Steric Effects in the Imidazole-Catalyzed Hydrolysis of Esters of N-Acetylserinamide and of *p*-Nitrophenol

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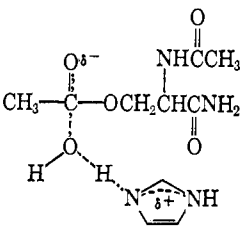
Abstract: The imidazole-catalyzed hydrolysis of a series of esters of N-acetylserinamide has been studied in H_2O at 95°. The acyl groups included acetyl, butyryl, isobutyryl, isovaleryl, and trimethylacetyl. Imidazole acts as a classical general base in this reaction. A plot of the logarithms of the second-order rate constants for imidazole catalysis at 95° *vs.* E_s, the Taft steric effects constant, is linear with a slope of 0.49. A similar plot using the rate constants for spontaneous hydrolysis has a slope of 0.47. The sensitivity of the imidazole-catalyzed reaction to steric effects is similar to that found for deacylation of acyl-α-chymotrypsins when the comparison is made at the same temperature, but is considerably less than for imidazole nucleophile-catalyzed hydrolysis of *p*-nitrophenyl esters. Activation parameters are reported for the imidazole-catalyzed hydrolysis of a series of esters of *p*-nitrophenol, where the acyl groups studied included acetyl, butyryl, isobutyryl, isovaleryl, and trimethylacetyl. Differences in ΔH^* are small, increased steric bulk in the acyl group primarily influencing ΔS^*.

The hydrolytic reaction catalyzed by α-chymotrypsin involves the formation of an acyl enzyme in which the acyl group is covalently bound to a group at the active site of the enzyme. It is very likely that this group is the serine hydroxyl.[2] The acylation and deacylation of this serine residue may be aided by a histidine residue which has been shown to be present at the active site.[2] A suitable model, therefore, for the deacylation of acyl-α-chymotrypsins would be the imidazole-catalyzed hydrolysis of esters of N-acetylserinamide. It has been well established that the role of imidazole in the hydrolysis of *p*-nitrophenyl acetate[3,4] and of other *p*-nitrophenyl esters[5] is that of a nucleophile, with attack by imidazole at the carbonyl carbon. Anderson, *et al.*,[6] on the other hand, have studied the reactions of N,O-diacetylserinamide and have established that the imidazole-catalyzed hydrolysis of that compound involves classical general base catalysis, with the most likely mechanism involving partial proton abstraction from a water molecule by the catalyzing base in the transition state.



General base catalysis, in which a proton is transferred in the transition state, appears generally to be only moderately affected by steric bulk in the catalyzing base,[7] whereas nucleophilic catalysis of ester hydrolysis is strongly retarded by large groups at the 2 position of imidazole.[8] Increased steric bulk in the acyl group also markedly inhibits the rate of nucleophile-catalyzed hydrolysis of *p*-nitrophenyl esters, in contrast to the small effects in the imidazole, general base catalyzed hydrolysis of N-acylimidazoles.[5]

Histidine must be acting as either a nucleophile or a classical general base in enzymatic acylation and deacylation. Since the usual methods for distinguishing between these mechanisms, such as the D_2O solvent isotope effect, may give results that are not easily interpretable when applied to enzyme reactions,[9] it was thought that a comparison of steric effects in the hydrolysis of nonenzymatic model systems with those seen in the enzyme reaction might provide an approach to deciding between these two mechanisms.

In a recent study[10] it was shown that in the deacylation of a series of acyl-α-chymotrypsins in which the acyl portion was varied with respect to steric bulk around the carbonyl, a linear plot of log (k_3/k_0) *vs.* E_s, the Taft steric effects constant,[11] was obtained having a slope of 1.1, whereas a similar plot of log (k_{Im}/k_0) *vs.* E_s for the imidazole, nucleophile-catalyzed hydrolysis of a series of *p*-nitrophenyl esters showed considerably greater sensitivity to steric hindrance, having a slope of 1.4. It was therefore of considerable interest to study steric effects in the imidazole, general base catalyzed hydrolysis of a series of esters of N-acetylserinamide.

Experimental Section

Materials. The imidazole used was from Eastman Kodak Co. (White Label). Acid chlorides and anhydrides were obtained from Matheson Coleman and Bell, with the exception of trimethylacetyl chloride (Aldrich Chemical Co.) and isovaleric anhydride (K & K Laboratories). Serine methyl ester hydrochloride was obtained from Aldrich Chemical Co. The *p*-nitrophenyl esters were the same as previously described.[5,10] Dioxane was purified by the method of Fieser.[12]

(1) This study represents part of the work to be submitted by J. B. Milstien in partial fulfillment of the requirements for the Ph.D. degree, University of Southern California, Los Angeles, Calif.

(2) For a summary of the evidence, see M. L. Bender and F. J. Kezdy, *J. Am. Chem. Soc.*, **86**, 3704 (1964).

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N-Acetylserinamide was prepared from serine methyl ester hydrochloride by the method of Rothstein.[13] N,O-Diacetylserinamide and N-acetyl-O-trimethylacetylserinamide were prepared by adding 0.05 equiv of the acid chloride dropwise with stirring to 0.05 equiv of N-acetylserinamide dissolved in 85 ml of pyridine. After refluxing the mixture at room temperature for 2 hr, the solvent was evaporated under reduced pressure. The residue was washed with ether and recrystallized first from acetonitrile, and then from a very small amount of 95% ethanol. N,O-Diacetylserinamide had mp 185–187° (lit.[6] mp 157–159°). *Anal.* Calcd for $C_7H_{12}N_2O_4$: C, 44.67; H, 6.43; N, 14.89. Found: C, 44.80; H, 6.66; N, 14.89. N-Acetyl-O-trimethylacetylserinamide had mp 191–194°. *Anal.* Calcd for $C_{10}H_{18}N_2O_4$: C, 52.16; H, 7.88; N, 12.17. Found: C, 51.97; H, 8.06; N, 12.03.

The other serinamides were prepared in a similar manner except that the acid anhydrides were used rather than the acid chlorides, and because of the difficulty encountered in recrystallization, the resulting white crystals were instead washed several times with ether. N-Acetyl-O-butyrylserinamide had mp 106–108°. *Anal.* Calcd for $C_9H_{16}N_2O_4$: C, 49.99; H, 7.46; N, 12.96. Found: C, 49.72; H, 7.41; N, 12.78. N-Acetyl-O-isobutyrylserinamide had mp 156–158°. *Anal.* Calcd for $C_9H_{16}N_2O_4$: C, 49.99; H, 7.46; N, 12.96. Found: C, 50.14; H, 7.61; N, 12.63. N-Acetyl-O-isovalerylserinamide had mp 127–130°. *Anal.* Calcd for $C_{10}H_{18}N_2O_4$: C, 52.16; H, 7.88; N, 12.17. Found: C, 51.99; H, 7.98; N, 12.01.

Kinetic Measurements. The kinetics of the imidazole-catalyzed hydrolysis of the esters of N-acetylserinamide were studied in imidazole buffers made up at a constant ionic strength of 1.0 *M* with KCl. The rates were measured by the hydroxamate method employed by Bruice and Bruno.[14] One milliliter of the appropriate buffer solution, 0.015 *M* in ester, was pipetted into each of ten Pyrex tubes. These were tightly closed with Teflon-lined screw caps and placed in a Haake NBe 65746 Ultrathermostat, which maintained the temperature at 95 ± 0.1°. Tubes were withdrawn at intervals and quenched in ice, and to each tube was added 2 ml of freshly prepared buffered hydroxylamine solution, consisting of one part 28% hydroxylamine solution in water, one part 14% NaOH, and two parts of buffer which was four parts 0.1 *M* sodium acetate and one part 0.1 *M* acetic acid. The tubes were then replaced in the bath for developing. After an appropriate length of time they were placed in ice and to each tube was added two ml of water, one ml of 3 *N* HCl, and one ml of 5% $FeCl_3$ in 0.1 *N* HCl. The color was read at 540 mμ on a Zeiss PMQ 11 spectrophotometer. Each rate was measured in duplicate. Duplicate values agreed to ±5%. Development time was determined for each compound under experimental conditions. At the end of each kinetic run, the solution was checked for β elimination by measuring the absorbance at 241 mμ. The amount of elimination was always less than 5%, and usually less than 2%, taking a molar extinction of 5000 for the dehydroalanine derivative.[15]

The imidazole-catalyzed hydrolysis of the *p*-nitrophenyl esters was followed at 400 mμ, on a Zeiss PMQ 11 spectrophotometer, in imidazole buffers of ionic strength 1.0 *M* with KCl. Each rate was measured in triplicate. Constant temperature (±0.1°) was maintained with a Precision Scientific LoTemptrol 154 circulating bath and a Zeiss constant-temperature cell holder. To 3.0 ml of the appropriate preequilibrated buffer solution was added exactly 0.05 ml of the ester solution in dioxane, so that the reaction mixture was always 1.64% in dioxane. The mixture was then stirred vigorously. Final concentration of ester in the cuvette was about 10^{-4} *M*.

Pseudo-first-order rate constants were calculated by means of an Olivetti-Underwood Programma 101 using a program designed to carry out regression and correlation analysis. The output of interest consisted of the regression coefficient (rate constant) of $\ln (OD_i - OD_\infty)/(OD_t - OD_\infty)$ *vs.* time, the intercept of the log function *vs.* time, the correlation coefficient, and the standard error of the estimate. Spot checks showed that the value obtained from the computer agreed well with those calculated by hand.

The pH of each solution was measured before and after each kinetic run at 30° on a Model 22 Radiometer pH meter and was found to remain constant.

The fraction of the imidazole buffer in the base form, $K_a/(K_a + a_H)$, was calculated at 30° employing the pH values measured for the appropriate buffers at that temperature and taking 7.10 as the pK_a of imidazole. This value of $K_a/(K_a + a_H)$ was then employed in calculating the reported second-order rate constants for imidazole-catalyzed hydrolysis of the N-acetylserinamide esters at 95°.

The pK_a of imidazole at 95° (6.11) was determined by the extrapolation of values given in the literature for the pK_a at various temperatures.[14,16–18] pK_a values at lower temperatures were determined directly. These determined values were as follows: 20°, 7.26; 40°, 6.94; 50°, 6.73.

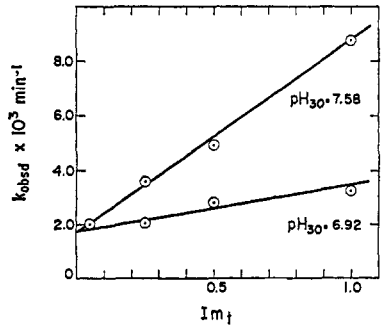

Figure 1. Plot of k_{obsd} for the imidazole-catalyzed hydrolysis of N-acetyl-O-butyrylserinamide *vs.* the total concentration of imidazole (Im + ImH^+) at 95°.

Results

It was found that the basic species of imidazole was the active form of the catalyst for the hydrolysis of each of the esters of N-acetylserinamide. This was determined by changing the ratio of the base form to the acid form of imidazole at 95°, and then plotting the observed pseudo-first-order rate constants *vs.* the total imidazole concentration. A typical plot is shown in Figure 1. It can be seen that the rate increases as the pH is increased; therefore, it is the basic form of imidazole that is catalytically active. The data are presented in Table I. To determine the second-order rate constant for imidazole catalysis, k_{obsd} was plotted *vs.* the expression $(Im)_t$ $(K_a/(K_a + a_H))$. The second-order rate constants are the slopes of the regression lines and are given in Table II. The intercept when the concentration of imidazole is equal to zero is that portion of the observed rate due to spontaneous hydrolysis by water and hydroxide ion. The values of k_s are also given in Table II.

A plot of $\log (k_{Im}/k_{Im}^0)$, where k_{Im}^0 is the second-order rate constant for the acetyl ester, *vs.* E_s, the Taft steric effects constant, for the esters of N-acetylserinamide is shown in Figure 2. The slope of this plot is 0.49 ± 0.18. The point for the isovaleryl ester deviates positively. A plot of $\log (k_s/k_s^0)$ *vs.* E_s is shown in Figure 3 where k_s^0 is the pseudo-first-order rate constant for the spontaneous hydrolysis of the acetyl ester. The slope of this plot is 0.47 ± 0.14, a value almost identical with that for the imidazole plot. The point for the isovaleryl ester fits the best line in this case.

It has already been established that imidazole acts as a nucleophilic catalyst in the hydrolysis of esters of *p*-nitrophenol, and that the basic species is the active form.[3–5] The values of k_{Im} at the different temperatures are given in Table III. Each second-order rate constant was calculated from rates measured at five different

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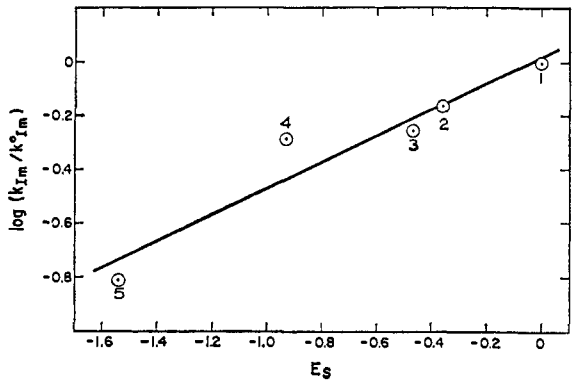

Figure 2. Plot of log (k_{Im}/k_{Im}^0) for imidazole-catalyzed hydrolysis of esters of N-acetylserinamide at 95° *vs.* E_s. The numbers pertain to the esters listed in Table II.

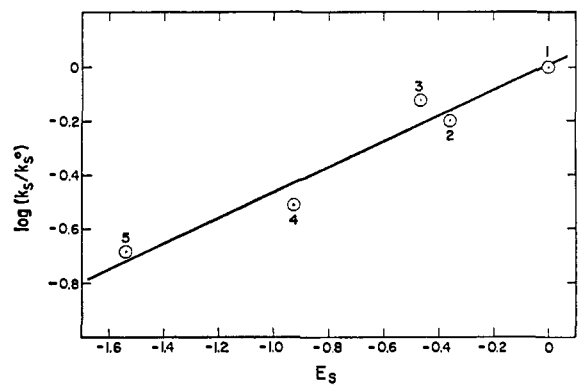

Figure 3. Plot of log (k_s/k_s^0) for spontaneous (water and hydroxide ion) hydrolysis of esters of N-acetylserinamide at 95°. The numbers pertain to the esters listed in Table II.

imidazole concentrations. The values of the activation parameters determined are given in Table IV for the esters of *p*-nitrophenol.

Table I. Pseudo-First-Order Rate Constants for the Hydrolysis of Esters of N-Acetylserinamide in Imidazole Buffers at 95°, μ = 1.0

Ester	$pH_{30°}$	Total imidazole, *M*	$k_{obsd} \times 10^3$, min^{-1}
Acetyl	7.58	1.0	14.9
		0.50	8.35
		0.25	5.52
		0.05	3.51
Acetyl	7.43	1.0	10.8
		0.50	7.42
		0.25	5.56
		0.05	3.92
Acetyl	6.92	1.0	6.32
		0.50	4.01
		0.25	2.26
		0.05	0.848
Butyryl	7.58	1.0	8.75
		0.50	4.94
		0.25	3.57
		0.05	2.01
Butyryl	6.92	1.0	3.24
		0.50	2.83
		0.25	2.08
Isobutyryl	7.58	1.0	6.79
		0.50	5.09
		0.25	3.41
		0.05	1.82
Isobutyryl	6.92	1.0	3.93
		0.50	2.06
		0.25	1.48
Isovaleryl[a]	7.43	1.0	5.68
		0.75	4.20
		0.50	3.29
Trimethylacetyl	7.58	1.0	2.12
		0.50	1.33

[a] Data was also obtained at pH 7.58, but the plot of k_{obsd} *vs.* Im_t, *M*, showed curvature.

Discussion

Comparison of the Taft steric effects plots for deacylation of acyl-α-chymotrypsins,[10] and for the two nonenzymatic systems involving nucleophilic[10] and classical general base catalysis, shows that all three are linear with differing slopes. The enzyme reaction is intermediate between nucleophilic catalysis by imidazole and general base catalysis by imidazole in its susceptibility to steric effects. Thus, the slope obtained from a plot of log (k_3/k_0) *vs.* E_s for deacylation of a series of acyl-α-chymotrypsins is 1.1,[10,19] while a slope of 1.4 was obtained for imidazole-catalyzed hydrolysis of *p*-nitrophenyl esters. In the present study a slope of

Table II. Second-Order Rate Constants for Imidazole Catalysis and Pseudo-First-Order Rate Constants for Spontaneous Hydrolysis of Esters of N-Acetylserinamide at 95° and μ = 1.0

No.	Ester	$k_{Im} \times 10^3$, l. mol^{-1} min^{-1}	$k_s \times 10^3$, min^{-1}
1	Acetyl	13.6 ± 2.2	2.62[a]
		11.0[b]	1.6[c]
2	Butyryl	9.34 ± 1.4	1.66[a]
3	Isobutyryl	7.57 ± 1.54	1.98[a]
4	Isovaleryl	7.02 ± 0.85	0.805[c]
5	Trimethylacetyl	2.10	0.543[a]

[a] $pH_{30°}$, 7.58. [b] Reference 6, 100°, μ = 0.9, and pH = 7.1. [c] $pH_{30°}$, 7.43.

Table III. Second-Order Rate Constants for Imidazole-Catalyzed Hydrolysis of Esters of *p*-Nitrophenol, μ = 1.0 *M* in 1.64% Dioxane–H_2O

Ester	Temp, °C	k_{Im}, l. mol^{-1} min^{-1}
Acetyl	20	28.2 ± 0.02
	30	49.5[a]
	40	73.3 ± 0.03
	50	102.0 ± 0.13
Butyryl	20	22.4 ± 0.02
	30	31.8[b]
	40	57.5 ± 0.14
	50	68.7 + 0.04
Isobutyryl	20	28.1 ± 0.04
	30	40.3[b]
	40	66.3 ± 0.08
	50	87.0 ± 0.11
Isovaleryl	20	5.52 ± 0.03
	30	6.02[a]
	40	13.0 ± 0.03
	50	16.2 ± 0.06
Trimethylacetyl	20	0.33 ± 0.01
	30	0.55[b]
	40	0.91 ± 0.06
	50	1.36 ± 0.05

[a] Reference 10. [b] Reference 5.

(19) Solution of the complete equation log (k_3/k_0) = $\rho^*\sigma^*$ + δE_s for values of ρ^* and δ that best fit the data gave a δ of 0.96 ± 0.01.

Table IV. Activation Parameters for the Imidazole-Catalyzed Hydrolysis of Esters of *p*-Nitrophenol, Calculated at 30°

Ester	ΔH^*, kcal/mol	ΔS^*, eu
Acetyl	7.4 ± 0.3	−34.5 ± 0.9
	7.1 ± 0.5[a]	−38.2[a]
	7.8[b]	−27.2[b]
Butyryl	6.9 ± 0.5	−37.2 ± 1.5
Isobutyryl	6.7 ± 0.2	−37.2 ± 0.8
Isovaleryl	6.9 ± 0.8	−40.4 ± 2.8
Trimethylacetyl	8.3 ± 0.1	−40.6 ± 0.3

[a] Reference 3, 5% dioxane, μ less than 0.02. [b] Reference 4, 28.5% ethanol–water (v/v), 0.2 *M* phosphate buffer, 30°.

0.49 for imidazole-catalyzed hydrolysis of esters of N-acetylserinamide was found from a plot of log (k_{Im}/k_{Im}^0) *vs.* E_s. This treatment, of course, ignores polar contributions by the acyl groups which would slightly reduce the slope. Such effects would be small due to the very small range of σ^* constants, and the enzymatic deacylation reaction and the hydrolysis of the N-acetylserinamide esters should be affected to the same extent.

It might be expected that steric effects would be less important in a general base catalyzed reaction where a proton is partially abstracted from a water molecule than in an imidazole, nucleophile-catalyzed reaction since an incipient hydroxide ion is considerably smaller than imidazole. The slope much less than unity found from the Taft plot for N-acetylserinamide esters is, however, surprising since the reaction might reasonably be expected to have nearly the same susceptibility to steric effects as the hydroxide ion or hydronium ion catalyzed hydrolysis of ethyl esters. The rates of enzymatic deacylation were measured at 25° and the hydrolysis of the *p*-nitrophenyl esters was studied at 30° so these two sets of data are directly comparable. The hydrolysis of the N-acetylserinamide esters was, however, studied at 95°. If significant differences exist in the activation energies for the various compounds in these reactions, then the slopes of the Taft plots would be markedly dependent upon temperature. The activation energies for hydrolysis of the *p*-nitrophenyl esters are, however, nearly constant (Table IV); therefore, the slope should be nearly 1.4 at any reasonable temperature. Extrapolation of the k_3 values for deacylation of acyl-α-chymotrypsins at 25°[10] to 95°, employing the known values of E_a, gives rate constants which provide a linear plot of log (k_3/k_0) *vs.* E_s with a slope of 0.7,[20] very similar to that found in the hydrolysis of esters of N-acetylserinamide. Consequently, the difference between the slopes of the experimentally determined Taft plots for deacylation and imidazole-catalyzed hydrolysis of N-acetylserinamide esters is mainly due to the experimental difference in temperature. The slopes are quite similar when compared at the same temperature but are much smaller than for the imidazole-catalyzed hydrolysis of *p*-nitrophenyl esters. The enzymatic reaction, therefore, much more nearly resembles a general base catalyzed reaction in regard to sensitivity to steric effects.

(20) A temperature of 95° is, of course, far above any experimentally feasible because of high-temperature effects on the protein. The correlation coefficient of the plot at 95° was 0.976. The point for the 3,3-dimethylbutyryl derivative showed a slight negative deviation from the best line and was not included in the correlation since that acyl group was not studied as part of the N-acetylserinamide ester series.

Evidence has thus been obtained supporting a general base role for the imidazole group at the active site of α-chymotrypsin. General base mechanisms have been previously postulated[2] mainly on the basis of the observed D_2O solvent isotope effect. It must be pointed out, however, that the enzymatic reaction does differ in certain important respects from the simple chemical model reactions. Bender and Heck,[21] for example, found that in the alkaline hydrolysis of N-acetyl-O-cinnamoylserinamide having the cinnamoyl carbonyl oxygen labeled with O^{18}, carbonyl oxygen exchange with the solvent takes place; whereas, in the deacylation of cinnamoyl-α-chymotrypsin, O^{18} exchange was not observed.

Several studies have been made of steric influences on the activation parameters in hydroxide ion and hydronium ion catalyzed ester hydrolysis. When compounds of the type CH_3COOR were studied, with steric hindrance varied in the alcohol portion of the ester,[22,23] a compensation in the activation parameters was noted with β, the isokinetic temperature, being around 300°K (270–330°K). It has been suggested[24] that this compensation may be due to steric interference with solvation since the transition state complex is an anion and since the value of β is in a range frequently associated with solvation effects. Variation of steric bulk in the acyl portion of the ester gives somewhat different results. In the acid-catalyzed esterification of various carboxylic acids in methanol,[25] ΔH^* is essentially constant, while ΔS^* varies over a range of only 4.4 eu. Highly branched carboxylic acids such as trimethylacetic and 3,3-dimethylbutyric are characterized by ΔH^* and ΔS^* values that are both more unfavorable than for straight-chain acids. In a study of the alkaline hydrolysis of a series of esters of the type RCO_2Et in 85% ethanol,[26] the differences in rate are due almost entirely to changes in E_a, except for compounds branched at the α-carbon which also show changes in ΔS^*. The variation in E_a is about 3.2 kcal over the entire series. In a study of the alkaline hydrolysis of the same esters in 70% acetone, ΔS^* is again fairly constant and E_a varies over about 4.5 kcal for the compounds studied, while in the case of acid-catalyzed hydrolysis, both activation parameters vary slightly with no obvious trend.[27] Thus, in studies made of the effect on the activation parameters of varying the steric bulk in the acyl portion of an ester, either ΔH^* or ΔS^* remains nearly constant or both parameters become more unfavorable. It is difficult, however, to be certain whether there is any real trend.[28]

The activation parameters for the imidazole-catalyzed hydrolysis of the series of *p*-nitrophenyl esters (Table IV) show the same type of behavior. ΔH^* is nearly constant, increasing by only 0.9 kcal, while ΔS^* becomes more negative over a range of 6.1 eu as steric hindrance

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is increased from acetyl to trimethylacetyl, The deacylation of acyl-α-chymotrypsins shows a marked compensation between the activation parameters with both ΔH^* and ΔS^* becoming more positive as steric bulk in the acyl group is increased.[10] The slope of the compensation plot for acyl groups that are not highly branched is 435°K.[10] Thus, again a pronounced difference can be observed between α-chymotrypsin deacylation and an imidazole, nucleophile-catalyzed reaction. Steric effects on the activation parameters of general base catalyzed hydrolysis reactions have yet to be determined.

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The Properties of Thyroglobulin. XVI. Energy Transfer to Iodoamino Acids

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Abstract: The quenching of tryptophan fluorescence by iodoamino acids in native bovine thyroglobulin and in iodinated thyroglobulin and human serum albumin has been measured. Ionization of the iodoamino acids has a greater quenching effect in human serum albumin than in thyroglobulin. From the change in tryptophan fluorescence with iodoamino acid ionization average distances have been computed between emitter and recipient residues. Critical energy transfer distances from tryptophan and tyrosine to the ionized and un-ionized forms of mono- and diiodotyrosine and thyroxine have been calculated by the Forster equation.

The fluorescent properties of proteins have been the subject of considerable recent interest both with respect to their fundamental nature and as a means of detecting structural modifications. The emission spectra of most proteins which contain tryosine and tryptophan residues resemble that of tryptophan with only a minor contribution from the tyrosyl residues.[1b,2] The quantum yields of tryptophan fluorescence in proteins show wide variations which are determined by both local (environmental) and long-range interactions. The latter arise from radiationless energy transfer processes[3] and have received comparatively less attention than the former. A notable exception is the study by Weber and Teale[4] of the quenching of tryptophan fluorescence by heme in several heme-containing enzymes and proteins.

Few studies have been directed toward evaluating energy migration between different residues in nonconjugated proteins since the conditions for energy transfer, as shown by Förster, are satisfied only from tyrosine to tryptophan and tryptophan to ionized tyrosine. Because of the difficulty in resolving tyrosine emission, only the transfer from tryptophan to ionized tyrosine has received confirmation and evaluation.[5,6] Due to the spectral shift that occurs with iodination of tyrosine, energy transfer is possible between tryptophan and iodinated tyrosyl derivatives.[7] Iodotyrosyl residues occur in native thyroglobulin. They can also be introduced into proteins without significant structural change by careful iodination.[8,9] Their influence on tryptophan emission has been assessed in native and iodinated thyroglobulin.

Native bovine thyroglobulin is a very large protein of 670,000 molecular weight and contains approximately 75 tryptophanyl, 110 tyrosyl, and 30 iodoamino acid residues (~1% iodine).[8,10] Due to the large number of tryptophans and iodoamino acids the possibilities of energy transfer are extensive. A second protein, human serum albumin (HSA), containing only a single tryptophanyl residue, was therefore selected for comparison. The molecular properties of this protein are well known from numerous investigations.[11] A recent study showed that the synthesis of several moles of monoiodotyrosine (MIT) and diiodotyrosine (DIT) by iodination did not affect its molecular properties.[12]

Materials

HSA, four times recrystallized, was obtained from Nutritional Biochemicals. This preparation gave a single symmetrical peak by velocity sedimentation and a weight-average molecular weight of 72,400 by sedimentation equilibrium by the meniscus depletion method ($\bar{v}$ = 0.733).

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