Deacylation Rates of Several Trifluoromethyl-Substituted Acylchymotrypsins*

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ABSTRACT: The rates of deacylation of 3-trifluoromethylbutyryl-, 4-trifluoromethylbutyryl-, 5-trifluoromethylvaleryl-, and 8-trifluoromethyloctanoyl acylchymotrypsins have been determined at pH 4.0 and 7.28. After approximate corrections for the inductive and steric effects of the trifluoromethyl group, it was found that the deacylation rates for the first-mentioned acyl enzyme are not unusual when compared with the corresponding unfluorinated alkyl derivative. The 4-trifluoromethylbutyryl compound is about nine times more reactive than anticipated from the behavior of the corresponding unsubstituted derivative while the last two materials react at about one-half the expected rate. It is suggested that these rate variations may, in part, arise from perturbations in the relative populations of various conformations of the acyl group that result from introduction of fluorine atoms into the alkyl chain.

Enzymic active sites have been extensively studied with the hope that the chemical features which distinguish one active site from another may be elucidated. Organic chemists have increasingly used the information that has thus become available to modify adroitly portions of active sites in specific ways (Baker, 1967; Singer, 1967). Such capabilities make possible further investigation of the active site if the modifying reagent has some detectable physical property that is sensitive to environment.

A number of elegant applications of this "reporter group" concept have been made to the investigation of the active site of the proteolytic enzyme, α-chymotrypsin. Ultraviolet and electron spin resonance spectroscopic techniques have been used with appropriately labeled materials to gain insight into the nature of binding and motional stability at the active site of this enzyme (Hille and Koshland, 1967; Sigman and Blout, 1967; Haughland and Stryer, 1967; Berliner and McConnell, 1966). An X-ray crystallographic study of the structure of this enzyme has been described recently so that comparisons between the conclusions reached from the reporter group studies and the structure of the protein in the crystalline state are now possible (Sigler *et al.*, 1968).

We are engaged in a program to determine the utility of nuclear magnetic resonance spectroscopy in the reporter group kind of experiment. The information that can, in principle, be obtained from a nuclear magnetic resonance experiment is complementary to that which is derived by the spectroscopic techniques mentioned above. Because of the great number of protons contained in the enzyme molecule itself, it appears unlikely that magnetic resonance experiments utilizing the signals from the protons of a reporter group will be highly productive; the resonance of interest will be obscured by the multitude of resonances from the enzyme. We are, therefore, resorting to the so-called "fluorine-labeling" technique that has been exploited by Roberts and coworkers in studies of conformational equilibration in carbocyclic molecules (Roberts, 1966; Lack and Roberts, 1968). The basic approach is to substitute one or more fluorine atoms into the molecule of interest and to observe the fluorine-19 spectrum of the resulting species. In addition to removing the nuclear magnetic resonance signal of interest from the resonances of the enzyme, the method also benefits from the extreme sensitivity of fluorine chemical shifts to environment (Evans, 1960; Muller and Birkham, 1967). This sensitive chemical shift effect is documented in recent reports which described nuclear magnetic resonance studies of experiments with fluorinated molecules and α-chymotrypsin (Spotswood, *et al.*, 1967; Zeffren and Reavill, 1968).

We have prepared a series of trifluoromethyl-substituted alkanoic acids and, by use of their *p*-nitrophenyl esters, hoped to prepare a series of stable acylated α-chymotrypsins which contained the trifluoromethyl reporter group at the active site (Bender *et al.*, 1964). Since these enzymes would be expected to have only limited stability with regard to deacylation, it was imperative that sufficient kinetic data be obtained for this process so that the optimum conditions for the envisioned nuclear magnetic resonance experiments could be selected. Unexpected deacylation rate variations were found in the series of compounds examined, and the results thus have some relevance to the question of how representative of the nonfluorinated acyl groups is the behavior of the fluorine-containing acyl enzymes. We wish to report herein these kinetic studies.

Experimental Section

5,5,5-Trifluorovaleric acid was prepared by the reaction of sulfur tetrafluoride with glutaric acid (Hasek *et al.*, 1960). In a stainless steel vessel of 45-ml capacity was placed 4.1 g (0.03 mole) of glutaric acid (Matheson Coleman and Bell) together with a magnetic stirring bar. The bomb was evacuated to about 1 mm and cooled to Dry Ice–acetone temperature. Sulfur tetrafluoride (6 ml, 0.1 mole) (Matheson and

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Co.) was distilled into a previously calibrated glass vessel and, from there, distilled into the bomb. An orange impurity in the sulfur tetrafluoride was removed by this prior distillation. After introduction of the sulfur tetrafluoride, the reaction vessel was sealed and warmed to 120° in an oil bath for 12 hr while the contents were stirred magnetically. The bomb was cooled to room temperature, the gases were vented, and the contents were made basic with 10% sodium hydroxide solution. The resulting two-phase system was heated to reflux. Alkali solution was added occasionally until the solution remained basic to litmus. The organic layer was separated and the aqueous layer was made acidic. An amber oil thereupon separated from the aqueous layer and was removed. The aqueous phase was extracted with methylene chloride. The extracts were combined with the amber oil and the resulting solution was dried over magnesium sulfate. After evaporation of the methylene chloride, a yield of 1.3 g (27%) of the crude acid was realized. Distillation under reduced pressure afforded the pure acid as a colorless liquid; the structure of this material was confirmed by conversion of the acid into the *p*-nitrophenyl ester, as described below, and by proton magnetic resonance spectroscopy.

6,6,6-Trifluorohexanoic acid was prepared in a similar manner, utilizing adipic acid (Matheson Coleman and Bell) and a reaction temperature of 150° for 12 hr to produce the desired acid in 11% yield. The proton magnetic resonance spectrum of the material and its conversion to the *p*-nitrophenyl ester establish the structure of this material.

9,9,9,-Trifluorononanoic acid was obtained in 10% yield by a procedure similar to that used for the valeryl compound. Azelaic acid monomethyl ester (Aldrich) was used as the starting material and the reflux under basic conditions was allowed to proceed overnight. The proton magnetic resonance spectrum of the product was consistent with the assigned structure.

p-Nitrophenyl 3-trifluoromethylbutyrate was prepared by a mixed anhydride method (Vaughn and Osato, 1952). To 0.2 g (1.3 mmoles) of racemic 3-trifluoromethylbutyric acid (Pierce Chemical Co.) was added 0.13 g (1.3 mmoles) of triethylamine and 1.5 ml of toluene. The mixture was cooled to −5° and stirred magnetically while 0.14 g (1.3 mmoles) of ethyl chloroformate (Eastman) in 2 ml of toluene was added dropwise. The reaction mixture was stirred 0.5 hr with no further cooling whereupon the contents of the reaction vessel were added dropwise to a slurry of 0.18 g (1.3 mmoles) of *p*-nitrophenol in 3 ml of toluene and 0.013 g (0.13 mmoles) of triethylamine. After 3 hr, the mixture was filtered, washed three times with 5% potassium carbonate solution, once with water, and dried over magnesium sulfate. Evaporation of the solvent and molecular distillation of the residue afforded the desired product in 45% yield (0.24 g). The proton and fluorine-19 magnetic resonance spectra were consistent with the assigned structure. *Anal.* Calcd for $C_{11}H_{10}F_3NO_4$: C, 47.7; H, 3.61. Found: C, 47.9; H, 3.62.

p-Nitrophenyl 3-methylbutyrate was prepared according to a procedure outlined by Fife (1965). *Anal.* Calcd for $C_{11}H_{13}NO_4$: C, 59.3; H, 5.83. Found: 59.5; 6.07.

p-Nitrophenyl 5,5,5-trifluorovalerate was prepared by allowing the corresponding acid chloride, prepared with thionyl chloride, to react with *p*-nitrophenol in pyridine solution at steam bath temperature for 10 min. The reaction mixture was added to ice water. The yellow crystals that resulted were taken up in methylene chloride and washed several times with 5% potassium carbonate solution, until the color of *p*-nitrophenol was discharged. After removal of the solvent, the resulting oil was distilled at 50 μ onto a cold finger to produce the desired product in 12% yield, mp 35–38°. The proton magnetic resonance spectrum was consistent with the assigned structure. *Anal.* Calcd for $C_{11}H_{10}F_3NO_4$: C, 47.65; H, 3.61. Found: C, 47.68; H, 3.78.

p-Nitrophenyl valerate was synthesized by the mixed anhydride procedure that was described above in 41% yield. *Anal.* Calcd for $C_{11}H_{13}NO_4$: C, 59.3; H, 5.83. Found: C, 59.3; H, 6.07.

p-Nitrophenyl 6,6,6-trifluorohexanoate was prepared by the acid chloride method in 20% yield. *Anal.* Calcd for $C_{12}H_{12}F_3NO_4$: C, 49.50; H, 4.13. Found: C, 49.25; H, 4.10.

p-Nitrophenyl hexanoate was obtained by the method outlined by Fife (Fife and Milstein, 1967).

p-Nitrophenyl 9,9,9-trifluorononanoate was synthesized by the mixed-anhydride sequence in 47% yield. *Anal.* Calcd for $C_{15}H_{18}F_3NO_4$: C, 54.10; H, 5.41. Found: C, 54.31; H, 5.57.

p-Nitrophenyl nonanoate was prepared by the mixed-anhydride method to afford the desired product in 10% yield, bp 159–160° (0.5 mm). *Anal.* Calcd for $C_{15}H_{21}NO_4$: C, 62.4; H, 7.28. Found: C, 63.9; H, 7.23. Proton and fluorine magnetic resonance spectra were determined on a Varian Associates A-60 or HA-100 spectrometer. Microanalyses were performed by Galbraith Laboratories or Chemalytics Inc.

Deacylation Kineties. The rates of deacylation of the various acylchymotrypsins produced when the enzyme was treated with the above-described *p*-nitrophenyl esters were obtained by monitoring the appearance of *p*-nitrophenol. All measurements were made with a Beckman Model DU spectrophotometer equipped with a Gilford 2000 recorder accessory. A temperature of 25° was maintained by circulating thermostated water through the cell compartment. At pH 4 the production of *p*-nitrophenol was followed at 330 mμ. At pH 7.8, optical density measurements were made at 400 mμ. Beer's law was shown to hold over the concentration range of interest at both pH values and extinction coefficients of 0.85 $\times 10^4$ and 1.16 $\times 10^4$ M^{-1}, respectively, were found at these wavelengths. Stock solutions of the *p*-nitrophenyl esters at 5.6 mM were prepared using reagent grade acetonitrile. Stock solutions of Worthington three-times recrystallized α-chymotrypsin in the appropriate buffer were prepared gravimetrically to give solutions nominally 0.2 mM in enzyme. The stock solutions were measured for activity using the irreversible acylating agent, 2-nitro-4-carboxyphenyl-*N*,*N*-diphenylcarbamate, purchased as part of a chymotrypsin assay kit from Mann Research Laboratories (Erlanger *et al.*, 1963). The active site concentration was 74–75% of the gravimetric concentration, in agreement with the observation of previous workers (Bender *et al.*, 1966). A succinic acid buffer at 0.01 *m* ionic strength was used at pH 4 while a phosphate buffer at 0.075 *m* ionic strength was used at pH 7.28 (Perin, 1963). The reaction was initiated by mixing 5.16 ml of buffer solution, 0.10 ml of acetonitrile, and 0.6 ml of enzyme stock solution to which was added 0.14 ml of stock ester solution. The final volume of solution was 6.00 ml of which 4% was acetonitrile. The enzyme concentration following mixing was 0.015 mM (corrected for the activity of the enzyme) while the substrate concentration was 0.13 mM. Replicate runs were

TABLE I: Rate Constants for Deacylation of Various Acyl-α-chymotrypsins at 25°.

Acyl Group	pH[a]	$k_3 \times 10^4$ (sec^{-1})[b]	Acyl Group	pH	$k_3 \times 10^4$ (sec^{-1})
$(CF_3)(CH_3)CHCH_2C(=O)$	4.0	0.73 ± 0.05	$(CH_3)_2CHCH_2C(=O)$	4.0	0.20 ± 0.02
Ia, 3-trifluoromethylbutyryl	7.28	14.6 ± 0.4	Ib, isovaleryl	7.28	6.68 ± 0.17
$CF_3(CH_2)_3C(=O)$	4.0	2.85 ± 0.17	$CH_3(CH_2)_3C(=O)$	4.0	0.36 ± 0.03
IIa, 4-trifluoromethylbutyryl	7.28	552 ± 14	IIb, valeryl	7.28	62.3 ± 1.5
$CF_3(CH_2)_4C(=O)$	4.0	0.82 ± 0.08	$CH_3(CH_2)_4C(=O)$	4.0	0.86 ± 0.08
IIIa, 5-trifluoromethylvaleryl	7.28	135 ± 6	IIIb, hexanoyl	7.28	153 ± 6
$CF_3(CH_2)_7C(=O)$	4.0	~0.1	$CH_3(CH_2)_7C(=O)$	4.0	~0.1
IVa, 8-trifluoromethyloctanoyl	7.28	22 ± 3	IVb, *n*-nonanoyl	7.28	45 ± 5

[a] Ionic strength at pH 4.0 = 0.01 M. Ionic strength at pH 7.28 = 0.075 M. The solvent contained 4% acetonitrile. [b] Average of at least two determinations.

usually reproducible to within 5%. The steady-state, zero-order appearance of *p*-nitrophenyl acetate after the initial acylation of the enzyme provides a direct measurement of the rate of deacylation provided that the condition $S_0/k_3 \gg K_M/k_2$ is satisfied (Kézdy and Bender, 1962). Plots of absorbance *vs.* time were linear to at least 50% reaction providing some indication that this restriction is met. However, no direct measurement of K_M was attempted. The limited solubility of of the nonanoyl esters made it necessary to follow the deacylation reaction at ester and enzyme concentrations one-fifth those mentioned above. The results for these two compounds are considered somewhat less reliable than those for the other esters studied. Rates for spontaneous hydrolysis of the esters were negligible under the conditions of these experiments.

Results

The rates of deacylation of the acylated chymotrypsin derived from the trifluoromethyl-substituted alkanoic acids mentioned above were determined at pH 4.0 and 7.28. To provide reference data, the deacylation rates for the analogous all-proton acids were also measured. The results are summarized in Table I. It may be noted that the data for the isovaleryl and hexanoyl compounds agree with the data of Fife and Milstein (1967) to within experimental error. This is an indication that it is legitimate to compare the remainder of these authors' data with the results of this study.

It is known that the deacylation of α-chymotrypsin is sensitive to the steric properties of the acyl group (Fife and Milstein, 1967). Inductive (or field) effects likely play a role in determining the rate of deacylation, also (Caplow and Jencks, 1962). Before making comparisons between the rate constants in Table I, it is necessary to take into account these factors. This can be done most conveniently within the framework of the Taft–Ingold equation

$$\log k_3 = \rho^*\sigma^* + \delta E_s - \log (k_3)_0 \qquad (1)$$

where σ^* and E_s are constants dependent on the inductive and steric attributes of the acyl group and the coefficients ρ^* and δ depend upon the sensitivity of the reaction to these attributes. The term in $(k_3)_0$ is the rate of the reference reaction, in this case, the deacylation of acetylchymotrypsin. Unfortunately, σ^* and E_s values are not available for all the alkyl groups listed in Table I and it was necessary to estimate these values by the methods described below.

A comparison of the σ^* values for propyl and isobutyl available from the literature (−0.115 and −0.125, respectively) suggests that the β position of a carbon chain has only a small effect on the inductive parameter. It was assumed that this is also the case for 2-trifluoromethylpropyl and σ^* for this group was taken to be about the same as that for 2-trifluoroethyl, +0.32 (Taft, 1956).

The available data suggest that the effect of trifluoromethyl substitution on the σ^* values for a normal alkyl chain falls off exponentially with increasing chain length, and it was estimated that σ^* for 3-trifluoromethylpropyl should be about 0.045. The inductive parameters for *n*-octyl and 7-trifluoromethylheptyl were assumed to be equal and were taken to be the same as those for several long-chain aliphatic groups, about −0.2.

We are unaware of any published values of E_s, the steric parameter, for any of the fluorinated materials used in this study. In order to estimate the magnitude of E_s for these groups, cognizance was taken of the fact that E_s depends upon the volume occupied by the substituent (Taft, 1956, p 601). A value for E_s was obtained by multiplying the change in E_s associated with the addition of a methyl group to a normal

TABLE II: Substitution Constants Used in Linear Free-Energy Correlations.[a]

Substituent	σ^*	E_s
Ib, $(CH_3)_2CHCH_2$	−0.125	−0.93
Ia, $(CF_3)(CH_3)CHCH_2$	(0.32)	(−1.50)
IIb, $CH_3(CH_2)_3$	−0.13	−0.39
IIa, $CF_3(CH_2)_3$	0.12	(−0.41)
IIIb, $CH_3(CH_2)_4$	(−0.14)	−0.40
IIIa, $CF_3(CH_2)_4$	(0.04)	(−0.40)
IVb, $CH_3(CH_2)_7$	(−0.2)	−0.33
IVa, $CF_3(CH_2)_7$	(−0.2)	(−0.33)

[a] Values in parentheses were estimated by methods described in the text. The remainder are from Taft's compilation.

aliphatic chain by the ratio of the volumes of the trifluoromethyl group and methyl group. A consideration of space-filling models indicates that the volume occupied by trifluoromethyl is about twice that occupied by methyl.[1] An example of the procedure follows. From Taft's compilation, E_s for a propyl group is −0.36 while E_s for 2-methylpropyl (isobutyl) is −0.93. If the difference between these values (−0.57) is due only to the additional volume of a methyl group, then the introduction of a trifluoromethyl group into the propyl system should change the E_s value about twice as much (−1.14). The calculated E_s value for the 2-trifluoromethylpropyl is −1.50. A value of E_s for 3-trifluoromethylpropyl was reckoned in a similar way while E_s for 4-trifluoromethylbutyl and 8-trifluoromethylheptyl were assumed to be equal to those for the corresponding nonfluorinated alkyl groups.

While the very approximate nature of the procedure is recognized, the errors involved are not likely to be significant for the longer normal alkyl chains, since the steric effect appears to be nearly constant as the length of the chain is varied from propyl to octyl (Taft, 1956). The values of σ^* and E_s for the alkyl groups used in this study are collected in Table II.

Fife and Milstein have found that the rates of deacylation of six aliphatic acylchymotrypsins are smoothly correlated by means of the equation $\log k_3/(k_3)_0 = 1.92\sigma^* + 0.96E_s$. The dominant effect of substituent observed in the deacylation rates was the steric effect as reflected by E_s. If we presume for the moment that the same value of ρ^* is applicable to the series of compounds examined in this work, then a plot of $\log k_3 - 1.92\sigma^*$ against E_s should be linear with a slope equal to δ. Such a plot has been prepared for the data at pH 7.28 as is reproduced in Figure 1. The plot includes the data of Fife and Milstein at this pH and the slope drawn corresponds to $\delta = 0.96$.

A number of the points including those for 4-trifluoromethylbutyl, valeryl, 5-trifluoromethylvaleryl, hexanoyl, and nonanoyl compounds deviate significantly from the correlation line.[2] Although there is doubtless some uncertainty in the location of several of the points on the plot due to errors made in estimating the substituent constants, no reasonable revision of these constants significantly improves the correlation. It is important to note that the point for 3-trifluoromethylbutyryl (Ia) lies near the correlation line; the estimated substituent constants for this compound are much more subject to error than those for the other fluorinated derivatives.

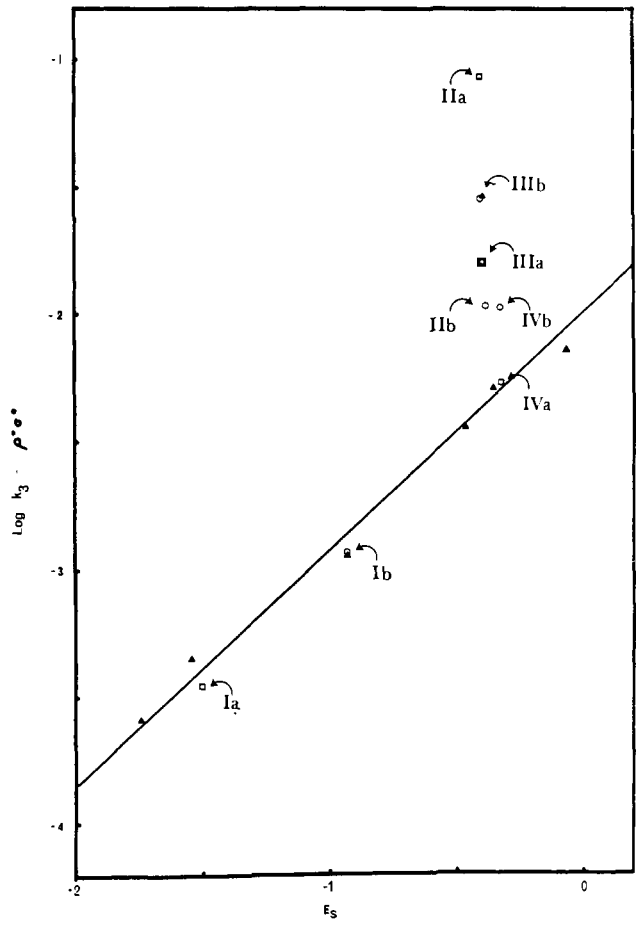


FIGURE 1: Plot of $(\log k_3 - \rho^*\sigma^*)$ *vs.* E_s for various acylchymotrypsins where k_3 is the observed first-order rate constant for deacylation at pH 7.28 and 25°. The filled triangles represent the data previously reported by Fife and Milstein. The open symbols are used for the data described in this paper; the open squares are used for fluorine-containing acyl groups. Numbers refer to entries in Table I.

Qualitatively the same order of reactivity for the various acyl enzymes is observed at pH 4.0.

Discussion

The observation that the exception is often more interesting than the rule is particularly applicable to linear free energy relations. As is demonstrated in Figure 1, the deacylation rates for several of the acylchymotrypsins used in this work lie close to the same regression line that has been previously reported by Fife and Milstein. These authors have commented

[1] Corey–Pauling–Koltun models were used. Similar conclusions are made if the van der Waals radii of the two groups are estimated from single-bond lengths and atomic van der Waals radii (Pauling, 1944).

[2] Fife and Milstein had previously noted the strong deviation of the hexanoyl compound in the correlation.

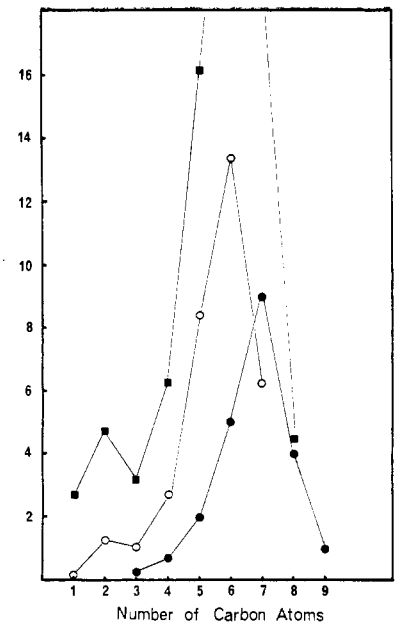


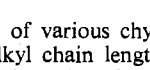
FIGURE 2: Rates of various chymotryptic-catalyzed hydrolyses as a function of alkyl chain length. The filled circles represent the data of Hofstee in units of μmoles/3 ml per 30 min, the open circles represent the data of Jones *et al.* in units of sec^{-1}, and the filled squares the data reported in Table I, in units of 10^{-4} sec^{-1}.

that the inductive and steric effects observed for this reaction are quite normal and doubtless are a result of steric hindrance to the approach of a nucleophilic species. The abnormally high rate of deacylation of hexanoyl-chymotrypsin was noted by these authors and was ascribed to some interaction of this alkyl group with the enzyme that leads to reduction in the free energy of activation for deacylation.

We have observed that, in addition to hexanoyl derivative, the valeryl-, 4-trifluoromethylbutyryl-, 5-trifluoromethylvaleryl-, and 8-trifluoromethyloctyl-chymotrypsins deacylate much more rapidly than would be anticipated from the normal steric or inductive effects of the substituents. Inductive effects should be very similar in all of these compounds and the *difference* in the steric effect, as measured by E_s, between a fluorinated compound and a hydrocarbon of the same chain length should be small. However, the data in Table III show that placement of three fluorine atoms at the terminal carbon of these alkyl chains can significantly increase, decrease, or have little effect on the rate of deacylation, depending upon the length of the alkyl chain in the acyl group.

The rate constant for deacylation of an acylated chymotrypsin is dependent on the apparent dissociation constant of an essential group near the catalytic site. It is possible that conformational changes in the enzyme induced by the acyl moiety could modify the pK of this essential group; large structural changes in the acyl group appear to have a small but real effect on this pK (Fife and Milstein, 1967). The comparisons in Table III are based on pairs of compounds of very similar structure and unless some unusual effect results from the properties of the fluorine–carbon bonds, it does not seem likely that an appreciable change in the pK of the essential group would result from the substitution of fluorine for hydrogen in these compounds. Further experimental work to clarify this point is desirable, however.

TABLE III: Effect of Fluorine Substitution on Deacylation of Various Acylchymotrypsins at pH 7.28.

Compd	Acyl Substituent	k_{corr}[a] × 10^3	k_{CF_3}/k_{CH_3}
I	$(CH_3)_2CHCH_2$	9.1	1.1
	$(CF_3)(CH_3)CHCH_2$	9.8	
II	$CH_3(CH_2)_3$	26	8.9
	$CH_3(CH_2)_3$	230	
III	$CH_3(CH_2)_4$	69	0.56
	$CF_3(CH_2)_4$	39	
IV	$CH_3(CH_2)_7$	23	0.48
	$CF_3(CH_2)_7$	11	
V		7.2[b]	1.2
		8.9[b]	

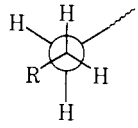
[a] The rate constant corrected for inductive and steric effects, *i.e.*, k_{corr} = antilog (log k_3 − $\rho^*\sigma^*$ − δE_s), where ρ^* = 1.92, δ = 0.96 and k_3 is the observed rate of deacylation. [b] Taken from the data of Caplow and Jencks at pH 7.07.

Our results combined with those of Fife and Milstein produce a pattern of rate effects that are quite reminiscent of similar observations reported in the past. Hofstee has noted that the α-chymotryptic hydrolysis rates in a series of alkanoate esters of *m*-hydroxybenzoic acid increase with increasing chain length, reaching a maximum rate with the octanoate ester (Hofstee, 1957). Niemann and coworkers have studied the hydrolysis of a series of *N*-acetyl-L-amino acid esters of the general form $CH_3CONHCH[(CH_2)_nH]CO_2CH_3$, where n = 1 to 6. The rate of the deacylation step in the reaction was found to be greatest when the number of carbon atoms in the acyl group (α-carbon plus side chain) was equal to six (Jones *et al.*, 1965). The data from these two investigations and the data from this work, combined with those of Fife and Milstein, are plotted schematically in Figure 2. Although a number of factors serve to modify any conclusion that would derive from comparison of these sets of data, it does seem clear that a rate optimum at an alkyl chain length of six to seven carbon atoms occurs in these systems and that this increase cannot be accounted for in terms of the ordinary inductive or steric effect associated with the alkyl chain. An additional interaction (or interactions) between the enzyme and the alkyl group is thus indicated.

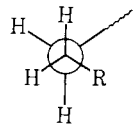
Study of a space-filling model of α-chymotrypsin suggests that contacts between a normal alkyl group and the walls of the enzymic active site become progressively more likely and more severe with chains greater than three carbon atoms in length. It is difficult to quantify the number of such possible interactions or to judge whether they will be attractive or repulsive in nature; we merely accept as a hypothesis the no-

tion that the interaction between the terminal portion of these alkyl groups and the enzymic active site leads to an acceleration in the rate of deacylation, possibly by a buttressing effect that increases the free energy of the ground state of the acylchymotrypsin.

The effect of fluorine substitution in the alkyl chain is roughly consistent with this hypothesis. The largest rate increase upon trifluoromethyl substitution at the terminus of a carbon chain occurs when there are four carbon atoms in the alkyl group (3-trifluoromethylpropyl, IIa). Conformations of this alkyl group that have the *gauche* arrangement about the central carbon–carbon bond (VI) would be expected to be considerably less favored for steric reasons when the terminal group is trifluoromethyl (R = CF_3) than when it is methyl (R = CH_3). If an extended conformation (VII) leads to more contact interactions with the active site



VI a, R = CF_3
b, R = CH_3



VII

then the trifluoromethyl compound should react more rapidly since this conformational state will be more heavily populated than when the terminal group is methyl. Addition of methylene groups to the alkyl chain permits greater flexibility of the chain with the result that the influence of trifluoromethyl substitution on the over-all conformational properties of the alkyl chain is diminished and at chain lengths greater than four carbon atoms, the effect of terminal trifluoromethyl substitution on the rate of deacylation should be smaller, as observed.

It will be of interest to correlate these rate effects with the results of a fluorine-19 magnetic resonance investigation of intermediate acyl enzymes; work is now in progress to obtain the data for such a correlation.

Acknowledgment

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