COMMUNICATION

Amino-cyclodextrins as biomimetics: catalysis of the Kemp elimination

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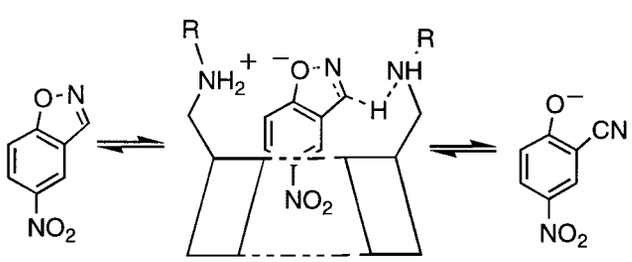
Synthetic amino-cyclodextrins (ACDs), perfunctionalized with pendant amines at the primary face, catalyse the Kemp elimination at physiological pH, *in simile* with proteins and synzymes.

Cyclodextrins (CDs) have provided the basis for numerous, important studies on enzyme models and molecular recognition.[1] Amino-CDs (ACDs) are homogeneous CD derivatives modified by persubstitution at the primary face with amino pendant groups which manifest compromised hydrophobic binding, but additional electrostatic binding of guest molecules relative to native CDs.[2,3] Potentiometric titration reveals that ACD nitrogen pK_a's are depressed by up to 4 units relative to parent amines, due to through bond and through space electrostatic interactions.[4,5] Thus ACDs, in contrast to simple mono- and disubstituted CDs, provide free amino groups at neutral pH, in mimicry of enzymes that provide catalytic lysine residues.[6]

The "Kemp elimination" of 5-nitrobenzisoxazole (NBI) is a concerted E2 elimination,[7] a model for biologically relevant proton transfers from carbon. This reaction provides a valuable test of ACDs, since it has recently been used as a probe of efficiency in: (i) "tailor-made" catalytic antibodies (34E4);[8] (ii) "off-the-shelf" proteins (BSA);[9] (iii) synzymes;[5] and (iv) a non-aqueous model system.[10] By using an efficient synthesis of the per-6-bromo-6-deoxy-βCD, a series of seven homogeneous ACD derivatives was synthesized and assayed as catalysts of the Kemp elimination.[11,†] These ACD derivatives demonstrate efficient catalysis by primary face amines at physiological pH, with poor substrate binding. Interestingly, at higher pH, the active site is switched to the CD secondary face with catalysis by the secondary face hydroxys (Scheme 1).



Scheme 1

In contrast to many nitrophenyl derivatives, NBI is not a good substrate for native βCD, showing a high K_M (≈20 mM) and modest rate acceleration (Table 1). ACDs demonstrated true catalysis at physiological pH and a more complex pH dependence (Fig. 1). Above the final pK_a inflection, the rate of base catalysis by the primary amine annulus of **βeACD** and **βpACD** intercepts the rate for catalysis by native βCD, confirming the novel observation of a switch to base catalysis by the secondary hydroxy annulus of the neutral ACDs.

ACDs catalyze the Kemp elimination with loose substrate binding. Saturation was only observed within solubility limits for **βNMeACD** and **βdACD**, the former under conditions of excess substrate. However, various inhibitors are able to bind competitively to ACD yielding 0–80% inhibition of catalysis.‡ The traditional hydrophobic cavity-binding inhibitors[12] showed modest inhibition with **βCD** and **βeACD** (≤28%) to poor inhibition of the reaction with **βNMeACD** (≤15%). Anionic polyvalent-binding inhibitors (*e.g.* phthalic, azaleic acids), expected to bind well to ACDs, showed modest inhibition with βCD (≤16%), potent inhibition with **βeACD**, **βACD** and **βpACD** (40–80%), but little inhibition of the reaction with **βNMeACD** (≤18%). The poor inhibitory effects of such compounds on **βNMeACD**, together with the observation of Michaelis–Menten catalysis for **βNMeACD** suggests that the combination of annulus and corona provides an alternate binding site to the cavity. Anionic and hydrophobic ligands are also inhibitors of the Kemp elimination catalyzed by BSA.[8,9]

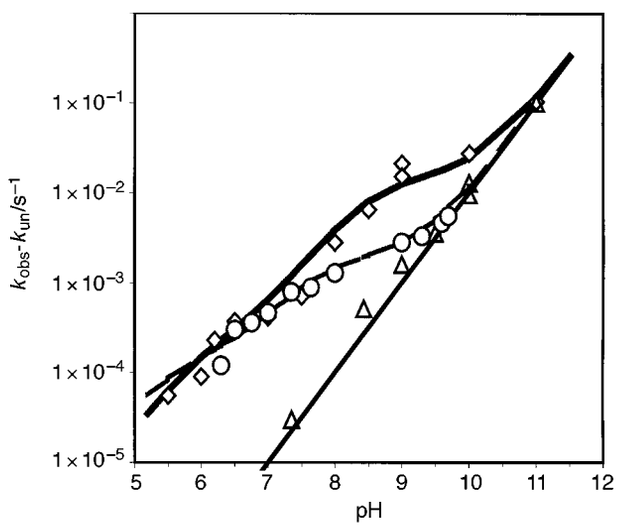


Fig. 1 pH–rate profile for Kemp elimination: △ = **βCD**; ◇ = **βeACD**; ○ = **βpACD**, at 20 °C in KCl (100 mM), ACD (5 mM), buffer (50 mM): sodium acetate (pH < 6); bis-tris (6 < pH < 7.4); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (7.5 < pH < 9); borate (8 < pH < 11); 2-amino-2-methylpropanol (9 < pH < 10). Data fit to $k_{cat} - k_{un} = k_{OH^-}[OH^-] + k_1[OH^-]/([OH^-] + K_w - K_{a1}) + k_7[OH^-]/([OH^-] + K_w - K_{a7})$, using terms for catalysis by $ACDH_6^{6+}$ (1) and ACD (7) only: $k_1 = 1.5 \times 10^{-4}$ s^{-1}, p$K_{a1} = 6.0$, $k_7 = 1.5 \times 10^{-2}$ s^{-1}, p$K_{a7} = 8.15$ (**βeACD**); $k_1 = 2.0 \times 10^{-4}$ s^{-1}, p$K_{a1} = 5.5$, $k_7 = 1.5 \times 10^{-3}$ s^{-1}, p$K_{a7} = 7.7$ (**βpACD**).

The simple rate enhancement ($(k_{obs} - k_u)/k_u$) of the Kemp elimination by ACDs, over the background reaction at physiological pH, also an amine-catalyzed elimination, surpassed βCD, ranging from 25–3700 (Table 1). Rate acceleration (r_{acc}) and effective molarity (EM)[13] give a more accurate assessment of proton transfer efficiency and allow comparison with Kirby's and Hilvert's work on catalytic antibodies, albumins and synzymes.[5,8,9] Rate accelerations (r_{acc}) for ACDs compared to parent amines§ are modest, but correcting of the benchmark amine rate to account for the low pK_a of the ACD amines (*in simile* with Kirby *et al.*;[5] Table 1), gives $r_{acc} = 10^2$–10^4, which compares with that for BSA ($r_{acc} = 4 \times 10^3$ at pH 8).[9] The best estimates of catalytic efficiency in the Kemp elimination can be

Table 1 Kinetic parameters for ACD catalyzed Kemp elimination

CD	pK_{a1} [a]	$(k_{obs} - k_u)/k_u$ [c]	k_{CD} [d]/M^{-1} s^{-1}	r_{acc} [f]	$r_{acc}(7.4)$ [g]	$r^{H}_{acc} = k^{H}_{CD}/k_B$ [h]	EM/M [i]
βCD	12.6	5.2	1.74 [e]	n/a	n/a	n/a	—
βeACD	6.0	433	0.48	24	1.5×10^3	1.0×10^3	—
βNMeACD	6.0	3670	5.46 [e]	140	1.7×10^4	—	2.3×10^3
βdACD	3.8	1140	1.03 [e]	10	3.1×10^3	—	1.9×10^2
βACD	7.0	25	0.03	46	0.9×10^2	—	—
βpACD	5.5	539	0.14	7.0	4.3×10^2	3.2×10^3	—
βMeACD	6.4	417	0.45	23	1.4×10^3	—	—
βsACD	6.4 [b]	250	0.27	14	8.2×10^2	—	—

[a] For amino-N from potentiometric titration [see ref. 4], or [b] by analogy with ACD congeners. [c] k_{obs} at 5 mM CD, bis-tris 50 mM, KCl 0.1 M, pH 7.4; $k_u = 5.4 \times 10^{-6}$ s^{-1} background buffer. [d] k_{CD} from plots of $k_{obs} - k_u$ *vs.* [ACD] at pH 7.4; or [e] $k_{CD} = k_{cat}/K_M$. [f] Rate acceleration relative to parent amine, k_{CD}/k_B(parent).§ [g] Rate acceleration k_{CD}/k_B relative to amine of pK_a 7.4, using Kirby's approximation [see ref. 5]. [h] Rate acceleration, where $k^{H}_{CD} = k_1$/[ACD] (Fig. 1), $k_B = k_B$(bis-tris)/$10^{\beta(pK_a - 6.5)}$ ($\beta = 0.73$ [see ref. 5]; k_B(bis-tris) = 6.7×10^{-5} M^{-1} s^{-1}). [i] EM = k_{cat}/k_B(bis-tris).

made for **βeACD** and **βpACD**, for which the second order rate constants for catalysis by ACD·$6H^+$ (and thence r^{H}_{acc}) can be calculated from fitting of the pH–rate profile, and for **βdACD** and **βNMeACD**, for which EM values may be calculated (Table 1).¶ Thus the rate acceleration is 10^3–10^4 and EM values are at the low end of the range for Kirby's synzymes ($1.2–5.1 \times 10^3$ M), which provide the most efficient artificial catalysts for proton transfer from carbon yet reported.[5] Comparison can also be made with Lehn's polyamine macrocycle, which catalyzes transphosphorylation at pH 7 ($r_{acc} = 500$) and is regarded as one of the most efficient enzyme mimics.[14,15]

The rate acceleration observed for ACD catalysis derives partly from provision of a basic amine group at neutral pH, in an annulus of cationic ammonium groups able to stabilize the anionic transition state. However, the hydrophobic microenvironment provided by the cavity and corona also has a role. The rate of amine catalysis of the Kemp elimination has been reported to be insensitive to solvent effects, based largely on work in MeCN.[7,8] But, in DMSO–water mixtures, used previously to model microenvironment effects in CD catalysis,[16] we have observed rate enhancements relative to aqueous solution, from ≤50-fold for simple alkylamines, to 210–680-fold for difunctional amines such as ethanolamine and 2-aminomethylpyridine. Thus loose binding and electrostatic stabilization of the highly-delocalized, anionic transition state by a combination of the cationic annulus and hydrophobic microenvironment of the ACD corona and cavity contribute to catalysis. In this respect, ACDs behave in an analogous fashion and with catalytic efficiency approaching polymeric synzymes, which have proven exceptionally efficient catalytic systems.[5] ACDs are able to catalyze reactions of anionic substrates[17] and in the Kemp elimination of a neutral substrate reacting *via* an anionic transition state.[18] ACDs provide basic free amine groups at neutral pH, and transition state stabilization by the cationic annulus, hydrophobic cavity and the corona of pendant groups, and can be expected to mimic an expanding range of enzyme reactions.

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Notes and references

† Per-6-(X)-6-deoxy-CD: **βeACD** X = $NH(CH_2)_2OH$; **βNMeACD** X = $NMe(CH_2)_2OH$; **βdACD** X = $NH(CH_2)_2NH_2$; **βACD** X = NH_2; **βpACD** X = $NHCH_2(2\text{-}C_6H_4N)$; **βMeACD** X = $NH(CH_2)_2OMe$; **βsACD** X = $NHCH(CH_2OH)_2$.

‡ Stock solutions of potential inhibitors (PI, 5 mM) in MeOH were added to buffered solutions equimolar in CD (10% v:v MeOH, 2,2-bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol (bis-tris) 50 mM, KCl 100 mM, pH 7.35). No inhibition of catalysis by free amines was observed.

§ *O*-Methyl-2-aminoethanol (pK_a 9.8) $k_B = 2.0 \times 10^{-2}$ M^{-1} s^{-1}; *O,N*-dimethyl-2-aminoethanol (pK_a 9.4) $k_B = 4.0 \times 10^{-2}$ M^{-1} s^{-1}; *N,N,N′*-trimethyl-1,2-diaminoethane (pK_a 10.2) $k_B = 9.9 \times 10^{-2}$ M^{-1} s^{-1}; 2-aminoethanol (pK_a 9.4) $k_B = 6.5 \times 10^{-2}$ M^{-1} s^{-1}; 2-aminomethylpyridine (pK_a 8.8) $k_B = 2.0 \times 10^{-2}$ M^{-1} s^{-1}; k_B 0.1 M KCl, at pH = pK_a(amine), $k_{obs} = k_{OH^-}[OH^-] + \frac{1}{2}k_B$[amine], $k_{OH^-} = 7.88$ M^{-1} s^{-1}.

¶ EM = k_{cat}/k_B, using k_B(bis-tris); k_{cat} from Lineweaver–Burke plots; **[βdACD]** = 1–10 mM, [S] = 28 μM, $k_{cat} = 43.4 \pm 16.5 \times 10^{-3}$ s^{-1}; **[βNMeACD]** = 3 μM, [S] = 0.08–1 mM, $k_{cat} = 15.3 \pm 2.3 \times 10^{-3}$ s^{-1}; pH 7.4, bis-tris 50 mM, KCl 0.1 M.

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