Spectator Catalysis by Alcohols in the Cleavage of an Aryl Ester by β-Cyclodextrin. Ternary Complexes and Structural Dependence

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Recently, we found that the cleavage of *p*-nitrophenyl acetate (pNPA) by β-cyclodextrin (β-CD)[1] in aqueous base[2-5] is *not* totally inhibited by competitive binding of alcohols and other potential inhibitors (PIs), unlike the case of *m*-nitrophenyl acetate.[4] Moreover, there is a strong correlation between the binding constants of PIs to β-CD and the rate constants for PI-mediated cleavage.[4b] These observations are consistent with the *p*-nitrophenyl group of pNPA being outside the β-CD cavity in the transition state, so that PIs may be inside. Conceivably, reaction could occur through a ternary (PI·CD·pNPA) complex, but no evidence was found for such.

We now report that the basic cleavage of *p*-nitrophenyl hexanoate (pNPH) by β-CD[5] is *catalyzed* by alcohols, and saturation kinetics[6] gives clear evidence of the formation of ternary complexes (Figure 1).[7] These findings were not anticipated since earlier work concluded that pNPH binds to CDs through its acyl chain, both in the initial state and in the transition state for acyl transfer.[5] Thus, inhibition was expected, and it has been observed for 1,6-hexanediol, suberate dianion, and perchlorate ion, but simple alcohols, alkanoate ions, and alkanesulfonate ions show catalysis.

Our results can be interpreted in terms of cleavage of pNPH (S) in the medium (eq 1), via a CD·S complex (eq 2), and through a ternary complex with a PI (eq 3):

$$S \xrightarrow{k_u} P \tag{1}$$

$$CD + S \underset{K_s}{\rightleftharpoons} CD{\cdot}S \xrightarrow{k_c} P \tag{2}$$

$$PI + CD{\cdot}S \underset{K_t}{\rightleftharpoons} PI{\cdot}CD{\cdot}S \xrightarrow{k_t} P \tag{3}$$

For low $[S]_0$ these pathways require that

$$k^{obsd} = \frac{k_u K_t K_s + k_c K_t [CD] + k_t [PI][CD]}{K_t K_s + K_t [CD] + [PI][CD]} \tag{4}$$

Since $k_u < k_c$, at high [CD] eq 4 approximates to[8]

$$k^{obsd} = \frac{k_c K_t + k_t [PI]}{K_t + [PI]} \tag{5}$$

which corresponds to simple saturation kinetics. Data for the cleavage of pNPH with various alcohols show just such behavior (Figure 1), and nonlinear fitting[9] affords estimates of K_t and k_t (Table I).

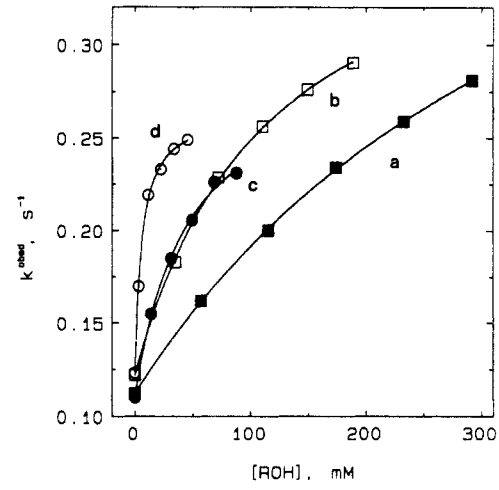

Figure 1. Variation of rate constants for the cleavage of *p*-nitrophenyl hexanoate in the presence of β-CD (15 mM) and alcohols:[6] (a) 2-propanol; (b) 2-butanol; (c) *tert*-butyl alcohol; (d) cyclohexanol. For these plots, [ROH] was corrected for the formation of β-CD·ROH complexes.[9]

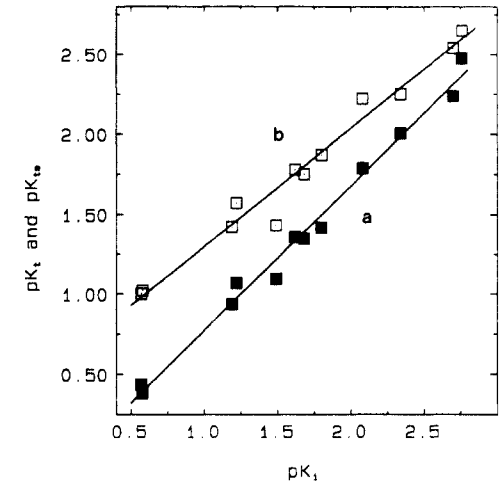

Figure 2. Initial-state and transition-state binding of the alcohols in Table I. (a) Correlation of pK_t with pK_i; slope = 0.90, and r = 0.994. (b) Correlation of pK_{ts} with pK_i; slope = 0.74, and r = 0.986.

Table I. Constants for Cleavage of *p*-Nitrophenyl Hexanoate in the Presence of β-Cyclodextrin and Alcohols, ROH[a]

R	K_i, mM	K_t, mM	k_t, s^{-1}	k_a, M^{-1} s^{-1}	K_{ts}, mM
n-Pr[b]	270	370	0.51	1.4	100
i-Pr	260	415	0.60	1.4	95
sec-Bu	65	116	0.41	3.6	38
n-Bu	60	86	0.43	5.1	27
2-Pen	32	81	0.30	3.7	37
i-Bu[b]	24	44	0.36	8.2	17
t-Bu	21	45	0.34	7.7	18
n-Pen	16	38	0.39	10	14
c-Pen	8.3	16	0.37	23	6.0
n-Hex	4.6	9.8	0.24	25	5.6
c-Hex	2.0	5.8	0.28	48	2.9
neo-Pen[b]	1.7	3.3	0.20	61	2.3

[a] At 25 °C. Kinetics in an aqueous phosphate buffer containing 15 mM β-CD.[6] The dissociation constants (K_i) of β-CD·ROH are from the literature.[3,10] Values of K_t and k_t were obtained by analysis in terms of eq 5. The other constants are as follows: $k_a = k_t/K_t$ (for CD·S + PI → P); $K_{ts} = k_c/k_a$ (see text).[12] [b] Solution contained 10 mM β-CD.

Values of K_t for the ternary complexes vary significantly, and they correlate strongly with K_i for dissociation of the β-CD·ROH complexes[3,10] (Figure 2a), whereas the rate constants k_t vary much less. Thus, the second-order rate constants k_a (for CD·S + PI

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(4) (a) Tee, O. S.; Hoeven, J. J. *J. Am. Chem. Soc.* **1989**, *111*, 8318. (b) Tee, O. S.; Hoeven, J. J.; Bozzi, M.; Gadosy, T. Unpublished results.
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(6) Cleavage of pNPH (0.025 or 0.050 mM) in a 0.2 M phosphate buffer (pH 11.6) containing β-CD (10 or 15 mM) and various [PI] was monitored by UV-visible spectrophotometry, with stopped-flow mixing. Cf.: Tee, O. S.; Takasaki, B. K. *Can. J. Chem.* **1985**, *63*, 3540. Reference 5b.
(7) Data analysis in terms of eq 5 of ref 4a gives curved plots, also indicative of saturation.
(8) (a) Under the reaction conditions: k_u = 0.045 s^{-1}, k_c = 0.14 s^{-1}, and K_s = 1.6 mM. (b) The terms in [CD] dominate the numerator and denominator of eq 4, and so [CD] effectively cancels out, even though increasing [PI] reduces the concentration of free CD. Hence, eq 5 is a reasonable approximation.
(9) Analysis used values of [PI] corrected for the formation of CD·PI.[4a]
(10) Matsui, Y.; Mochida, K. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 2808.

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→ P) increase with the strength of binding of ROH to β-CD, as was found with pNPA.[4] Using an approach discussed elsewhere,[5b,11] we can estimate apparent dissociation constants (K_{ts}) for the transition states containing ROH.[12] Values of K_{ts} also parallel K_i (Table I), and there is a reasonable correlation between pK_{ts} and pK_i (Figure 2b), even though the alcohols include different structural types. These trends are consistent with modes of binding of ROH in the ternary complexes *and* in the transition states for cleavage that are not too dissimilar from those in the β-CD·ROH complexes.

Values of k_t are 1.4-4.3 times larger than $k_c = 0.14\ s^{-1}$ for CD·pNPH[8a] since the catalysis by ROH is only modest. Nevertheless, they must mean that the presence of an alcohol in the β-CD cavity can stabilize the transition state relative to the initial state.[11] Presumably, the alcohols act as inert spacers,[13] improving the fit of the acyl chain of pNPH in the cavity of β-CD in the transition state for ester cleavage.[14] This behavior may be considered a novel type of "spectator catalysis".[15] It will be of interest to see how this form of catalysis varies with the substrate, the CD, and the structure of PI.[16]

(11) (a) Tee, O. S. *Carbohydr. Res.* **1989**, *192*, 181. This paper discusses the binding of transition states to CDs. It employs an approach pioneered by Kurz[11b] and used by enzymologists.[11c] (b) Kurz, J. L. *J. Am. Chem. Soc.* **1963**, *85*, 987. (c) Lienhard, G. E. *Science (Washington, D.C)* **1973**, *180*, 149. Jencks, W. P. *Adv. Enzymol.* **1975**, *43*, 219. Schowen, R. L. In *Transition States in Biochemical Processes*; Gandour, R. D., Schowen, R. L., Eds.; Plenum: New York, 1978; Chapter 2. Kraut, J. *Science (Washington, D.C.)* **1988**, *242*, 533.

(12) Using Kurz's approach:[11] $k_c = Q[TS]/[CD\cdot S]$ and $k_a = Q[TS\cdot PI]/[CD\cdot S][PI]$, where $Q = (k_BT/h)$ and TS is the transition state in reaction 2. Thus, $K_{ts} = [TS]\cdot[PI]/[TS\cdot PI] = k_c/k_a$. In the present case, PI = ROH.

(13) For another study invoking a spacer, see: Ueno, A.; Moriwaki, F.; Osa, T.; Ikeda, T.; Toda, F.; Hattori, K. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3109.

(14) Rate increases in esterolysis have also been brought about by modifying β-CD with flexible caps: Emert, J.; Breslow, R. *J. Am. Chem. Soc.* **1975**, *97*, 670. Breslow, R.; Czarniecki, M. F.; Emert, J.; Hamaguchi, H. *J. Am. Chem. Soc.* **1980**, *102*, 762.

(15) For a different kind of spectator catalysis, see: Kershner, L. D.; Schowen, R. L. *J. Am. Chem. Soc.* **1971**, *93*, 2014.

(16) Our work is supported by grants from the Natural Sciences and Engineering and Research Council of Canada.

Structural Model of a Short Carboxyl-Imidazole Hydrogen Bond with a Nearly Centrally Located Proton: Implications for the Asp-His Dyad in Serine Proteases

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The cornerstone of the hypothesis[1] on the orientation of carboxylate in general base catalysis is that there is more electron density in the syn direction than in either anti.[2] Consequently, when carboxylate hydrogen bonds to an acid with a pK_a comparable to that of carboxyl, the position of the proton should depend on the directionality of the hydrogen bond to carboxylate.[3] If anti, the proton will be closer to the weak base; if syn, closer to the carboxylate. As ΔpK_a between donor and acceptor approaches 0, the hydrogen bond becomes equidistant and the distance between the heavy atoms decreases.[4]

(1) Gandour, R. D. *Bioorg. Chem.* **1981**, *10*, 169-176.

(2) Wiberg, K. B.; Laidig, K. W. *J. Am. Chem. Soc.* **1987**, *109*, 5935-5943. Siggel, M. R. F.; Streitwieser, A., Jr.; Thomas, T. D. *J. Am. Chem. Soc.* **1988**, *110*, 8022-8028. Slee, T.; Larouche, A.; Bader, R. F. W. *J. Phys. Chem.* **1988**, *92*, 6219-6227.

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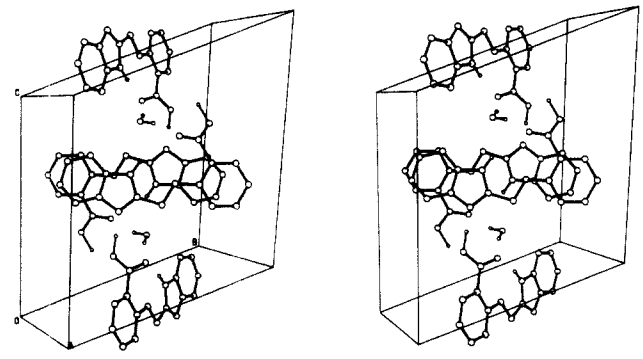


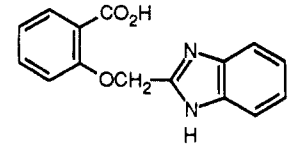
Figure 1. Stereoview of the packing diagram of 2-(2-benzimidazolylmethoxy)benzoic acid.

Table I. Hydrogen-Bonding Parameters[a]

atoms	O···N, Å	O-H, Å	O-H···N, deg
O2A-N1B	2.590 (2)	1.18 (3)	175 (3)
O2B-N1A	2.594 (2)	1.16 (2)	177 (2)
mean	2.592 (2)	1.17 (2)	176 (2)

[a] Standard deviations are in parentheses.

Single-crystal X-ray analysis of small-molecule models of biomolecular hydrogen bonds reveals structural trends[5,6] that parallel the trends in proteins.[7,8] The 100-fold improvement in resolution in the smaller structures enables a more precise picture of these trends.



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In the crystal structure of **1**·1/2H_2O (see Figure 1), two crystallographically independent molecules form intermolecular hydrogen bonds between carboxyl and benzimidazolyl as chains along the direction of the *c* axis.[9] The two hydrogen bonds are independent measures of the same interaction (Table I). The resulting O···N contacts are shorter than the mean O···N distances of imidazolium-carboxylate couples.[10] A water bridges the two molecules by hydrogen bonding to the carboxyls, with O···O distances 2.756 (3) and 2.793 (3) Å.

The strong *inter*molecular hydrogen bonding between a syn-oriented carboxyl and a benzimidazolyl, rather than anti-oriented *intra*molecular hydrogen bonding, emphasizes the importance of orientation. Hydrogen bonds between carboxyl and imidazole in small molecules[11] and in proteins[8] strongly prefer the syn orien-

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(9) Diffraction data were collected on an Enraf-Nonius CAD4 diffractometer, equipped with Cu Kα radiation: $C_{15}H_{12}N_2O_3\cdot{}^1/_2H_2O$, FW = 277.3, triclinic space group $P\bar{1}$, $a = 8.2883$ (7) Å, $b = 12.582$ (2) Å, $c = 14.205$ (2) Å, $\alpha = 69.24$ (1)°, $\beta = 80.25$ (1)°, $\gamma = 72.46$ (1)°, $V = 1317.6$ (3) Å^3, $Z = 4$, $\lambda = 1.54184$ Å, $D_{calcd} = 1.398$ g cm^{-3}, $R = 0.047$ for 4160 observed data with $\theta < 75°$, 475 variables. Hydrogen atoms were refined. Structural details are in the supplementary material.

(10) (a) Gorbitz[6] reports two means: 2.664 (17) Å for (His)$HN_\pi$$^+$···$^-$OOC-(Asp/Glu) and 2.736 (35) Å for (His)$HN_\tau$$^+$···$^-$OOC-(Asp/Glu). The crystal structure of histidinium trimesate[10b] has a shorter (His)$HN_\pi$$^+$···$^-$OOC-bond (2.568 (7) Å; $R = 0.057$), but the hydrogen positions were not accurately determined. (b) Herbstein, F. H.; Kapon, M. *Acta Crystallogr., Sect. B* **1979**, *B35*, 1614-1619.

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