Multiple Pathways in Cyclodextrin-Catalyzed Hydrolysis of Perfluoroalkylamides[1]

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Abstract: The hydrolysis of *p*-nitroanilide of perfluoroalkanoic acids, $CF_3(CF_2)_nCO^-$, with n = 1, 2, 3, 5, 6, and 7, **1a−f**, was studied in the presence of β-cyclodextrin (CD). All reactions were catalyzed by CD through the formation of a 1:1 and 1:2 inclusion complexes. The association equilibrium constants for the 1:1 complexes were dependent on the number of carbons of the fluoroalkyl chain, whereas those of the 1:2 complexes were almost independent. These results indicate that, in the former case, the perfluoroalkyl chain is included, while in the latter, the CD unit encloses the aryl ring. For compounds **1a,b** both complexes were more reactive than the substrate itself. The ratio of the reaction of complexed to uncomplexed substrate had its highest value for **1a** in the case of the 1:1 complex, and for **1b**, the 1:2 complex. This is attributed to the geometry of the complexes. Although compounds **1c−e** reacted at the same rates in the free or 1:1 complexed form, CD accelerated the reactions because of an increase of the pK_a of the substrate, which results in a higher concentration of the neutral reactive substrate at the same pH. Compound **1f** formed aggregates even at 10^{-6} M concentration, and CD-induced deaggregation resulted in catalysis of the reaction.

Introduction

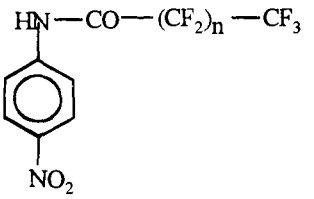
Cyclodextrins are cyclic oligomers of α-D-glucose which are produced by enzymatic hydrolysis of starch. Compounds with 6, 7, and 8 glucose units are called α-, β-, and γ-cyclodextrin.[2] These compounds have been used as enzyme models for proteases and have proved to be good catalysts for the hydrolysis of esters.[3] On the other hand, the hydrolysis of amides has been little studied in the presence of cyclodextrins.[4−6]

The effect of cyclodextrins on the hydrolysis of *p*-nitrophenyl alkanoates of different chain length has been studied in several laboratories,[7−9] and it was found that the kinetic parameters varied, as the acyl chain length changed, consistent with the change in the mode of binding of the ester from aryl to alkyl group inclusion.[9]

The hydrolysis of *m*-nitrotrifluoroacetanilide and trifluoroacetanilide is inhibited by β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HPCD), whereas the reaction of *p*-nitrotrifluoroacetanilide is catalyzed.[10] This contrasting behavior of amides and esters is attributed to different rate-limiting steps in the mechanism of product formation of the catalyzed pathway, i.e., in the hydrolysis of esters the nucleophilic attack of CD on the carbonyl carbon of the ester is the rate-determining step, whereas in the hydrolysis of amides leaving of the amine from the tetrahedral intermediate is partially rate-determining.

In the reactions of *p*-nitrotrifluoroacetanilide in the presence of β-CD, complexes of 1:1 and 1:2 stoichiometry were formed, and both reacted faster than the substrate itself, but it was not possible to determine whether the 1:1 complex involves trifluoromethyl or *p*-nitrophenyl group inclusion. We considered it of interest to study the effect of changing the alkyl chain on the hydrolysis of amides in the hope that the analysis of the change in the kinetic parameters with the length of the alkyl chain might shed some light onto the nature of the complexes involved in the reactions. Also, the reactivity of perfluorinated compounds has great intrinsic interest due to the increasing use of these compounds in medicine[11] and other areas of technological interest.[12]

We report here results on the hydrolysis of compound **1** in the presence of cyclodextrins. These reactions show some special features derived from the high hydrophobicity of the perfluoroalkyl chain.[13]



1a (n = 1), **1b** (n = 2), **1c** (n = 3), **1d** (n = 5), **1e** (n = 6), **1f** (n = 7).

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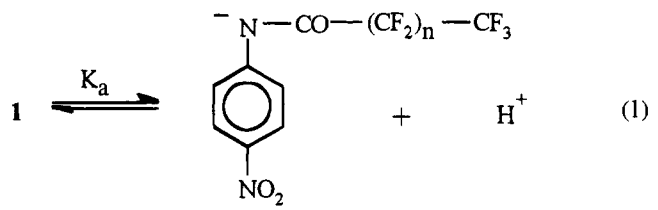
Table 1. Calculated Rates and Equilibrium Constants for the Hydrolysis of **1** at 25 °C

substrate	PNT[b]	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**3**
$K_{S1} \times 10^{-3}$ M^{-1}	0.100	2.9 ± 0.1[c]	3.2 ± 0.1[c]	1.6 ± 0.1[c]	63 ± 1[d]	100 ± 10[e]	84 ± 9[n] 24 ± 3[o]	
K_{S2}, M^{-1}	16 ± 6	41 ± 5[c]	86 ± 6[c]	23 ± 3[c]	100 ± 10[d]	20		
k_0, M^{-1} s^{-1}	340 ± 10[f]	276 ± 8[f]	156 ± 7[f]	136 ± 7[f]	130 ± 6[f]	134 ± 7[f,g]		
$K_{SH1} \times 10^{-3}$, M^{-1}	0.07 ± 0.004	0.47 ± 0.04[h]	2.0 ± 0.1[h]	6.2 ± 0.1[i]	180 ± 10[j]	240 ± 10[k]		1.0 ± 0.1[e]
K_{SH2}, M^{-1}	60 ± 10	182 ± 30[n]	49 ± 8[n]	30 ± 7[i]	100	20		90 ± 30[e]
$k_1 \times 10^{-2}$ M^{-1} s^{-1}	10	18.8 ± 1.8[l]	3.4 ± 0.4[l]	1.00 ± 0.06[l]	1.37 ± 0.13[l]	1.34		
$k_2 \times 10^{-2}$, M^{-1} s^{-1}	9	6.8 ± 0.6[l]	16 ± 4[l]	3.0 ± 0.2[l]	2.4 ± 0.5[l]	4.3 ± 0.1[m]		
k_1/k_0	2.9	6.8	2.2	0.76	1.0	1.0		
k_2/k_0	2.6	2.5	7.0	2.2	1.9	3.2		

[a] Solvent is water with less than 2% acetonitrile. [b] Data from ref 10, PNT = *p*-nitrofluoroacetanilide. [c] Determined spectrophotometrically from eq 5, $[S]_0 = 5 \times 10^{-5}$ M, pH = 10. [d] Idem note *c*, but $[S]_0 = 10^{-5}$ M. [e] Idem note *c*, but $[S]_0 = 3 \times 10^{-6}$ M. [f] Second-order rate constant in the solution without CD. [g] $[S]_0 = 3 \times 10^{-6}$ M. [h] Determined spectrophotometrically, $[S]_0 = 5 \times 10^{-5}$ M, pH = 3, using eq 5. [i] Idem note *h*, $[S]_0 = 7.9 \times 10^{-6}$ M. [j] Obtained from the change in pK_a in the presence of CD. [k] Obtained from the fit to eq 12. [l] Obtained from the fit to eq 11. [m] Obtained from eq 13. [n] Apparent equilibrium constant $[S]_0 = 3 \times 10^{-6}$ M. [o] Apparent equilibrium constant $[S]_0 = 2 \times 10^{-5}$ M.

Results

The UV−visible spectrum of compounds **1a−d** at 2×10^{-5} and **1e** at 3×10^{-6} M concentrations shows maximum absorptions at 334 nm at pH = 10 and at 304 nm at pH = 3. This behavior is similar to that of *p*-nitrotrifluoroacetanilide,[14] indicating that the substrate ionizes according to eq 1. The species with $\lambda_{max} = 334$ nm is identified as the anion **2**, while **1** has $\lambda_{max} = 304$ nm.



(1)

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The pK_a of **1a** and **1c** was determined as 7.7 and 7.6, respectively, from the change in absorption with the pH in the range from 6.1 to 8.9. On the other hand, **1f** at pH = 10 and 2×10^{-6} M substrate concentration shows a maximum absorption at 271 nm. Besides, the spectrum of **1f** is almost the same in pure water (pH ≈ 6) and in water containing 0.5 M NaCl. A similar spectrum is obtained with **1e** at 1×10^{-5} M or higher concentration. With **1e** at 2×10^{-5} M there is a change in the spectrum from $\lambda_{max} = 271$ nm at pH 8.5 to $\lambda_{max} = 334$ at pH 11.9. From the change in absorbance with pH at 334 nm the apparent p$K_a = 10.5 \pm 0.1$ was determined.

The hydrolysis of all the substrates produces *p*-nitroaniline, and the observed rate constant was measured at pH = 10 (Table S1).[15] Since it is known that the anion of *p*-nitrotrifluoroacetanilide is unreactive[14] it is likely that the ions derived from **1** are also unreactive, therefore, the value of k_{obs} must be corrected for ionization according to eq 2.

$$k_{corr} = k_{obs}\frac{K_a + [H^+]}{[H^+]} \qquad (2)$$

The observed rate constants for the reactions of **1a−d** are independent of the substrate concentration in the range 3×10^{-6} to 2×10^{-5} M. In the case of **1e**, it decreases from 5.1×10^{-5} to 6.5×10^{-6} s^{-1} when the substrate concentration increases from 3×10^{-6} to 2×10^{-5} M (Table S2).[15] The rates of the reactions of **1f** at 2×10^{-6} and 3×10^{-6} M are the same within experimental error, and the pseudo-first-order rate plots are linear up to 80 and 60%, respectively, despite the fact that the spectrum of the substrate indicates that the substrate is aggregated at this concentration.

Effect of Cyclodextrin on the Spectrum. The spectrum of **1a−d** at pH 10 shows a shift to longer wavelengths upon addition of CD. The spectra were recorded with the substrate concentration at 4.5×10^{-5} M for **1a−c**, 2×10^{-5} M for **1d**, and CD in the range 0.02−10 mM; no isosbestic point is observed. These results were interpreted in terms of the formation of inclusion complexes (see below) of 1:1 and 1:2 stoichiometry (substrate:cyclodextrin), eqs 3 and 4 (S represents substrate **1** or its anion **2**).

$$S + CD \overset{K_1}{\rightleftharpoons} S{\cdot}CD \qquad (3)$$

$$S{\cdot}CD + CD \overset{K_2}{\rightleftharpoons} S{\cdot}(CD)_2 \qquad (4)$$

The absorbance data (A) at the wavelength of maximum difference with that of the substrate in water (A_0), namely, 380 nm for **1a−c** and 400 nm for **1d**, were fitted[16] with eq 5, and the equilibrium constants for the two types of complexes were obtained.[17]

$$A = A_0 + \frac{(\Delta\epsilon_{11}K_1[CD] + \Delta\epsilon_{12}K_1K_2[CD]^2)[\mathbf{1}]_0}{1 + K_1[CD] + K_1K_2[CD]^2} \qquad (5)$$

In eq 5 $\Delta\epsilon_{11}$ and $\Delta\epsilon_{12}$ are the differences between the molar extinction coefficients of the complexes of 1:1 and 1:2 stoichiometry and the substrate. K_1 and K_2 are the corresponding equilibrium constants for the formation of the two types of complexes, and CD represents the free CD concentration which is considered to be equal to the stoichiometric concentration when $[CD]_0 > 10[\mathbf{1}]_0$ or is calculated as indicated in the Appendix. Since the pK_a of **1** is below 8, the values of K_1 and

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(15) Supplementary material.
(16) Nonlinear adjustment was carried out using the following: *Sigmaplot*, version 6.1; Jandel Corporation: 1993.
(17) Equation 5 is derived assuming that the system can be described as a mixture of independently absorbing species. In this case the optical density of the solution (A) is the sum of the contribution by the free molecules and the two inclusion complexes

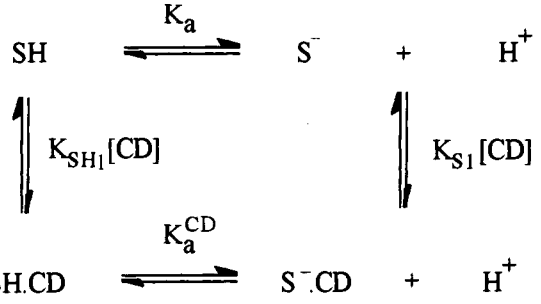
$$A = \epsilon_s[S] + \epsilon_{11}[S{\cdot}CD] + \epsilon_{12}[S{\cdot}(CD)_2]$$

The concentrations of S, S·CD, and S·$(CD)_2$ are related to the added concentrations, $[S]_0$ and $[CD]_0$, of guest and host, respectively, by the equilibrium constants

$$K_1 = \frac{[S{\cdot}CD]}{[S][CD]} \qquad K_2 = \frac{[S{\cdot}(CD)_2]}{[S{\cdot}CD][CD]}$$

and the initial condition that the sum of the respective concentrations is constant.

Scheme 1



K_2 determined at pH = 10 represent the association constant of the anion **2** with CD, K_{S1}, and K_{S2} (Table 1).

At pH = 3 where the substrate must be in its neutral form, the spectrum also changes with the addition of CD, and there is no isosbestic point. The measured absorbances were fitted with eq 5, and the values of the equilibrium constants $K_{SH1} = K_1$ and $K_{SH2} = K_2$ were calculated (Table 1) for compounds **1a–c.**

Compounds **1d–f** are quite insoluble in acid media, so it was not possible to determine K_{SH1} and K_{SH2} as we did for the shorter chain compounds. To determine the value of K_{SH1} for **1d** we measured the change in absorption with pH at 334 nm in a solution containing 2×10^{-5} M **1d** and 8×10^{-4} M CD. The inflection point of the plot of absorption vs pH occurs at pH = 8.13 ± 0.04. Considering the thermodynamic cycle shown in Scheme 1 eq 6 follows.

$$K_a K_{S1} = K_{SH1} K_a^{CD} \quad (6)$$

Since there is a small decrease in pK_a when the value for **1a** is compared with that of trifluoroacetanilide, but the pK_a values of **1a** and **1c** are about the same, it is likely that an additional CF_2 group does not change the pK_a significantly. Using pK_a = 7.6, the value of pK_a^{CD} = 8.13 determined in the presence of CD, and K_{S1} determined using eq 5, the K_{SH1} can be obtained from eq 6. The pK_a of **1d** was also determined in the presence of 0.01 M CD and gave the same value as that obtained at lower CD concentration. Raising the CD concentrations increases the fraction of 1:2 complex which should result in different pK_a^{CD} if K_{S2} is different from K_{SH2}. The fact that the pK_a^{CD} does not change indicates that $K_{SH2} \approx K_{S2}$.

The maximum absorption of **1e** at 2×10^{-5} M with CD from 0.5×10^{-5} to 7×10^{-5} M changes from 271 to 334 nm (Figure 1), and there is an isosbestic point at 310 nm. At higher concentrations of CD, up to 0.9×10^{-2} M, the wavelength maximum changes to 350 nm.

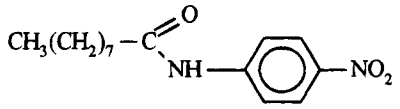
It is important to note that, in solutions of **1e** at 2×10^{-5} M or **1f** at 3×10^{-6} M, the addition of α- or γ-cyclodextrin or lactose (1.6×10^{-4}, 1.8×10^{-4}, and 5×10^{-4} M, respectively) does not produce any change in the spectrum. On the other hand, hydroxylpropyl-β-cyclodextrin (HPCD) produces changes similar to those of CD at the same concentration.

The solutions of **1e** and **1f** with CD > 8×10^{-4} M become cloudy with time, therefore the equilibrium constants were determined using lower CD concentrations. Under these conditions the square term in eq 5 is very small so only the values for the complex of 1:1 stoichiometry could be determined. Also, solubility reasons preclude measurements in acid solutions.

Since **1f** is aggregated even at 3×10^{-6} M, the measured equilibrium constant corresponds to eq 7, and it is not directly comparable with the binding constants for the other substrates.

$$SH_x + xCD \rightleftharpoons xS{\cdot}CD + H^+ \quad (7)$$

To compare the behavior of the compound with the longest perfluorinated alkyl chain studied, **1f**, with the hydrocarbonated analog, compound 3, was prepared. This compound had λ_{max}



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at 317 nm, and its spectrum does not change when the concentration increases from 3×10^{-6} to 2×10^{-5} M; however, the absorbance does not follow the Lambert–Beer law, indicating some degree of association. On the other hand, in the presence of a 10 mM CD concentration, the change in absorbance is linearly correlated with concentration. The spectrum of **3** at 3×10^{-6} M shows a small bathochromic shift when CD is added, and there is an increase of the intensity of absorption. The absorbance values at 317 nm were fitted by eq 5, and K_1 and K_2 were calculated as 10^3 and 90 M^{-1}, respectively.

Effect of CD on the Rate. The hydrolysis rate of all the substrates increases upon addition of cyclodextrin in a nonlinear fashion (Figure 2 is representative). The relative increase in rate, i.e., the ratio of the rate constant at the maximum concentration of CD to that without CD, increases as the length of the perfluoroalkyl chain increases, from example k_{corr}(CD=8mM)/k_{corr}(CD=0) is 1.27, 2.25, and 3.78 for **1a**, **1b**, and **1c**, respectively (Table S1).[15] For substrate **1f**, the addition of 3×10^{-5} M CD to a solution of the substrate 3×10^{-6} M changes the observed rate constant from 0.68×10^{-5} s^{-1} to 7.5×10^{-5} s^{-1}. Also, changing the concentration of the substrate under these conditions does not produce any change in rates.

Discussion

The hydrolysis of *p*-nitrotrifluoroacetanilide takes place with the formation of a tetrahedral intermediate, and, at pH 10, its formation is rate-determining.[18,19] Since it is not likely that the relative rate of formation and decomposition of the intermediate changes with the chain length, the rates measured for **1** must represent the rate of formation of the intermediate.

The changes in the spectrum of **1** with the addition of cyclodextrin indicate that there is some sort of interaction between CD and the substrates. This interaction is probably the formation of inclusion complexes and not just an unspecific medium effect as evidenced by the fact that α- and γ-cyclodextrin[20] or a linear sugar do not produce changes in the spectrum of **1**. In the inclusion of ligands by cyclodextrins in solution, a major factor in the stability of the resulting complex is thought to be the closeness of fit of the guest molecule within the CD cavity. Thus, for example, the binding constant values of adamantane carboxylate are 10^5 M^{-1} in β-CD but only 10^2 M^{-1} in α-CD and 10^3 M^{-1} in γ-CD,[21] and those of sodium perfluorooctanoate with γ-, β-, and α-CD are 640, 4500, and essentially zero.[22]

It can be seen in Table 1 that the association constant for the 1:1 complex increases as the chain length increases for the neutral and anionic substrate as well. A reasonable good

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(19) Huffman, R. W. *J. Org. Chem.* **1980**, *45*, 5004.
(20) α-, β-, and γ-cyclodextrins have six, seven, and eight glucose units joined in a torus. The sizes of their cavities differ in width, but not in depth.
(21) Cromwell, C.; Bystrom, K.; Eftink, M. R. *J. Phys. Chem.* **1985**, *89*, 326.
(22) Palepu, R.; Reinsborough, V. C. *Can. J. Chem.* **1989**, *67*, 1550.

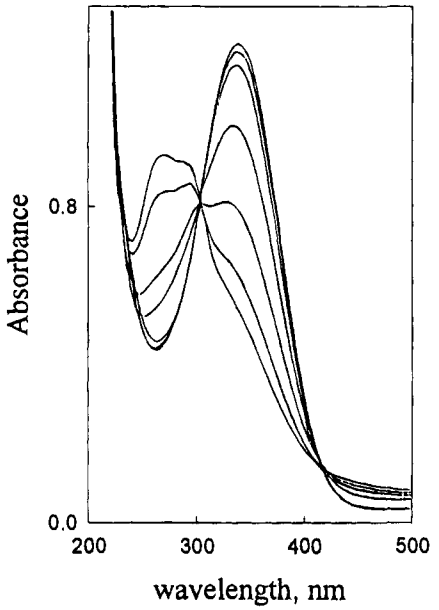


Figure 1. Spectrum of **1e** as a function of CD concentration. [CD], M = 0 (λ_{max} = 270 nm), 5.2 × 10^{-6}, 9.4 × 10^{-6}, 2.6 × 10^{-5}, 3.5 × 10^{-5}, 5.3 × 10^{-5}, and 7 × 10^{-5} (λ_{max} = 334 nm).

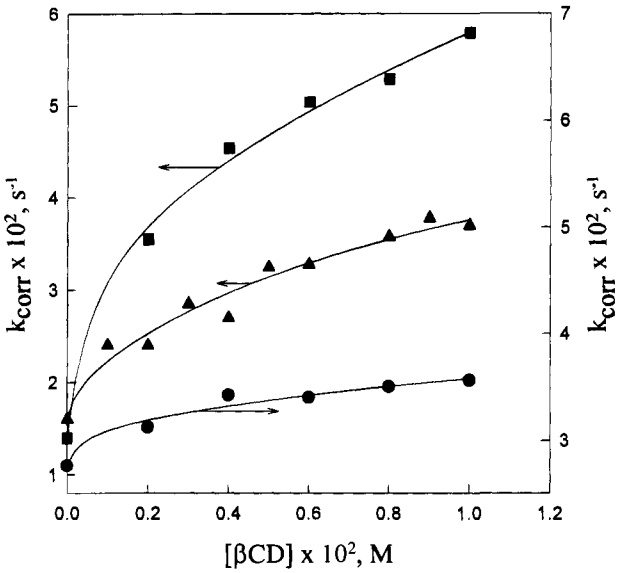


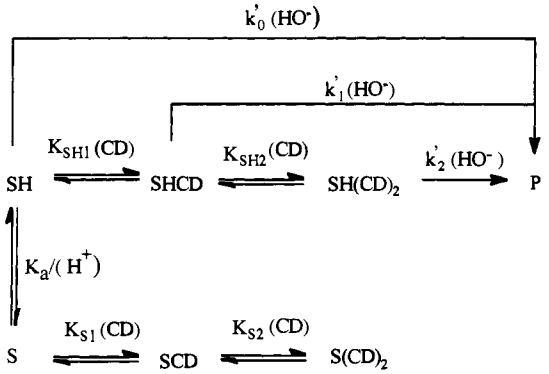
Figure 2. k_{corr} vs [CD]: ●, **1a** (right ordinate); ▲, **1b**; ■, **1c** (left ordinate).

correlation of the form of eq 8 is obtained using the equilibrium constants for the neutral substrate. For the anions **2**, the correlation is poorer, but it is evident that there is less sensitivity of the association constant with the number of carbons in the perfluoroalkyl chain, *N*.

$$\log K = 1.91 + 0.66N \qquad r^2 = 0.987 \qquad (8)$$

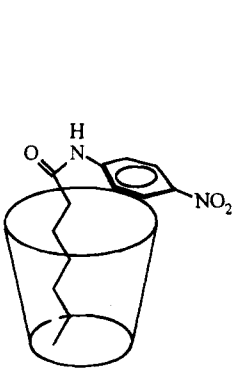
The binding of organic substrates to cyclodextrins is largely determined by their hydrophobicity and size.[23,24] For linear aliphatic molecules, both of these properties increase linearly with the number of carbons in the alkyl chain.[25] The association equilibrium constant of *p*-nitrophenyl esters of *n*-alkanoic acids with β-CD correlate with the number of carbon atoms in the alkyl chain with slope of 0.194 (≡ 0.266 kcal/mol) and that of

Scheme 2



acetate of *p*-alkyl phenol with a slope of 0.371 (≡ 0.509 kcal/mol).[9] The free energy for association of alkyl sulfonates with CD also correlates with the number of carbons in the alkyl chain, and there is a decrease in 0.64 kcal/mol for each methylene group.[26] These values indicate that the nature of the moiety bonded to the alkyl chain is also important in determining the degree of dependence of *K* with the number of atoms in the alkyl chain. The free-energy difference of transferring one mole of CH_2 groups from an aqueous environment to a micellar aggregate is estimated as 0.639 kcal/mol for ordinary surfactants and 0.946 kcal/mol for CF_2.[27] Considering that the correlation of log *K* ($2.303\Delta G°/RT$) with *N* is a linear free-energy relationship, the value of 0.66 (≡ 0.808 kcal/mol) for the slope appears to be in agreement with the results mentioned above. The driving force for inclusion to form the 1:1 complex is largely determined by a favorable interaction of the highly hydrophobic perfluoroalkyl chain. This is evident from the values of the equilibrium constants for **3** and **1f**. The comparison of the association constant of sodium perfluorooctanoate, namely, 4.5 × 10^3 M^{-1}, with that of substrate **1e**, 2.41 × 10^5 M^{-1}, which has the same chain, indicates that there must be additional interactions with the aryl head group which favor complexation. Probably, the phenyl ring of the guest acts as a cap for the cone-shaped cavity of β-CD making the microenvironment more hydrophobic.[28,29]

The equilibrium constant for the complex of 1:2 stoichiometry is nearly independent of the alkyl chain length. This result indicates that the 1:1 complex has the structure indicated in **4**, while the 1:2 complex can be represented as in **5**.



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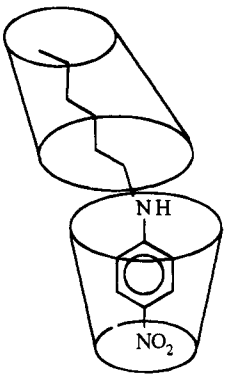

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Considering the formation of the two types of complexes,

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(24) Schneider, H.-J.; Kramer, R.; Simova, S.; Schneider, V. *J. Am. Chem. Soc.* **1988**, *110*, 6442.
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(29) (a) Breslow, R.; Zhang, B. *J. Am. Chem. Soc.* **1992**, *114*, 5882–5883. (b) Tong, L.-H.; Hou, Z.-J.; Inoue, Y.; Tai, A. *J. Chem. Soc., Perkin Trans 2* **1992**, 1253–1257.

the mechanism of hydrolysis can be represented as in Scheme 2. The observed rate constant for this mechanism is given by eq 9.

$$k_{obs} = [k_0 + K_{SH1}k_1[CD] + K_{SH1}K_{SH2}k_2[CD]^2] \Big/ \left[1 + \frac{K_a}{H^+}(1 + K_{S1}[CD] + K_{S1}K_{S2}[CD]^2) + K_{SH1}[CD] + K_{SH1}K_{SH2}[CD]^2\right] \quad (9)$$

$$k_0 = k_0'[HO^-]$$

$$k_1 = k_1'[HO^-]$$

$$k_2 = k_2'[HO^-]$$

Correcting k_{obs} (eq 9) as in eq 2 leads to eq 10.

$$k_{corr} = [k_0 + K_{SH1}k_1[CD] + K_{SH1}K_{SH2}k_2[CD]^2] / [F + (1 - F)(1 + K_{S1}[CD] + K_{S1}K_{S2}[CD]^2) + F(K_{SH1}[CD] + K_{SH1}K_{SH2}[CD]^2)] \quad (10)$$

$$F = \frac{H^+}{K_a + H^+}$$

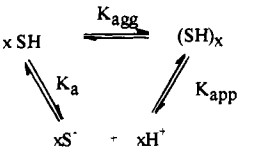
Since all the reactions were studied at pH = 10, $F \ll 1$ and eq 10 simplifies to eq 11.

$$k_{corr} = \frac{k_0 + K_{SH1}k_1[CD] + K_{SH1}K_{SH2}k_2[CD]^2}{1 + K_{S1}[CD] + K_{S1}K_{S2}[CD]^2} \quad (11)$$

All the equilibrium constants values of eq 11 for **1a–c** were determined spectrophotometrically (see Results). Using these values and nonlinear fitting of the data to eq 11, k_2 and k_1 were obtained, and they are collected in Table 1. It can be seen that the 1:1 complex reacts faster than the free substrate only in the members of the series of shorter chain length (up to three carbon atoms). The ratio k_1/k_0 has the higher value for **1a**, and then it decreases until it reaches 1. On the other hand, the ratio k_2/k_0 is about 2–3 for all the compounds, only for **1b** it is 10. The fact that the 1:1 inclusion complex of **1a** and the 1:2 complex of **1b** shows the highest reactivity in the series probably reflects the most favorable geometry of these complexes for the reaction. The length of **1a** from the carbonyl carbon to the terminal trifluoromethyl group is 3.56 Å and that of **1b** is 4.88 Å, the distance of the carbonyl carbon to the nitro group is 7 Å.[30] Although the ratio k_1/k_0 is approximately 1 for **1d**, the reactions of these compounds are faster in the presence of CD (Table S1)[15] due to an increase in the fraction of neutral substrate since the pK_a of the complexed substrate is higher than that of the substrate. Similar results are obtained with **1e** and **1f**.

The change observed in the spectrum of **1e** and **1f** with concentration as well as the rate dependence with the concentration indicates that these substrates are aggregated.[31] It is remarkable that the self-association of **1e** and **1f** should occur at such low concentrations. Considering that the cmc (critical micellar concentration) of *n*-$C_7F_{15}COOH$ is 9×10^{-3} M and that of *n*-$C_7F_{15}COONa$ is 3.6×10^{-2} M,[27] it follows that the change of an OH group to a *p*-nitrophenylamino group increases the tendency to aggregation by more than two orders of magnitude. These results are in agreement with the well-known importance of the hydrophilic group in amphiphilic compounds for determining the cmc.[32] Besides, similar results are observed with hydrocarbon analogs: for example, the cmc of $C_{12}H_{25}$-COOK is 12.5 mM,[33] whereas *p*-nitrophenyl dodecanoate is aggregated already at 4×10^{-6} M[34]. Comparison of the spectrum of the aggregated species with that of the monomer in acidic and basic solution indicates that the aggregate is formed mainly by the neutral species. The amount of deprotonated substrate increases with the pH of the solution, and it is not aggregated at 2×10^{-5} M concentration. The effect of replacing a proton by a sodium ion is similar to that observed with carboxylic acid and the salt (*vide supra*).

Scheme 3



The effect of the pH change on the aggregation equilibrium can be represented as in Scheme 3. The value of pK_{app} for **1e** was determined as 10.5 at 2×10^{-5} M, and the value of pK_a can be estimated to be the same as that of the shorter member of the series, namely, 7.6. These differences are the consequence of the higher thermodynamic stability of the aggregate when compared with the free substrate.

The aggregation number is probably very small since the association appears to be complete at concentrations as low as 10^{-5} M. A value of 7 was determined for the aggregation number of sodium perfluorooctanoate at 0.5 M[35] and it is known that the aggregation number is dependent on the concentration.[36]

The value of the rate constant for the hydrolysis of **1e** changes approximately 10 times when the concentration of the substrate increases from 3×10^{-6} M, where it is mainly a monomer, to 2×10^{-5} M, where it is mainly in the aggregated form. The latter is the highest possible concentration in solution. When the concentration of the substrate and CD are both 2×10^{-5} M, the observed rate constant is the same as that of the free substrate. The change in rate under these conditions is attributed to the deaggregation of the substrate as a consequence of the formation of an inclusion complex with CD. The increase in critical micellar concentration of surfactants in the presence of CD is known,[37] as is the effect of CD on the aggregation of dyes.[38] When the concentration of CD increases from 2×10^{-5} to 10^{-3} M, the rate of hydrolysis of **1e** increases and shows a saturation effect. At this low CD concentration only the 1:1 complex was expected to be formed and, considering that $k_0 \approx k_1$, eq 11 simplifies to eq 12. From this equation and the k_{corr}

$$k_{corr} = \frac{k_0(1 + K_{SH1}[CD])}{1 + K_{S1}[CD]} \quad (12)$$

values at different CD concentration was calculated K_{SH1}. The effect of CD on the rate of hydrolysis of this substrate is also

(30) These values were calculated using PCM, Serena Software.
(31) Jiang, X.-K. *Acc. Chem. Res.* **1988**, *21*, 362.
(32) Myers, D. *Surfactants Science and Technology*, 2nd ed.; VCH Publishers: 1992; p 114.
(33) Reference 32, p 115.
(34) Jiang, X.-K.; Xing-Ya, L.; Huang, B.-Z. *Proc. Indian Acad. Sci., Chem. Sci.* **1987**, *98*, 408.
(35) Turro, N. J.; Lee, C. C. P. *J. Phys. Chem.* **1982**, *86*, 3367.
(36) Berr, S. S.; Jones, R. R. M. *J. Phys. Chem.* **1989**, *93*, 2558.
(37) Funasaki, N.; Yodo, H.; Hada, S.; Neya, S. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 1323.
(38) Herkstroeter, W. G.; Martic, P. A.; Farid, S. *J. Am. Chem. Soc.* **1990**, *112*, 3583–3589.

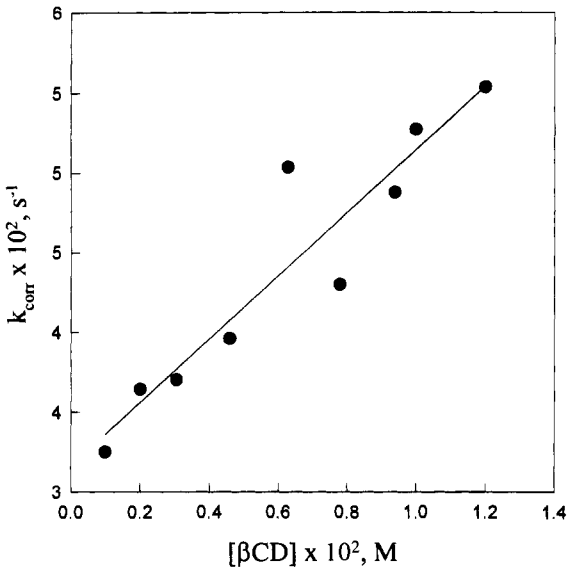


Figure 3. k_{corr} vs [CD] for the hydrolysis of **1e**.

attributed to an increase in the pK_a of the included substrate as a consequence of $K_{S1} < K_{SH1}$.

When the CD concentration is in the range 10^{-3} to 1.2×10^{-2} M, there is a linear dependence of the rate with CD concentration (Figure 3). Considering that $K_{S1} = 10^5$ M and that K_{SH2} and K_{S2} should be about 20, eq 11 simplifies to eq 13, which predicts linear dependence of k_{corr} with CD concentration, provided that the first term is very small.

$$k_{corr} = \frac{k_0}{K_{S1}[CD]} + \frac{K_{SH1}}{K_{S1}}k_1 + \frac{K_{SH1}}{K_{S1}}K_{SH2}k_2[CD] \quad (13)$$

From the intercept and slope of the plot of k_{corr} vs CD it can be inferred that the 1:1 complex has approximately the same reactivity as the substrate and that the 1:2 complex is approximately three times more reactive than the free substrate.

Conclusions

The hydrolysis of perfluoroalkylamides **1** is catalyzed by β-cyclodextrin through four different mechanisms: formation of complexes of 1:1 and 1:2 stoichiometry, decrease in the acidity of the NH group, and deaggregation of the substrate induced by specific complexation with β-CD. Complexes of 1:1 stoichiometry are formed with inclusion of the perfluorinated chain in the hydrophobic cavity of cyclodextrin, and they react about twice as fast as the free substrate only for the members of shorter chain length (up to three carbons). The 1:2 complex involves one cyclodextrin enclosing the perfluoroalkyl chain and another one binding the *p*-nitrophenyl moiety. This complex reacts faster than the substrate for all the members of the series. With compounds where the 1:1 complex is as reactive as the free substrate, the reactions are accelerated by CD because of an increase in the concentration of the reactive species, as a consequence of an increase in the pK_a of the substrate induced by complexation.

The compounds with longer perfluorinated chains (8 or 9 carbons), **1e,f**, have a greater tendency to aggregate even at low concentrations ($\geq 10^{-6}$ M for **1f**), and cyclodextrin induces deaggregation of them even at equal molar concentration, indicating that the cyclodextrin−substrate binding constant is very high.

Table 2. Yields and Physical Properties of the Synthesized Compounds

		elem. anal.						
		found (%)			calcd (%)			
compd	yield (%)	C	H	N	C	H	N	mp (°C)
1a	80	37.85	1.47	10.04	38.02	1.76	9.86	122−123
1b	40							84−85 (lit.[a] 85−86)
1c	75	34.15	1.20	7.42	34.37	1.30	7.29	95−96
1d	60	31.86	0.74	6.26	32.23	1.03	5.78	98−99
1e	60	3.97	0.66	5.85	31.46	0.94	5.24	105−106
1f	60	30.63	0.80	4.67	30.82	0.86	4.79	119−120
2	50	64.32	8.28	10.0	64.75	7.91	10.07	79−80

[a] Sawicki, E.; Hauser, T. R.; Stanley, T. W. *Anal. Chem.* **1959**, *12*, 2063−2065.

Experimental Section

Aqueous solutions were made up from water purified in a Millipore apparatus. Acetonitrile Merck HPLC grade was used as received.

α-Cyclodextrin (Aldrich), β-cyclodextrin (Roquette),[39] γ-cyclodextrin (FDS publications (Chinoin Pharmaceutical and Chemical Works, Ltd, Budapest), Hungary), HPCD (Roquette),[39] and soluble starch (Sigma) were used as received, but purity was periodically checked by UV spectroscopy. HPCD has an average of one hydroxypropyl group per glucose unit. Elemental analyses were carried out by Galbraith Laboratories, U.S.A.

The substrates **1** were prepared by the reaction of the appropriate perfluoroalkyl acid (Kanto Chemical Co)[40] with thionyl chloride (Merck) and then adding a solution of 4-nitroaniline in acetonitrile. The mixture was refluxed for 2−6 h in the dark with stirring, and the product was precipitated by pouring the mixture onto ice. The solid was filtered off, washed with aqueous bicarbonate and then water, and dried under vacuum. Substrate **3** was prepared from *p*-nitroaniline dissolved in dried benzene added dropwise to nonanoyl chloride. The mixture was stirred at room temperature during 1 h, and the yellow precipitate was filtered out and washed with benzene. The purity of all the substrates was checked by thin-layer chromatography, and the spectrum of a completely hydrolyzed solution was compared with a mock solution of the corresponding aniline. The yields, elemental analyses and melting points of the compounds are given in Table 2.

Kinetic Procedures. The reactions were initiated by adding the substrate dissolved in acetonitrile to a solution containing all the other constituents. The total acetonitrile concentration was always less than 2%.

All reactions were run at 25 °C and at constant ionic strength (0.5 M), using NaCl as the compensating electrolyte. The observed rate constants were determined by following the appearance of the *p*-nitroaniline at 380 nm. The change in absorbance during a kinetic run was recorded on a Shimadzu 260 recording spectrophotometer with a thermostated cell compartment. The reactions were followed up to 80−90% conversion. We did not detect any deviation from pseudo-first-order behavior in these conditions.

The pH of the solutions containing varying concentrations of cyclodextrin was adjusted by adding a drop of dilute acid or base. At pH 3−7 the buffer was $NaPO_4H_2$ with the appropriate amount of NaOH or HCl, and at pH 8−10 the buffer was CO_3^{2-}/CO_3H^-. The total buffer concentration was in all cases 0.01 M. Below pH 3 and above pH 12, HCl and NaOH were used, respectively.

The pK_a of **1a**, **1c**, and **1e** were determined by measuring the change in absorbance at 300 and 334 nm with pH. Values found were 7.7 for **1a** and 7.6 for **1c**. The pK_a of **1d** was determined in the presence of 8×10^{-4} M and 10^{-2} M β-cyclodextrin and gave the same value within experimental error, i.e., 8.13 ± 0.05.

The association equilibrium constants with β-CD were determined from the difference spectrum of solutions containing the appropriate concentrations of CD and the substrate. For **1a−c** a 1 cm cell was

(39) This compound was gift from the pharmaceutical company Ferromet S. A., Buenos Aires, Argentina.

(40) This compound was generously provided by Dr. Takaaki Sonoda, Kyushu University, Japan.

used, but for **1d–f** a 5 cm cell was chosen because of the low concentration of the substrates due to their solubility. These solutions were filtered through a 0.45 μm filter.

Appendix

The free CD concentration was calculated following Connors methodology.[41] The absorbance A of the solution is measured as a function of the total CD concentration, $[CD]_{0i}$, the system is described by eqs 14 and 15,where η_j and λ_k are parameters and $[CD]_i$ is the free cyclodextrin concentration. The form of the functions and the nature of the parameters depend on the system being studied and on the assumed stoichiometric model. The parameters include the desired stability constant, K.

$$Y_i = f([CD]_i, \eta_j) \tag{14}$$

$$[CD]_{0i} = g([CD]_i, \lambda_i) \tag{15}$$

We use the expression of the measured absorbance for the Y function and the mass balance for $[CD]_{0i}$, assuming that the complex formed has 1:1 stoichiometry. This assumption is valid provided that the concentration of the ligand is not higher than 10^{-4} M. Equations 14 and 15 thus become eq 16 and 17. $\Delta A/b$ is the change in absorbance per centimeter when the total [CD] changes from 0 to 0_i, K_{11} represents the 1:1 association constant, $[S]_0$ is the total concentration of the substrate, and $\Delta\epsilon$ is the difference in molar absorptivities of the substrate and complex.

$$\frac{\Delta A}{b} = \frac{\Delta\epsilon K_{11}[S]_0[CD]_i}{1 + K_{11}[CD]_i} \tag{16}$$

$$[CD]_{0i} = [CD]_i + \frac{K_{11}[CD]_i[S]_0}{1 + K_{11}[CD]_i} \tag{17}$$

Equation 15 can be expanded into a Taylor's series, which is truncated to give an expression that can be solved explicitly for $[CD]_i$. The Taylor's series of eq 15 is eq 18,

$$[CD]_{0i} = g([CD]_{0i}) + g'([CD]_{0i})([CD]_i - [CD]_{0i}) + \frac{g''([CD]_{0i})}{2}([CD]_i - [CD]_{0i})^2 + \tag{18}$$

where $g([CD]_{0i})$ is given by eq 17 and $g'([CD]_{0i})$ and $g''([CD]_{0i})$ are the first and second derivatives of eq 17 with respect to $[CD]_i$ evaluated for $[CD]_i = [CD]_{0i}$. Replacing $g([CD]_{0i})$ and $g'([CD]_{0i})$ in eq 18 truncated at the linear term and solving for $[CD]_i$, eq 19 is obtained.

$$[CD]_i = [CD]_{0i} - \frac{1}{g'([CD]_{0i})}\frac{K_{11}[S]_0[CD]_{0i}}{(1 + K_{11}[CD]_{0i})} \tag{19}$$

$$g'([CD]_{0i}) = 1 + \frac{K_{11}[S]_0}{1 + 2K_{11}[CD]_{0i} + K_{11}^2[CD]_{0i}^2}$$

The concentration of free CD was calculated for each i concentration of total CD; starting from an estimated value of K_{11}, a group of CD_i is thus calculated, and from this iteratively new values of K_{11} are calculated until convergency is found.

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Supplementary Material Available: Table S1 listing the observed rate constants for the hydrolysis of compounds **1** vs cyclodextrin concentration and Table S2 containing the observed rate constant for **1e** vs substrate concentration (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA9426204

(41) Connors, K. A. *Binding constants: The measurement of molecular complex stability*; John Wiley and Sons: New York, 1987.