^{19}F Magnetic Resonance Spectroscopic Investigation of the Binding of *N*-Trifluoroacetylated Amino-acids by Chymotrypsin

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Summary The binding of *N*-trifluoroacetyl-D-tryptophan by chymotrypsin causes a downfield shift in the ^{19}F n.m.r. signal in the pH range 6·33—8·12 and that of *N*-trifluoroacetyl-D-phenylalanine and of *N*-trifluoroacetyl-L-tryptophan causes a downfield shift at pH 6·34.

THE shift in the ^{19}F n.m.r. signal of the sodium salt of *N*-trifluoroacetyl-D-tryptophan in the presence of chymotrypsin may be used to measure the dissociation constant of the complex formed by this inhibitor and the enzyme (K_I). Some of the values obtained are given in the Table. At all pH values there was a variation in δ of more than 4 Hz over the concentration range 5×10^{-2}—5×10^{-3}M with the concentration of chymotrypsin 2×10^{-3}M. At some pH values, *e.g.* 6·33, this variation was as large as 13·4 Hz. The direction of the shift was always downfield. The large increase in K_I at pH *ca.* 8 is similar to that reported by Johnson and Knowles[1] for the binding of *N*-acetyl-D-tryptophan to chymotrypsin, measured by equilibrium dialysis. Within experimental error ($\pm$ 0·1 Hz) the chemical shift was independent of concentration in the absence of enzyme. Care was taken to keep the pH constant over the whole concentration range studied (always to within $\pm$ 0·05). The values of K_I in the Table were calculated by the method of Nakano, Nakano, and Higuchi[2] and are in moderate agreement with those determined kinetically for the hydrolysis of *N*-furylacroyl-L-tryptophanamide.

TABLE

Binding of N-*trifluoroacetyl*-D-*tryptophan to chymotrypsin*

pH	6·33	6·89	7·72	8·12
10^2K_I (M)[a] ..	0·65	1·59	2·86	7·2
Δ(Hz)[b] ..	112	182	148	255
K_I (M)[c] ..	*ca.* 10^{-3}		*ca.* 10^{-2}	

[a] Dissociation constant of chymotrypsin–*N*-trifluoroacetyl-D-tryptophan complex determined from ^{19}F-resonance results at 94·1 MHz; trifluoroacetic acid was used as external standard. [b] Difference in chemical shift between bound and unbound *N*-trifluoroacetyl-D-tryptophan. [c] Kinetically determined inhibition constant.

There have been two previous investigations of the binding of *N*-trifluoroacetylamino-acids to chymotrypsin by ^{19}F resonance. Zeffren and Reavill reported that in citrate buffer (0·1M), at pH 6·0, there was a downfield shift of the ^{19}F signal of one enantiomer of *N*-trifluoroacetyl-DL-phenylalanine in the presence of chymotrypsin[3] but Sykes reported an upfield shift in the ^{19}F signal of *N*-trifluoroacetyl-D-phenylalanine in 0·1M-tris-HCl buffer at pH 7·8.[4] However the shifts observed were very small. Thus, the reported variation in shift for a concentration range 4×10^{-3} M—$3{\cdot}6 \times 10^{-2}$ M was less than 1 Hz with an experimental error of $\pm$ 0·1 Hz.[4] In our experiments the difference in pH between a solution of 4×10^{-3} and $3{\cdot}6 \times 10^{-2}$ M-*N*-trifluoroacetyl-D-phenylalanine in the presence of chymotrypsin (2×10^{-3} M) in a 0·1 M-tris-HCl buffer of nominal pH 7·88 was over one pH unit. The difference between chemical shifts in the presence and absence of chymotrypsin was 4·5 $\pm$ 0·2 Hz for all concentrations of *N*-trifluoroacetyl-D-phenylalanine and varied randomly. The signal moved to lower field in the presence of chymotrypsin. The ^{19}F signal of the sodium salt of *N*-trifluoroacetyl-D-phenylalanine at pH 7·96 $\pm$ 0·01 moved to lower field in the presence of chymotrypsin (2×10^{-3}M) but the shift was constant at 3·2 $\pm$ 0·2 Hz over the concentration range 5×10^{-3}—4×10^{-2}M. At pH 6·34 ($\pm$ 0·05) there was a downfield shift in the presence of chymotrypsin which varied with concentration. The value of K_I was calculated to be $4{\cdot}3 \times 10^{-2}$M, in quite good agreement with that determined kinetically (3×10^{-2}).

The ^{19}F signal of *N*-trifluoroacetyl-L-tryptophan at pH 6·34 shows a concentration-dependent downfield shift in the presence of chymotrypsin but this is too small (< 4 Hz) to allow a reliable value of K_I to be determined.

It has been concluded[5] from the reports that the ^{19}F signal of *N*-trifluoroacetyl-D-phenylalanine shows a downfield shift in the presence of chymotrypsin at pH 6·0[3] and an upfield shift at pH 7·8[4] that there is a 'major change in the magnetic environment of the fluorine atoms of the bound inhibitor with pH'. The results obtained in this investigation suggest that if there is such a change the effect on the ^{19}F-shift of the bound inhibitor is not so large as previously supposed.

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