PROTON NUCLEAR MAGNETIC RESONANCE OF HISTIDINE RESIDUES IN REINDEER PANCREATIC RIBONUCLEASE

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1. Introduction

The pK values of the four histidines at positions 12, 48, 105, and 119 in bovine pancreatic ribonuclease have been determined from the titration behaviour of the $C_{(2)}$ proton resonances in the n.m.r. spectrum of this protein [1–3]. Similar experiments performed on rat pancreatic ribonuclease showed many differences between the n.m.r. properties of the histidines in bovine and rat ribonuclease [4].

A study of the histidine resonances in chinchilla and coypu pancreatic ribonucleases led to a tentative assignment of these resonances to the four histidines at positions 12, 48, 80, and 119 in these two enzymes [4]. A titration curve with a high pK value (Coypu: 8.0; chinchilla: 7.2) has been tentatively assigned to histidine 80 in both enzymes. Pancreatic ribonuclease from most mammalian species contain a histidine residue at position 80 [5–7, unpublished]. Bovine and rat ribonuclease are two of the few exceptions. Ribonucleases from deer species differ relatively little in amino acid sequence from bovine ribonuclease [6, unpublished] and possess a histidine at position 80. So, an investigation of the histidine resonances in a deer ribonuclease can give us more information about the properties of a histidine residue at position 80.

In this article we describe the n.m.r. properties of the histidine residues in the pancreatic ribonuclease from reindeer (*Rangifer tarandus*). The amino acid sequence of this enzyme has been determined recently (unpublished). Histidines occur at positions 12, 48, 80, 105, and 119.

2. Materials and methods

Reindeer pancreatic ribonuclease was isolated by affinity chromatography [8]. The sample used was desalted several times by gel filtration on Sephadex G-25 in 0.1 M acetic acid before it was used for the n.m.r. experiments.

All experimental procedures were as described before [4]. The pH values are direct meter readings without D_2O correction. Proton n.m.r. spectra were obtained at 31°C with approx. 2.0 mM ribonuclease solutions in 99.75% D_2O containing 0.2 M NaCl in 12 mm sample tubes at 100 MHz with a Varian XL100-15 spectrometer. A signal-to-noise enhancement was obtained by multiscan averaging with a Varian C1024 computer of average transients.

3. Results

Fig. 1A and 1B show the results of the titration experiments. In the presence of 8mM 3′-CMP* (4-fold excess) five titration curves could be distinguished and from the similarity of four of these curves to the titration curves of the histidines in the complex of bovine ribonuclease with 3′-CMP [4,9] we arrived at the assigments given in fig. 1B.

Interpretation of the results in the absence of 3′-CMP was more difficult. A detailed comparison of the

* *Abbreviations:* p.p.m.–parts per million of the magnetic field; 3′-CMP–cytidine-3′-monophosphate; 2′-CMP–cytidine-2′-monophosphate.

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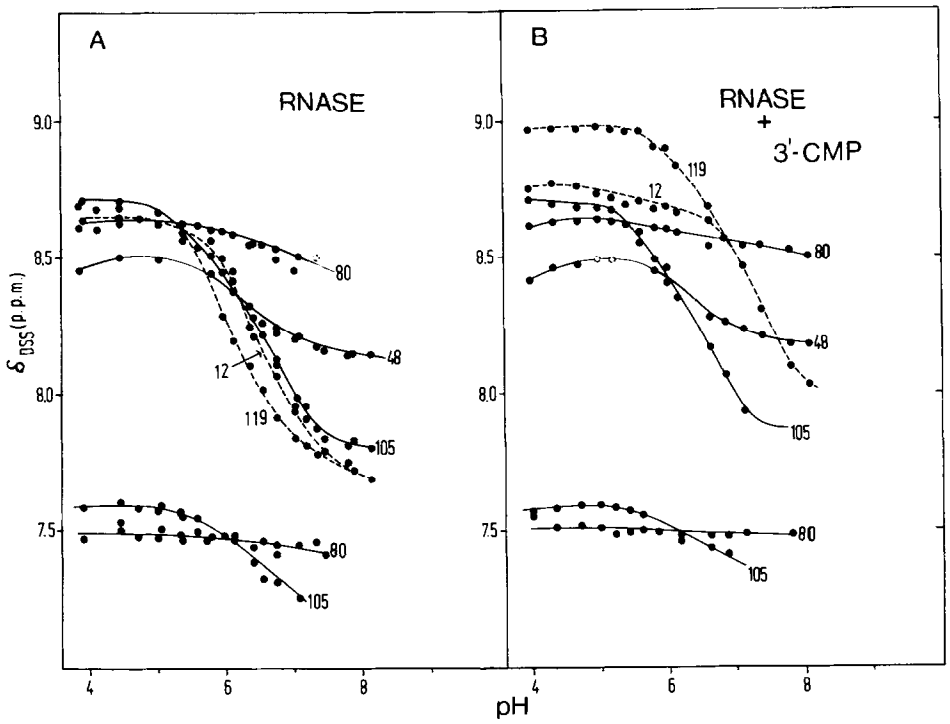

Fig. 1. Titration curves of histidines in reindeer ribonuclease in the absence (fig. 1A) and presence (fig. 1B) of excess 3′-CMP. The lower curves correspond to two $C_{(4)}$ proton resonances and the upper curves to $C_{(2)}$ proton resonances of the five histidines. Dashed lines indicate the titration curves of the active site histidines 12 and 119. The peaks of histidine-48 broaden and disappear in the pH region 5.0–6.5 and those of histidine 80 at pH values higher than 7.5. DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate.

n.m.r. spectra of reindeer ribonuclease with and without 3′-CMP obtained at each pH value has been made. Peaks which show large down-field shifts in the presence of 3′-CMP have been assigned to the active site histidines 12 and 119. The other peaks, which are essentially unaffected by 3′-CMP, can be assigned to the other three histidines. After making this discrimination between the active site histidines and the other histidines, we were able to draw the titration curves for reindeer ribonuclease as shown in fig. 1A. The curves of the active site histidines are indicated by dashed lines.

Discrimination between the curves of histidine 12 (pK 6.5) and 119 (pK 6.1) could be obtained from correspondence with bovine ribonuclease by following the positions of the peaks of these two histidines at pH 6.0 with increasing amounts of inhibitor up to about fourfold excess.

The $C_{(2)}$ proton curve with a pK value of 6.5, which corresponds with a $C_{(4)}$ curve with the same shape at higher field, has been assigned to histidine 105, because it shows a similar behaviour at titration as the curve of histidine 105 in the bovine enzyme. The curve with a pK value of 6.3 has been assigned to histidine 48. This histidine shows a characteristic up-field shift at low pH values, a down-field shift at high pH values and broadening and disappearance of the peaks at intermediate pH values, similar to the homologous histidine in the bovine enzyme [10,11]. This leaves the remaining fifth curve for histidine 80. The peaks of this histidine broaden and disappear at pH values higher than 7.5.

Table 1 summarizes the pK values of the histidine residues in reindeer ribonuclease and compares these values with those obtained earlier for other ribonucleases [4]. Near 7.5 p.p.m. two curves have been obtained for $C_{(4)}$ resonances. The normal curve already has been assigned to histidine 105. The other one,

which is influenced very little by pH, corresponds to the $C_{(2)}$ curve of histidine 80.

4. Discussion

The amino acid sequences of reindeer (unpublished), red deer [6] and bovine [12] pancreatic ribonuclease are shown in fig. 2. Bovine and reindeer ribonuclease differ at 14 positions. Five involve changes in charge. The total excess of positive charges in three less than in the bovine enzyme. This may explain the higher pK values of the active site histidines in reindeer ribonuclease as compared to the bovine enzyme (table I).

The n.m.r. properties of histidine 48 in reindeer ribonuclease differ from those of the homologous histidine in the bovine enzyme in that sharp peaks are produced at pH values higher than pH 6.5 and that there is no clear influence of the addition of 3′-CMP on the position of the peaks in the entire pH region investigated.

Table 1
pK values of histidine residues in the pancreatic ribonucleases from reindeer, cow, rat, chinchilla and coypu

Position	pK values				
	Reindeer	Cow	Rat	Chinchilla	Coypu
12	6.5	6.3	6.6	6.0–6.1	6.3
119	6.1	5.8	6.2		
48	6.3	6.4	7.6	4.9	5.8
73	–	–	6.1	–	–
80	> 7.0	–	–	7.2	8.0
105	6.5	6.7	6.3	–	–

The assignment of the titration curves with high pK values to histidine 80 in the ribonucleases from chinchilla and coypu [4] has been confirmed by the

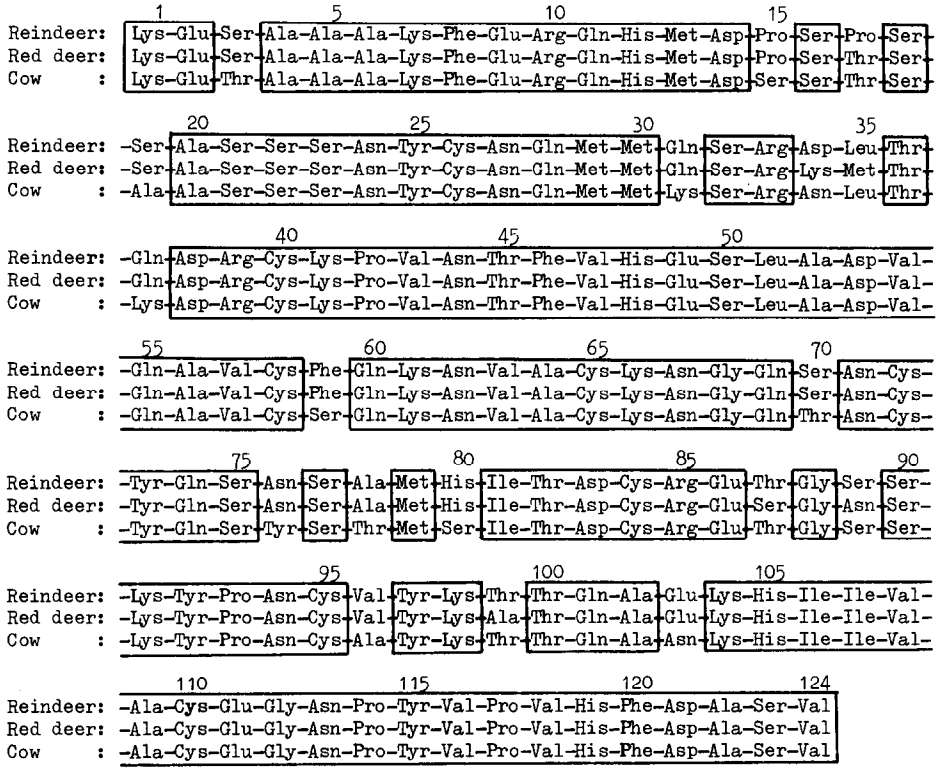

Fig. 2. Amino acid sequences of the pancreatic ribonucleases from reindeer (unpublished), red deer ([6]; sequence 15–23 corrected (unpublished)) and beef [12]. Identical residues in the three ribonucleases are enclosed in blocks.

results with reindeer ribonuclease. In reindeer, like in chinchilla and coypu, this histidine also exhibits a $C_{(4)}$ proton peak down-field of the aramatic envelope.

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