THE N-FORMYL-L-TRYPTOPHAN-CHYMOTRYPSIN COMPLEX IN SOLUTION

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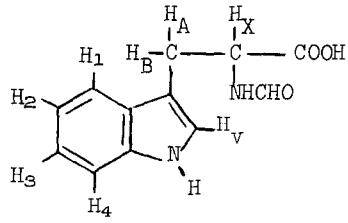
Summary. A high-resolution proton magnetic resonance study of the reversible complex formed between N-formyl-L-tryptophan and the proteolytic enzyme α-chymotrypsin has been carried out. The data indicate that the signals from the aromatic protons of the inhibitor are shifted upfield considerably in the enzyme-inhibitor complex but that the vinyl, alkyl and formyl resonances are only slightly affected. These results are consistent with the proposition that the complex has essentially the same structure in aqueous solution as determined for the crystalline state by x-ray methods.

X-ray diffraction studies of crystalline enzymes have provided remarkable insights into the nature of enzyme-substrate and enzyme-inhibitor complexes (1,2,3). When considering these results, one must constantly keep in mind that they apply strictly only to the solid state and that for each system it must be demonstrated that the descriptions of protein structure obtained by x-ray methods are applicable to solution phase experiments. Nuclear magnetic resonance spectroscopy promises to be a powerful tool for determining the correspondence between the crystalline and solution structural properties of proteins,(4) especially now that high-resolution n.m.r. studies of solid proteins may be possible (5).

Blow and coworkers have recently described an x-ray crystallographic study of the complex formed between N-formyl-L-tryptophan and the enzyme, α-chymotrypsin(2,6). We wish to report here preliminary results from a p.m.r. investigation which bear on the question of the structure of this complex in solution relative to the known solid state structure.

N-formyl-L-tryptophan was prepared by the procedure of Dalgliesh(7). The p.m.r. spectrum of this material (40 mM in D_2O solution, phosphate buffered at apparent pH 6.2) was easily interpretable in terms of previous

work(4,8) and multiplets could be assigned to each of the aromatic (H_1 - H_4) and vinyl (H_v) protons of the tryptophanyl moeity. The aliphatic protons



of the sidechain gave rise to the expected ABX spectrum. However, a considerable amount (~15%) of a second ABX pattern was in evidence, indicating that both the cis and trans rotational isomers of the amide (peptide)bond are present(9). Appreciable chemical shift and line broadening effects were observed when α-chymotrypsin (Worthington, 3X recrystallized, Lot # CPI 8LK) was present in the samples. The position of each signal in the spectrum (relative to a trace of sodium acetate included as an internal reference) was determined as a function of enzyme concentration. The resulting data were analyzed according to equation [1] as described previously(10). Here Δ represents the observed change in the chemical

$$\Delta = \frac{E_o \delta_{EA}}{A_o + K_A} \qquad [1]$$

shift of a given signal, E_o and A_o are the total concentrations of enzyme and inhibitor, respectively, K_A is the dissociation constant for the enzyme-inhibitor complex and δ_{EA} is the chemical shift of the nucleus under consideration within this complex. The chemical shift for each proton of N-formyl-L-tryptophan in the chymotrypsin complex estimated in this way are listed in Table I. Very small chemical shift effects were observed for the vinyl (H_v), formyl or AB signals; the tertiary proton (X) is located too close to the water peak to permit accurate measurements of the chemical shift of this nucleus. However, the chemical shifts of the four aromatic protons (H_1 - H_4) are substantially shifted to higher field in the enzyme-bound environment.

Table I

Observed and Calculated Chemical Shift Effects for the N-Formyl-L-tryptophan-α-Chymotrypsin Complex[a]

Proton	δ_{EA}, obs.[b]	δ_{EA}, corr.[b,c]	δ_{EA}, calc.[b,d]
H_1	0.64	0.85	0.16, -0.05
H_2	0.74	0.99	0.40, 0.15
H_3	0.55	0.73	0.10, 0.06
H_4	0.37	0.49	0.0, -0.11
H_V	0.01	0.01	~0, ~0
H_A	0.10	0.13	~0, -0.04
H_B	0.13	0.17	~0, -0.09
Formyl	-0.04	-0.05	~0, ~0

[a] pH 6.6, 31°C

[b] In p.p.m., estimated accuracy ± 0.1 p.p.m.

[c] Corrected to 100% enzyme activity

[d] The first number is the computed aromatic ring current effect while the second refers to a crude estimate of the effect of the anistropy of the carbonyl groups at ser-190, try-215, ser-189 and ser-214.

We began our interpretation of these large chemical shifts by noting that in the crystalline state the aromatic side chain of the tyrosine-228 residue of the enzyme lies moderately close to the tryptophan ring of the inhibitor in the complex, as crudely depicted in Figure 1. Using the published atomic coordinates for the complex(6), we estimated the ring current effect at protons H_1 - H_4 that should arise from this side chain (11). These estimates are included in Table I. Minor adjustments(12) in the position of the acyltryptophan relative to the tyrosine ring can improve the agreement between the observed and calculated effects. However, since

the quantitative validity of the Johnson-Bovey model for the ring current effect at positions perpendicular to the plane of an aromatic system has

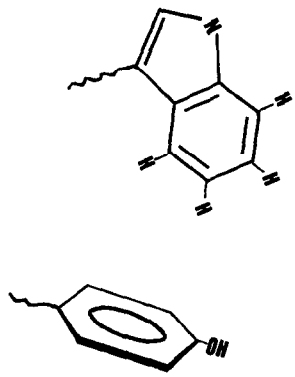


Figure 1

not been established(4), and because the influence of substituents on this effect are uncertain, only the qualitative conclusion that the ring-current from the tyrosine-228 residue can produce chemical shifts of the correct magnitude and direction at each aromatic proton of the inhibitors molecule can be made at this point. We also attempted to estimate the effect of the amide carbonyl groups that are in the vicinity of the inhibitor molecule in the complex by using the diagrams provided by Jackman and Sternhell(13). These estimated contributions to the shieldings of the various nuclei of N-formyl-L-tryptophan in the enzyme-bound state do little to improve the agreement between observed and calculated chemical shifts. The quantitative aspects of the Pople model for anisotropic shielding effects for the carbonyl group are not completely reliable and we do not as yet regard these discrepancies as serious.

The computed aromatic proton shifts for the inhibitor are about 0.5 p.p.m. too low. We have observed that tosylchymotrypsin binds N-formyl-L-tryptophan and produces nearly equal upfield chemical shifts of about this magnitude at the aromatic protons of the inhibitor. The presence of the

tosyl group at the serine-195 residue of the enzyme should prevent interaction of the acylamino acid at the active site but should not prohibit binding at other localities on the protein. In the crystalline enzyme, there are four other binding sites per molecule that apparently have a high affinity for formyl-L-tryptophan and it is presumably these sites that are responsible for the chemical shift effects observed with the tosylated enzyme (6). A part of the discrepancy between calculated and observed chemical shifts in the native enzyme system can, therefore, be ascribed to additional protein-inhibitor interactions of the same nature. Also, the crystallographic study indicates that the binding locus for the tryptophan ring of the inhibitor is lined with hydrocarbon side chains and it is possible that a solvation effect on chemical shifts arises when this ring is transferred from an aqueous to a water-poor environment. This effect should be relatively nonspecific and could result in upfield shifts of this magnitude (14,15,16). Thus, a position-specific ring current effect acting in concert with non-specific influences derived from binding at protein sites other than the active center and from solvation changes can account for the direction and order of the enzyme-induced chemical shifts of the various aromatic proton of N-formyl-L-tryptophan if one assumes that the chymotrypsin-inhibitor complex has virtually the same structure in aqueous solution as that found in the solid state. No appreciable chemical shift effects at the vinyl, aliphatic or formyl positions would be expected on this basis since these nuclei are quite removed from the tyrosine side chain and appear to be well-exposed to the solvent. Our n.m.r. data, while not yet entirely satisfactory in a quantitative way, thus are consistent with the proposition that the enzyme-inhibitor complex formed between α-chymotrypsin and N-formyl-L-tryptophan has similar, if not identical, structures in the solid and aqueous solution phases.

Acknowledgements

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