 (s, 1H, CH aldehyde), 8.9 (s, 1H, CH oxime), 8.8 (d, 1H, phen Ar), 8.6 (d, 1H, phen Ar), 8.3 (d, 1H, phen Ar), 8.2 (d, 2H, phen Ar), 8.1 (d, 1H, phen Ar), 7.8 (m, 4 H, Ph), 7.45 (m, 6H, Ph), 1.2 (s, 9H, t-Bu). MS (CI) : 490 (M+1) 507 (M+18)
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7. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.0 (s, 1H, CH oxime), 7.8-8.6 (m, 6H, phen Ar), 5.5-6.5 (m, 13H, 2° CD-OH), 4.7-5.0 (m, 7H, CD anomeric), 4.3-4.6 (m, 7H, CD 1°OH), 3.5-4.0 (m, 28H, CD), 3.0-3.5 (m, 14+, CD+dmso). MS (FAB) : 1370 (M+1)
8. ^{1}H NMR (DMSO-d_6 (**6**•HOAc), 400 MHz): δ 11.9 (s, 1H, =NOH oxime), 8.55 (d, 2H, phen Ar), 8.5 (s, 1H, CH oxime), 8.2 (d, 1H, phen Ar), 8.0 (m, 2H, phen Ar), 7.9 (d, 1H, phen Ar), 5.6-5.9 (m, 14H, 2° CD-OH), 4.7-5.0 (m, 7H, CD anomeric), 4.0-4.5 (m, 6H, CD 1°OH), 3.5-4.0 (m, 28H, CD), 3.0-3.5 (m, 14+, CD+dmso), 1.9 (s, HOAc). MS (FAB) : 1370 (M+1)
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