AN NMR STUDY OF A TYROSINE AND TWO HISTIDINE RESIDUES IN THE STRUCTURE OF PORCINE PANCREATIC COLIPASE

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1. Introduction

Pancreatic colipase is a small protein of approx. mol. wt 11 000 [1]. Its primary structure and the position of three of the five disulfide bridges have been determined, while there is still ambiguity about the position of the additional two [2,3]. The crystal structure of colipase has not been reported.

Colipase participates in the hydrolysis of triglycerides in the duodenal lumen as a cofactor to the active enzyme, pancreatic lipase [4]. Its primary function is to counteract the desorbing effect of bile salts on lipase by anchoring the lipase molecule to the triglyceride surface [5,6]. The interactions between colipase and the triglyceride surface in the presence of bile salts has been shown to be of polar nature [7]. It is thus of interest to investigate the ionizable side-chains in colipase which might be responsible for the colipase–triglyceride interaction.

NMR can be a powerful tool in the studies of the conformation of proteins in solution [8] and in the determination of the pK_a values of specific amino acid residues in proteins, such as histidines, tyrosines and lysines [9]. This paper is the first in a series of high resolution NMR investigations on the structure and properties of colipase. In this investigation we have determined the pK_a values of the two histidines in colipase and made a tentative assignment of the resonances to the amino acids in the sequence. We also conclude that one of the tyrosines and one histidine residue are closely related in space.

2. Experimental

Porcine pancreatic colipase II was prepared as described [10]. Samples were dissolved in 99.8% D_2O (Stohler Isotope Chemicals) and exchangeable protons in colipase were replaced by deuterons by heating at 50°C for several minutes. The pH was measured with a Radiometer pH meter with a glass-electrode. The pH was adjusted with 1 M solutions of KOD and DCl. Proton NMR spectra were recorded using a Bruker WH-270 FT NMR spectrometer with a Nicolet 1085 computer. Chemical shifts are quoted in ppm downfield from DSS (2,2-dimethyl-2-silapentan-5-sulphonate).

3. Results and discussion

The NMR spectrum of colipase was presented [11]. The colipase molecule studied in that investigation contained 84 amino acid residues. The colipase molecule studied in the present investigation contained 5 additional amino acid residues in the N-terminal

and 11 in the C-terminal. This difference in amino acid composition does not seem to have any major effect on the colipase NMR spectrum, although differences in the chemical shift values of some resonances in the aromatic region are observed.

3.1. *Histidine titrations*

The aromatic region (δ_{DSS} 6–9 ppm) of the NMR spectrum of colipase at different pH values is shown in fig.1. The chemical shift of the C(2) proton resonance of histidine normally varies with pH within the range 7.7–8.7 ppm. The exact position of the resonance is dependent on the ionization state of the imidazole ring and a titration curve may be obtained when the chemical shift is plotted as a function of pH [9]. Resonance I and II show a titration behavior characteristic for C(2) protons and the pK_a values were determined by a best-fitting procedure of the curves in fig.2. The apparent pK_a value of histidine I, resonance I, was thus determined to be 7.8. We ascribe the somewhat high pK_a value to the proximity of negatively-charged groups [12]. There is a slight inflexion of the titration curve at low pH. This could be due to changes in ionization state of an acid side-chain in the proximity of histidine I having a pK_a value of about 5.2, e.g., glutamic acid which would be consistent with the observation above.

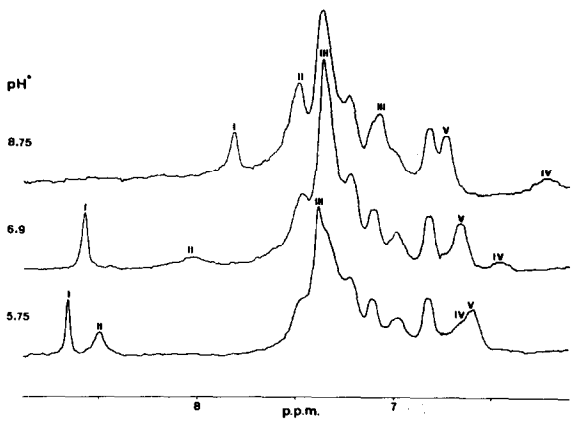

Fig.1. Aromatic region (δ_{DSS} 6–9 ppm) of colipase II. The colipase concentration was 4 mM in D_2O and 150 mM NaCl at a pH* (pH meter reading in D_2O) of 8.75, 6.9, 5.75. The resonances are labelled I–V as described in the text. 500 scans were recorded in 8 K memory with a repetition time of 0.7 s.

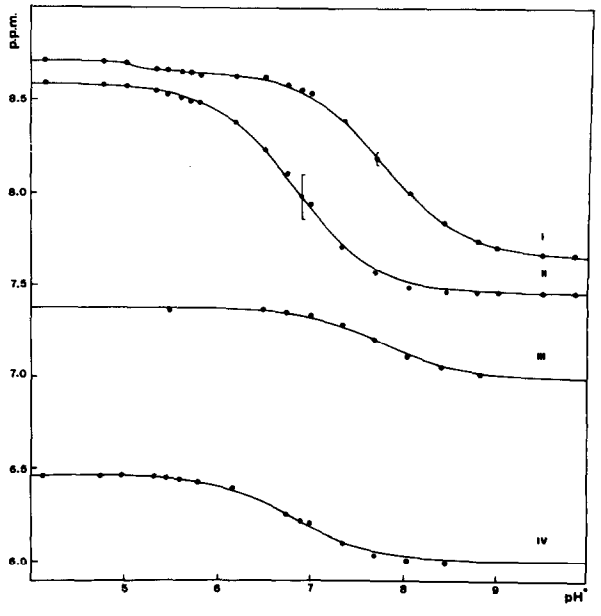

Fig.2. pH titration curve of the C(2) and C(4) proton resonances of the two histidyl residues of colipase (resonance I–IV). The brackets indicate the line-width of the resonances.

Histidine II was found to have an apparent pK_a value of 6.9. As is evident in fig.1 the C(2) proton resonance (II) broadens considerably at intermediate titration levels. This is interpreted as due to a slow protonation–deprotonation rate of the imidazole ring [13].

The C(4) proton of a histidine titrates in the same manner as the C(2) proton although at higher field and within a smaller chemical shift range, 6.9–7.5 ppm [9]. The C(4) proton resonance of histidine I, although difficult to resolve, titrates within a normal chemical-shift range. The C(4) proton of histidine II, resonance IV, on the other hand has a considerably lower chemical-shift value in the pH range studied. The simplest explanation for this upfield shift, 0.8 ppm, is that there is a ring current-induced shift arising from a nearby aromatic side-chain.

3.2. *pH effects on tyrosine resonance*

The resonance at 6.8 ppm and a resonance at 7.4 ppm are shown by mutual decoupling to be a pair of doublets (fig.3). These signals change chemical shift with pH with a pK_a value of ~10.5 and from

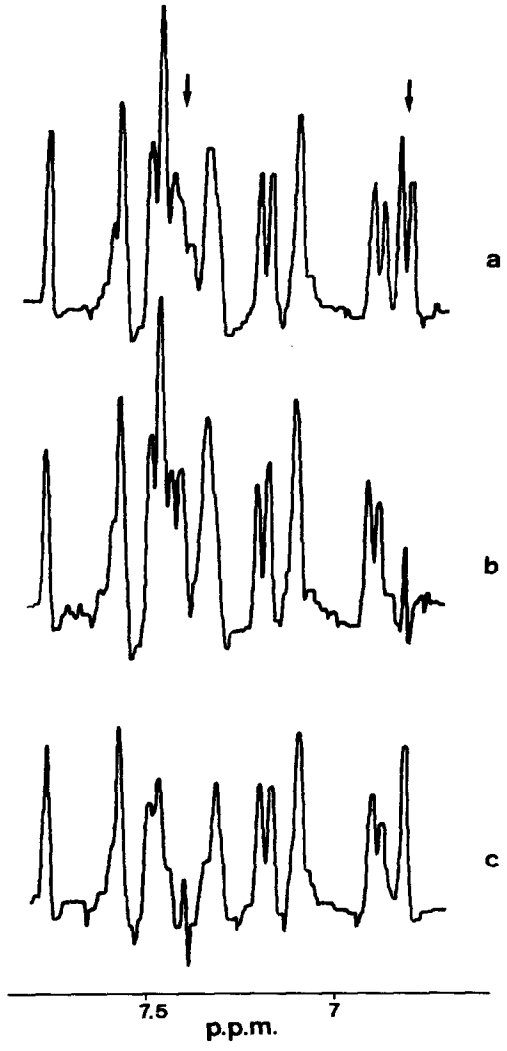

Fig.3. 270 MHz spectra of 2 mM colipase at pH* 10.0 and temp. 40°C showing the spin decoupling of the resonances of a tyrosine residue. The resolution has been enhanced by using the convolution difference technique [15]. (a) Undecoupled spectrum. (b) Irradiation at 6.8 ppm causes decoupling at 7.4 ppm. (c) Irradiation at 7.4 ppm causes decoupling at 6.8 ppm.

this information it is clear that they correspond to the *meta* and *ortho* protons of one of the three tyrosine residues in colipase. The chemical shift of the resonance from the *ortho* protons of this tyrosine also shows a strong pH dependence at low pH (fig.1). No observable shifts of the *meta* protons were noticed.

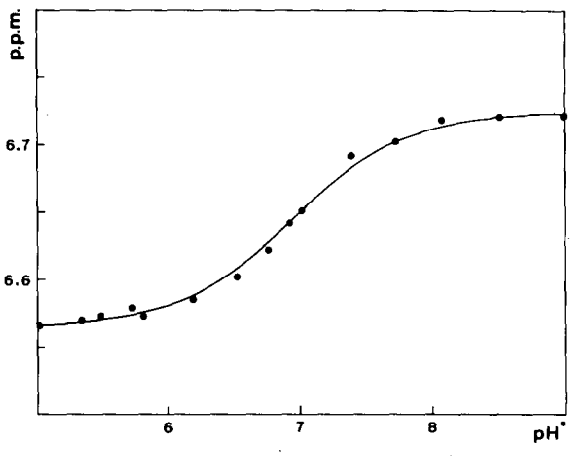

Fig.4. The pH dependence of chemical shift of the *ortho* proton resonance of one of the tyrosines in colipase (resonance V in fig.1).

If the chemical shift of the *ortho* protons is plotted against pH the resulting curve may readily be fitted to a titration curve as above (fig.4). The value of the inflexion point was calculated to be 6.9. The shift of this resonance is thus clearly correlated to the ionization state of histidine II. We interpret this upfield shift as being due to the deshielding effect of a local magnetic field within a cone on either side of the protonated histidine ring (fig.5). According to simple ring current calculations [14] the *ortho* protons of the tyrosine should be positioned within this cone at a distance from the imidazole ring of 4–6 Å. The lack of shifts of the *meta* protons suggests that the *meta* protons are positioned near an angle of 55° to the normal of the plane of the histidine ring, where the ring current field is negligible.

The upfield-shifted position of the histidine II C(4) proton resonance might be a consequence of the

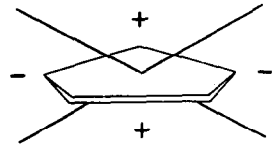

Fig.5. Schematic drawing of a protonated histidine imidazole ring with space region in which protons will experience different magnetic fields causing upfield (+) or downfield (–) shift of the NMR-signal.

ring current effect from this tyrosine, although contributions from other aromatic side-chains cannot be neglected. Whatever the case, the normal chemical-shift value of the C(2) proton could be explained in a similar manner as was done for the *meta* protons of the tyrosine residue.

3.3. *Spatial arrangement of the histidines*

It is very difficult to state from the sequence information, which of the two histidines corresponds to a specific residue in the amino acid sequence. It seems, however, likely that histidine I corresponds to His 30. This histidine has close negatively-charged groups which could account for effects on the titration curve mentioned above [12]. Furthermore, histidine II and a tyrosine must be closely related in space in order to cause the above-mentioned shifts. If the ring current effects discussed above are of mutual nature the distance between the two side-chain rings should be in the order 4–6 Å, with the planes parallel or close to parallel.

Addition of the bile salt, taurodeoxycholate, causes significant shifts and line-broadening effects on the tyrosine I and histidine II resonances. It seems likely therefore, that the suggested arrangement of these two residues forms a part of the binding area for bile salts.

Acknowledgements

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