ORGANIC LETTERS

1999
Vol. 1, No. 6
829−832

Catalysis and Acceleration of Acyl Transfer by Aminocyclodextrins: A Biomimetic System of Use in Enzyme Modeling and Drug Design

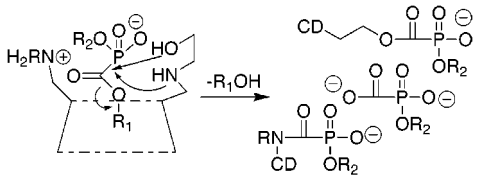
Colin G. Ferguson and Gregory R. J. Thatcher*

Department of Chemistry, Queen's University, Kingston, Ontario K7L 3N6, Canada

thatcher@chem.queensu.ca

Received April 28, 1999

ABSTRACT

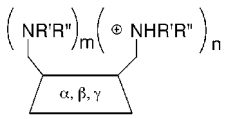


Aminocyclodextrins (ACDs), perfunctionalized at the 6-position with amino groups, bind phosphonoformate (PFA) diesters and accelerate acyl transfer reactions with high efficiency at neutral pH. Aminolysis and esterolysis are accelerated and hydrolysis of PFA diesters is catalyzed by ACDs. PFA diesters have significant antiviral activity. The rapid reactions observed with ACDs show that biological nucleophiles may undergo facile covalent modification by PFA esters at physiological pH, which has significant implications for prodrug and drug design strategies.

Cyclodextrins (CDs) are naturally occurring, toroidal cyclomaltooligosaccharides, which may bind organic molecules of appropriate size within the hydrophobic cavity. Substrate binding and subsequent reaction, accelerated or catalyzed by functional groups at the primary or secondary annulus, has provided the basis for many enzyme models,[1] although in many cases catalytic efficiency is very low.[2] CDs remain attractive for study as enzyme mimics because of advantages over other model systems: (i) the active site has well-defined geometry and (ii) substrate binding and product release in aqueous solution are incorporated within the catalytic mechanism.

CD derivatives, obtained by functionalization of 1 or 2 of the 18−24 hydroxyl groups, have frequently been studied as enzyme models, but monofacially substituted derivatives have received much less attention.[1,2] Aminocyclodextrins (ACDs), synthetically accessible, homogeneous CD derivatives persubstituted at the primary face with amino pendant groups, represent interesting biomimetic host molecules (Scheme 1).[3] Two important features common to biological host molecules are provided by ACDs: a hydrophobic binding site in the cavity which is linked cooperatively with the electrostatic binding site of the cationic primary annulus. Importantly for catalysis, the pK_a of the ACD amino groups is reduced by up to 4 units relative to that of the parent

Scheme 1



α: m + n = 6; β: m + n = 7, γ m + n = 8
α, β**eACD:** R' = $HO(CH_2)_2$; R" = H
α, β**MeACD:** R' = $MeO(CH_2)_2$; R" = H
β**NMeACD:** R' = $HO(CH_2)_2$; R" = Me
α, β, γ**ACD** R' = H; R" = H

(1) Breslow, R. *Acc. Chem. Res.* **1995**, *28*, 146, and references therein. Breslow, R. H.;Dong, A. *Chem. Rev.* **1998**, *98*, 1997, and references therein. Tee, O. S. *Adv. Phys. Org. Chem.* **1994**, *29*, 1, and references therein. D'Souza, V. T.; Bender, M. L. *Acc. Chem. Res.* **1987**, *20*, 146.

(2) Kirby, A. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 707, and references therein.

(3) McCracken, P. G.; Ferguson, C. G.; Vizitiu, D.; Walkinshaw, C. S.; Wang, Y.; Thatcher, G. R. J. *J. Chem. Soc., Perkin Trans. 2* **1999**, 911.

10.1021/ol9906211 CCC: $18.00
Published on Web 08/21/1999

amines, providing both free amine and ammonium groups at the primary annulus at neutral pH.[4,5] This pK_a effect potentially allows for nucleophilic and general base catalysis by ACDs at neutral pH, in contrast to many CD-amine derivatives, but catalysis of acyl transfer by ACDs, a benchmark reaction for biomimetic catalysts, has not been reported.[3,6] Herein we provide the first report of ACDs acting as enzyme mimics in acyl group transfer with phenyl and unactivated ethyl esters as substrates, displaying large rate accelerations and effective molarities (EMs). The substrates used are anionic phosphonoformate (PFA) esters.

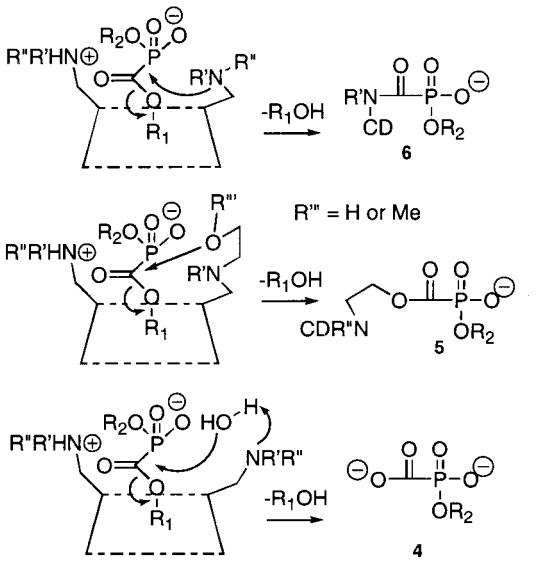
PFA esters are of therapeutic importance, and studies on their reactivity under biomimetic conditions are of significance in antiviral drug and prodrug design. PFA (Foscarnet) is an antiviral agent, effective against many viruses including HIV, HCMV, and HSV, that is in use clinically in AIDS therapy.[7] However, since PFA is trianionic at physiological pH, cell membrane permeability is poor, leading to several attempts to circumvent poor bioavailability by synthesis of PFA esters as prodrugs.[8,9] Mechanistic studies have shown that PFA triesters are remarkably reactive toward reaction at P and that competitive reaction at the acyl C can lead to C−O or P−C bond cleavage.[9] Monoanionic PFA diesters have shown antiviral activity in studies on Foscarnet prodrugs[8] and are ideal substrates for ACD, since the phosphonate and phenyl moieties permit cooperative electrostatic and hydrophobic binding. Study of the reactivity of PFA diesters with ACD derivatives yields information of use in drug design and demonstrates the application of ACDs as biomimetics, since ACDs are models of biomolecules containing cationic and amino groups.

The reactivity of three PFA diesters, **1**−**3**, was studied in aqueous solution, 5.6 ≤ pH ≤ 7.2, in the presence of eight ACD derivatives (Schemes 1 and 2).[10]

The simple phenyl ethyl PFA diester (**1**) underwent aminolysis by **αACD** (Scheme 2).[11] No reaction was observed with **βACD**, **γACD**, or native CDs. The reactivity patterns for **1** and the ethanolamine-ACDs were more complex. Reaction with **αeACD** gave both the hydrolysis product **4** (<10%) and the aminolysis product **6** (>90%) (Scheme 2). Reaction with **βeACD** was even more complex, giving in addition a transesterification product, **5**, from *O*-acylation of the ethanolamine tendril, which was the major product at higher **βeACD** concentrations (Scheme 2).[12] Masking of the tendril hydroxyls in α- and **βMeACD** resulted in complete hydrolysis and the absence of any ACD-acylation products.

Each ACD derivative showed saturation kinetics for breakdown of substrate (**1**) (Figure 1; Table 1).[13]

Scheme 2



PFA Derivatives:
1 R_1 = Ph, R_2 = Et ; **2** R_1 = Et, R_2 = Et; **3** R_1 = Ph, R_2 = $(CH_2)_2OH$

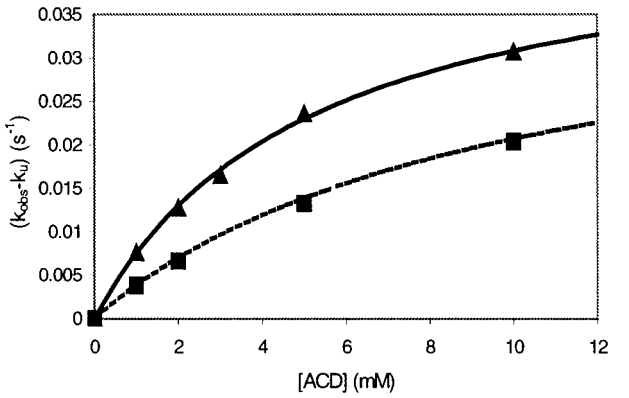


Figure 1. 1 Representative saturation curves from pseudo-first-order rate data (pH 5.6; see Table 1 for conditions; curves fitted with data in Table 1): **αeACD+1** (▲); **βeACD+1** (■).

Comparable K_m values in the millimolar range were obtained, suggestive of a similar mode of binding for each ACD (Table 1). The higher K_m obtained for **βeACD** is reflective of a high apparent K_m (>10 mM) for the trans-

Table 1. Kinetic Parameters for Reactions of **1**

ACD	pK_{a1}	pH	k_{cat} (s^{-1})[a]	K_m, mM	r_{acc}[b]	EM (M)[c]
αACD	7.4	5.6	2.74×10^{-3}	6.93	3.6×10^5	2.10×10^3
αACD		6.4	1.80×10^{-2}	6.19		
αACD		7.2	4.39×10^{-2}	4.71		
αeACD	6.3	6.4	4.68×10^{-2}	5.18	2.0×10^6	1.04×10^4
βeACD	6.4	6.4	3.91×10^{-2}	9.49	1.7×10^4	1.15×10^2
αMeACD	6.4	6.4	2.39×10^{-3}	4.64	1.4×10^2	0.63
βMeACD	6.4	6.4	4.94×10^{-4}	5.34	25	0.13

[a] 50 mM bis-Tris, 0.5 M KCl, 37 °C, 100 μM **1**, monitoring at 271 nm, [ACD]= 1−10 mM. k_{cat} and K_m from Eadie-Hofstee plots of $k_{obs} - k_{un}$ vs [ACD], where k_{un} is the background rate in bis-Tris buffer (5.2×10^{-6} s^{-1}, pH 5.6). [b] $r_{acc} = (k_{cat}/K_m)^H/k_B^{corr}$, where k^H_{cat} is the value obtained by fitting k_{cat} to pK_{a1}; $k_B^{corr} = k_B \times 10^{\Delta pK_a\beta}$, where both k_{cat} and the intermolecular aminolysis rates are corrected for an *N*-nucleophile of identical pK_a = pK_{a1}(ACD), using $\beta = \beta_{Nu} = 0.9$; k_B(OEM) **1** = 1.16×10^{-2} M^{-1} s^{-1} ; k_B(BTP) **1** = 1.74×10^{-2} M^{-1} s^{-1}; k_B(bis-Tris) **1** = 6.1×10^{-4} M^{-1} s^{-1}, k_B is the second-order rate constant obtained from plots of k_B/f_B at 7 < pH < 8.9. [c] EM = k^H_{cat}/k_B^{corr}.

esterification reaction, which suggests a binding mode with the substrate external to the ACD cavity for transesterification.

ACD aminolysis is significantly inhibited by polyvalent inhibitors such as α,ω-octanedioate compatible with involvement of the cavity in substrate binding.[14] Moreover, hydrophobic binding of the phenyl group in cooperation with electrostatic binding of the phosphonate moiety in the transition state is suggested to be important for aminolysis, because breakdown of the diethyl ester **2** by α**ACD** leads to catalysis of hydrolysis but not aminolysis.[15] Electrostatic and hydrophobic interactions have both been shown to be at play in binding of aryl phosphate anions to ACD receptors.[4]

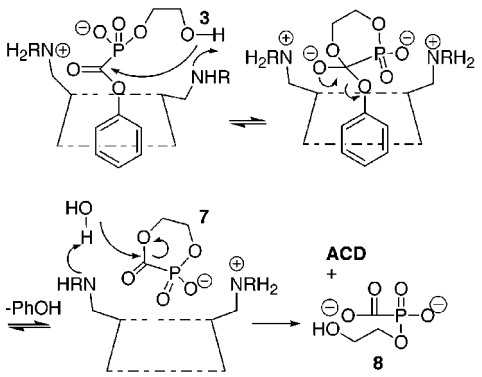
The pH−rate data for α**ACD** can be fit to the first pK_a of 7.4, giving second- and first-order rate constants for aminolysis by α**ACD·5H**$^+$ ((k_{cat}/K_m)^H = 30 M^{-1} s^{-1} and k^H_{cat} = 0.2 s^{-1}, respectively). A similar treatment may be applied to the other ACDs (Table 1). To assess the efficiency of ACDs for aminolysis, rate acceleration (r_{acc}) and effective molarity (EM) were benchmarked against the second-order rate constant for aminolysis of **1** in *O*-methyl-2-aminoethanol (OME) buffer. Quantitative comparison requires that the difference in pK_a, between the *N*-nucleophiles, ACD, and OME, be accounted for (using a treatment similar to that used for synzymes; for details see Table 1). The resulting rate accelerations, of a millionfold for aminolysis by the αACDs, are very large but not unreasonable for a pseudo-intramolecular reaction (Table 1). Kirby has pointed out that low EMs are a crucial failing of artificial catalysts designed as enzyme models, whereas intramolecular models, with high EMs, lack the enzymic preassociation step.[2] The ACDs display large EM values of up to 10^4, comparable to some rigid intramolecular enzyme models, but in addition ACD reactions require preassociation of substrate and host in aqueous solution.

EM and rate acceleration can be calculated for catalysis of hydrolysis by α**MeACD** and β**MeACD** benchmarking against buffer-catalyzed hydrolysis in 1,3-bis[tris(hydroxymethyl)methylamino]propane (BTP; Table 1). Similarly, estimates can be made for tranesterification by β**eACD** using the intermolecular transesterification reaction of **1** with bis-Tris buffer as a benchmark (Table 1).

Substrate **3** may undergo aminolysis, transesterification, or hydrolysis (Scheme 3), but in fact, in contrast to the reactions of **1**, hydrolysis is observed as the dominant reaction for **3** (>90% of reaction products). Also in contrast to the reactions of **1**, masking of the hydroxyls of the α**eACD** tendrils in α**MeACD** has no effect on reaction rates or products for **3**, compatible with hydrolysis resulting from intramolecular nucleophilic catalysis by the PFA-glycol group, rather than reaction with the N or O of the ethanolamine ACD-tendril. This is confirmed by reaction of **3** with equimolar ACD, a self-quenching reaction due to product inhibition, which yields both **7** and **8** as products. Thus the ACDs provide acid/base catalysis of transesterification and catalysis of subsequent hydrolysis, with cyclic ester **7** detected and characterized as the transesterification product and the hydrolysis reaction intermediate[16].

ACD catalysis of transesterification may reasonably be benchmarked against either the transesterification/cyclization

Scheme 3



(4) Vizitiu, D.; Thatcher, G. R. J. *J. Org. Chem.* In press.
(5) Eliseev, A. V.; Schneider, H. J. *J. Am. Chem. Soc.* **1994**, *116*, 6088.
(6) Three examples of β**ACD** catalysis have been reported in the literature, for H/D exchange [Binder, W. H.; Menger, F. M. *Tetrahedron Lett.* **1996**, 8963] and for imine formation [Tagaki, W.; Yano, K.; Yamanaka, K.; Yamamoto, H.; Miyasaki, T. *Tetrahedron Lett.* **1990**, 3897. Breslow, R.; Czarniecki, M. F.; Emert, J.; Hamaguchi, H. *J. Am. Chem. Soc.* **1980**, *102*, 2, 762].
(7) Oberg, B. *Pharmacol. Ther.* **1989**, *40*, 213.
(8) For example, see: (a) Noren, J. O.; Helgstrand, E.; Johansson, N. G.; Misiorny, A.; Stenning, G. *J. Med. Chem.* **1983**, *26*, 264. (b) Gorin, B. I.; Ferguson, C. G.; Thatcher, G. R. J. *Tetrahedron Lett.* **1997**, 2791. (c) Briggs, A. D.; Camplo, M.; Freeman, S.; Lundstromm, J.; Pring, B. *Tetrahedron* **1996**, *52*, 14937, and references therein.
(9) (a) Krol, E. S.; Thatcher G. R. J. *J. Chem. Soc., Perkin Trans. 2* **1993**, 793. (b) Krol, E. S.; Davis, J. M.; Thatcher, G. R. J. *J. Chem. Soc., Chem. Commun.* **1991**, 118. (c) Mitchell, A. G.; Nichols; D. Irwin, W. J.; Freeman, S. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1145. (d) Thatcher G. R. J.; Krol, E. S.; Cameron D. R. *J. Chem. Soc., Perkin Trans. 2* **1994**, 683.
(10) All ACDs homogeneous and fully characterized: (a) Gorin, B. I.; Riopelle, R. J.; Thatcher, G. R. J. *Tetrahedron Lett.* **1996**, 4647. (b) Vizitiu, D.; Walkinshaw, C. S.; Gorin, B. I.; Thatcher, G. R. J. *J. Org. Chem.* **1997**, *62*, 8760.
(11) The aminolysis product shows a ^{31}P NMR shift similar to other PFA amides (δ ~0.7 ppm) [Ferguson, C. G.; Thatcher, G. R. J. *Synlett* **1998**, 1325], is differentiated from the hydrolysis product (**4**; δ ~1.7 ppm), and has an ES-MS spectrum consistent with a monoacylated ACD ((M + 1)$^+$ = 1103.4 Da/e).
(12) By ^{31}P NMR: the hydrolysis and aminolysis products were formed in almost equivalent amounts while the ester product was the major product, the relative yield of which increased with the proportion of ACD to substrate. The assignment of the two different ACD−PFA adducts was based upon their chemical shift proximity to known PFA amides and alkyl diesters and ES-MS results.
(13) UV kinetics were carried out using a Beckman DU7400 diode array spectrophotometer equipped with a thermostated cell holder. Stock solutions of **1** and **3** were prepared in MeOH at 10 mM. Unless otherwise specified, all reactions were carried out in 50 mM bis-Tris buffer, with 500 mM KCl to maintain constant ionic strength, at the appropriate pH where indicated. All measurements were performed at 37.0 ± 0.1 °C. Reactions were initiated by adding 10 μL of a substrate solution to 1 mL of the buffer solution (which was incubated at 37 °C for approximately 10 min) followed by brief shaking. Final substrate concentration was 100 μM while [ACD] = 1−10 mM. All reactions were monitored at 271 nm for at least 3 half-lives. First-order rate constants (k_{obs}) were calculated by software on the spectrometer.
(14) At concentrations equimolar with ACD (2 mM), partial inhibition (20%) of the aminolysis reaction between **1** and α**ACD** was observed with traditional cavity binding inhibitors (e.g. heptanediol, see: Tee, O. S.; Donga, R. A. *J. Chem. Soc. Perkin Trans. 2* **1996**, 2763), as expected from the weak binding of hydrophobic molecules in the ACD cavity. Greater inhibition (50%) was observed with the polyvalent binding inhibitors, α,ω-nonanedioate and α,ω-octanedioate.
(15) Hydrolysis of **2**, at pH 7.0, is catalyzed by α**ACD** (5 mM) at a rate ($t_{1/2}$ = 66 min) 3.5 × 10^3 fold greater than the background aminolysis in Tris buffer (Tris, 500 mM; KCl, 500 mM, 25 °C).

observed for **3** in bis-Tris buffer or the transesterification of **1** in bis-Tris (Table 2). The resulting rate enhancements of

Table 2. Kinetic Parameters for Reaction of **3**

ACD	k_{cat} (s^{-1})[a]	K_m (mM)	k_{cat}/K_m (M^{-1} s^{-1})	r_{acc}[b]	EM (M)[c]	EM_c (M)[d]
αACD	2.07×10^{-2}	8.15	25.1	2.0×10^{3}	16	350
αeACD	9.63×10^{-3}	5.01	2.00	8.0×10^{2}	4.0	91
αMeACD	1.13×10^{-2}	3.94	28.6	1.3×10^{4}	5.1	110

[a] 50 mM bis-Tris, 0.5 M KCl, pH 5.8, 37 °C, 100 μM **3**, monitoring at 271 nm; [ACD]= 1−10 mM. k_{cat} and K_m from Eadie-Hofstee plots of $k_{obs} - k_{un}$ vs [ACD], where k_{un} is the background rate in bis-Tris buffer = 8.53×10^{-4} s^{-1}. [b−d] See Table 1 for definitions; $\beta = 0.7$. (*b* and *c*) k_B(bis-Tris) **3** = 1.28×10^{-2} M^{-1} s^{-1}. (*d*) k_B(bis-Tris) **1** = 6.1×10^{-4} M^{-1} s^{-1}.

up to 10^4 and EM values of 4−350 are at the higher end of the range observed for general acid/base catalysis in intramolecular models and are large for a system that provides preassociation of substrate in aqueous solution.[17]

Catalysis of the hydrolysis of esters **1** and **3** by ACDs represents clear and simple mimicry of the action of serine protease enzymes, the combination of ACD and the PFA ester providing (1) hydrophobic and electrostatic binding of the substrate, (2) a nucleophilic hydroxyl activated by a nitrogen base, and (3) electrostatic complementarity to the anionic tetrahedral transition state. The efficiency of ACDs in accelerating and catalyzing the reactions of PFA esters demonstrates their potential as enzyme models and biomimetics and, furthermore, yields important information on PFA reactivity and drug design.

Simple PFA esters, related to compounds **1**−**3**, designed as Foscarnet prodrugs, have shown antiviral activity equivalent to Foscarnet.[8] Since there are many protein sites, rich in lysine residues, mimicked by ACD,[18] it can be predicted that (i) de-esterification of the *C*-ester group in vivo is unlikely to be a problem in release of Foscarnet from prodrugs and (ii) covalent modification of biomolecules by reaction at the carbonyl C of PFA diesters is quite possible. Thus, targeting of enzyme inhibitors containing the PFA moiety to active site enzyme nucleophiles represents a novel strategy for design of new therapeutic agents and biological probes.

Acknowledgment. NSERC Canada is thanked for financial support.

OL9906211

(16) Compound **7** was identified by spiking with an authentic sample (^{31}P NMR δ ~−7.8 ppm): Ferguson, C. G.; Thatcher, G. R. J. *Synlett* **1998**, 1325.

(17) Kirby has reported high EM values for amine catalysis of proton transfer in the Kemp elimination by a synzyme (Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *J. Am. Chem. Soc.* **1997** *119*, 9578) and lower values for catalysis by serum albumins [ref 18a].

(18) For example, serum albumins and glycosaminoglycan sulfate binding domains: (a) Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *Nature* **1996**, *383*, 60. Carter, D. C.; Ho, J. X. *Adv. Prot. Chem.* **1994**, *45*, 153. (b) Borrajo, A. M. P.; Gorin, B. I.; Dostaler, S. M.; Riopelle, R. J.; Thatcher, G. R. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1185.