Reassignment of the Histidine ^1H Nuclear Magnetic Resonances of Ribonuclease-A

By J. HOWARD BRADBURY* and JOO SENG TEH
(*Chemistry Department, Australian National University, Canberra, A.C.T., Australia*)

Summary The previous assignment of two of the four H-2 histidine resonances in ribonuclease-A to residues 12 and 119 is shown to be incorrect; at pH > 5 the 119 residue resonates downfield of the 12 residue.

THE ^1H n.m.r. spectrum of the H-2 histidine protons of ribonuclease-A was first observed as separate resonances at 60 MHz[3] and then at 100 MHz.[4] The basis of the assignment of the four resonances to particular residues in the protein[1] involved an extrapolation of the results obtained with ribonuclease-S to ribonuclease-A. This extrapolation was questioned[5] and another method used to check the original assignment.[2] However subsequent studies[6–9] have indicated inconsistencies in the previous data.[2] A re-investigation described here shows the source of the error and consequently the assignment of residues 12 and 119 is reversed.

TABLE

Areas of H-2 histidine resonances relative to large aromatic resonance

Protein sample		Numbering of histidine residues 12	48	105	119
Ribonuclease-A	Control	1·01	0·86	0·96	0·96
	Deuteriated	0·73	0·89	0·27	0·45
S-Peptide	Control	1·04		—	—
	Deuteriated	0·75	—	—	—
S-Protein	Control	—	0·87	1·10	0·90
	Deuteriated	—	0·86	0·44	0·66

Phosphate-free ribonuclease-A (Worthington Biochemical Corp.) was partially deuteriated at pH 7·15 for 6 days at 35 °C.[2,9] Native and partially deuteriated products were converted into the S-protein and S-peptide using an established procedure,[10] except that the phosphate was removed from ribonuclease-S by using Sephadex G-25 in 0·1 M acetic acid (pH 3) in order to prevent back exchange. For this reason also the column separation of ribonuclease-S at pH 6·5 was performed at 4 °C instead of 25 °C. All ^1H n.m.r. measurements were made in D_2O with no added salt at the pH meter readings indicated.[11]

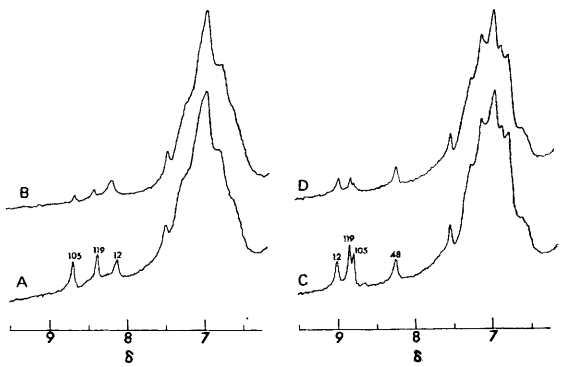

FIGURE 1. ^1H n.m.r. spectra at 100 MHz in D_2O of the aromatic region of native (A,C) and partially deuteriated (B,D) ribonuclease-A at pH 5·6 (A,B) and pH 3·1 (C,D). The new assignment of peaks is shown in A and B at pH 5·6; the crossover of peaks as the pH is reduced to 3·1 (C,D) has been shown by partial deuteriation[11] and agrees with other workers.[7,8]

The assignments of the H-2 protons of histidine 105 and 48 as shown in Figure 1 is firmly established from previous work.[1,2] The point of controversy surrounds the resonances labelled 12 and 119 in Figure 1A. In the partially deuteriated spectrum (Figure 1B) it is seen that histidine 119 is deuteriated much more rapidly than histidine 12. We would therefore expect the deuteriated S-peptide (which consists of residues 1—20) to show one residual histidine resonance of comparable area to that of histidine 12 in Figure 1B. This is indeed the case (see Figure 2B and Table). In addition, the areas of the three H-2 histidine protons (48, 105, and 119) in the deuteriated S-protein (Figures 3B and 3D) should be consistent with the areas in Figures 1B and 1D. Although the area measurements on

the S-protein are open to considerable error, owing to the broadness of the peaks and overlaps,[9] the results in the Table confirm the correctness of the new assignment.

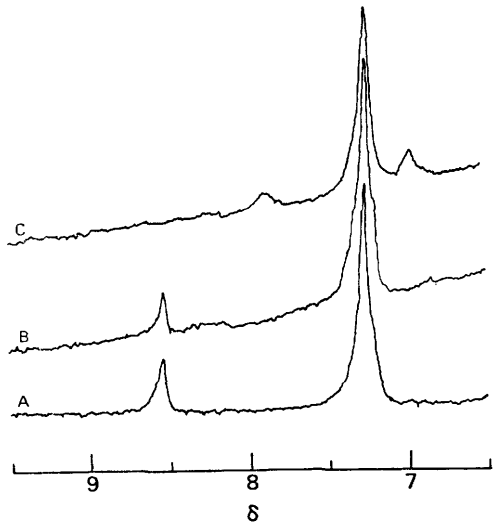

FIGURE 2. ^{1}H n.m.r. spectra at 100 MHz in D_2O of the S-peptide of ribonuclease-A: (A) undeuteriated, (B) deuteriated sample at pH 4 and 10 °C, and (C) deuteriated sample at pH 7 and 10 °C.

Figure 2C shows the deuteriated S-peptide spectrum recorded at pH 7 and the H-2 peak at δ 7·91 corresponds with a small broad peak in the previous spectrum (Figure 4B of ref. 2) at *ca.* 310 Hz from HOD. This is now seen as the residual H-2 resonance from the partially deuteriated S-peptide, but was not recognized as such in the previous work,[2] because of its breadth and upfield position compared

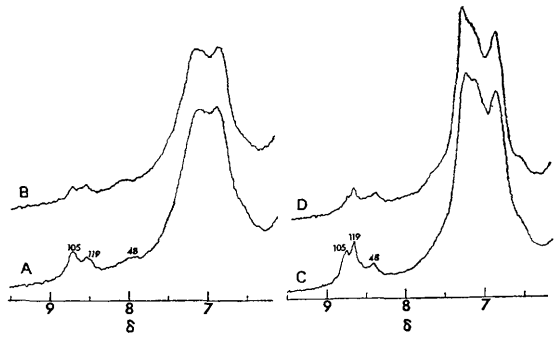

FIGURE 3. ^{1}H n.m.r. spectra at 100 MHz in D_2O of the S-protein of ribonuclease-A: undeuteriated S-protein (A,C), deuteriated S-protein (B,D) at pH 5·5 (A,B) and pH 3 (C,D). The labelling of peaks is based on relative rates of exchange of H–2 protons of histidines 105 and 119 and will be confirmed later.[9]

with the H-2 resonance of Figure 4A of ref. 2, which was erroneously thought to have been recorded at pH 7; in fact it was recorded at pH 5·25.[1] Further confirmatory experiments will be reported subsequently.[9] We conclude that the previous assignment[1,2] of the H-2 histidine resonances of residues 12 and 119 of ribonuclease-A should be reversed.

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