Histidine H-2 N.M.R. Resonances of Sperm Whale Oxy-, Carbonyl-, and Met-myoglobin

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Summary Seven H-2 histidine n.m.r. resonances of sperm whale metmyoglobin and eleven resonances of both carbonyl- and oxy-myoglobin are assigned and pK' values determined; there are six titration curves from surface histidines which are similar in the three derivatives, three others which are similar in the carbonyl and oxy derivatives, and one other (the distal histidine) which has a low pK' in the former and a normal pK' in the latter derivative.

SPERM whale myoglobin has twelve histidine residues.[1] The proximal histidine (residue 93) is directly bound to the iron atom of the haem and thus does not titrate.[2] In earlier n.m.r. studies on paramagnetic metmyoglobin[3–6] seven H-2 resonances were observed and assigned, in diamagnetic oxymyoglobin ($Fe^{II}O_2Mb$) nine were observed,[7] and in diamagnetic carbonylmyoglobin ($Fe^{II}COMb$) we observed all eleven titrating H-2 and H-4 histidine resonances.[2] We report here the observation of eleven titrating H-2 histidine resonances in oxy- and carbonyl-myoglobin and the firm assignment of eight of these.

Sperm whale iron(III) myoglobin (Sigma) was converted into $Fe^{II}COMb$[8] and $Fe^{II}O_2Mb$ (by modification of a previous method[9]) and spectra were obtained at 270 MHz in ^2H_2O at various pH values uncorrected for deuterium isotope effects.

The titration curves (not shown) and pK' values for the seven observable histidine residues of metmyoglobin agree well with those obtained previously.[3–5] Furthermore, six of these titration curves are reproduced quite closely in the carbonyl- and oxy-myoglobin spectra (see Figures 1 and 2). Thus using the previous assignments[3–5] the first six entries in the Table are assigned to histidine residues 81, 12, 48, 116, 119, and 113. These are at some distance from the iron atom and therefore their titration characteristics are relatively insensitive to either the type of ligand or whether the derivative is diamagnetic or paramagnetic.

TABLE. Assignments of the H-2 histidine resonances and pK' values of histidines in myoglobins.

	Metmyoglobin	$Fe^{II}COMb$ (Figure 1)	$Fe^{II}O_2Mb$ (Figure 2)	Histidine residue number
Curve	1	1	1	81
pK'	6·36	6·11	6·32	
Curve	2	2	2	12
pK'	6·52	6·20	6·45	
Curve	3	3	3	48
pK'	6.70	6·49	6·68	
Curve	4	4	4	116
pK'	6·88	6·69	6·88	
Curve	5	7	7	119
pK'	5·48	5·56	5·72	
Curve	6	8	8	113
pK'	5·50	5·87	5·60	
Curve	7	5	5	36
pK'	8·03	6·72	6·85	
Curve		10	10	97[a]
pK'		6·20	6·28	
Curve		11	11	64
pK'		4·53[b]	6·65	
Curve		9	9	82[a]
pK'		6·57	6·60	
Curve		6	6	24[a]
pK'		5·96	5·71	

[a] Tentative assignments. [b] This value is appreciably lower than our previous result,[2] but agrees better with other work.[9]

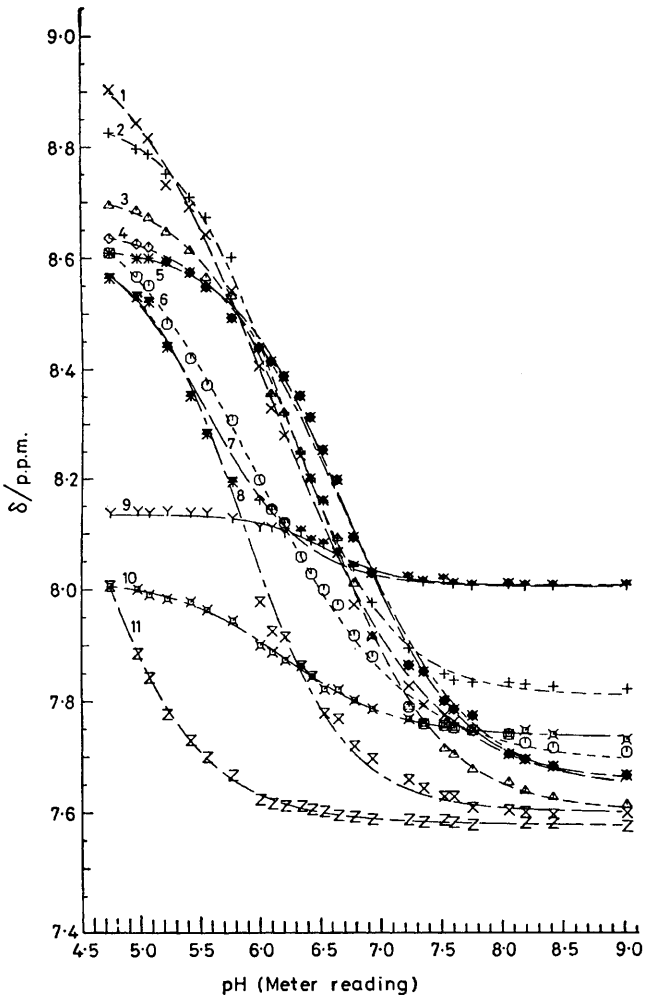

FIGURE 1. ^1H N.m.r. titration curves at 270 MHz and 40 °C for H-2 histidine resonances of $Fe^{II}COMb$.

Curve 7 in metmyoglobin was previously assigned to histidine 36; its upfield position was attributed to proximity to Phe-106 and the high pK to proximity to Glu-38.[5,6] We do not observe such a titration curve in $Fe^{II}O_2Mb$ at 20 °C (in contrast to Ohms *et al.*[7]) or in $Fe^{II}COMb$ at 40 °C[2] or 20 °C. Upon treatment of metmyoglobin in ^2H_2O–

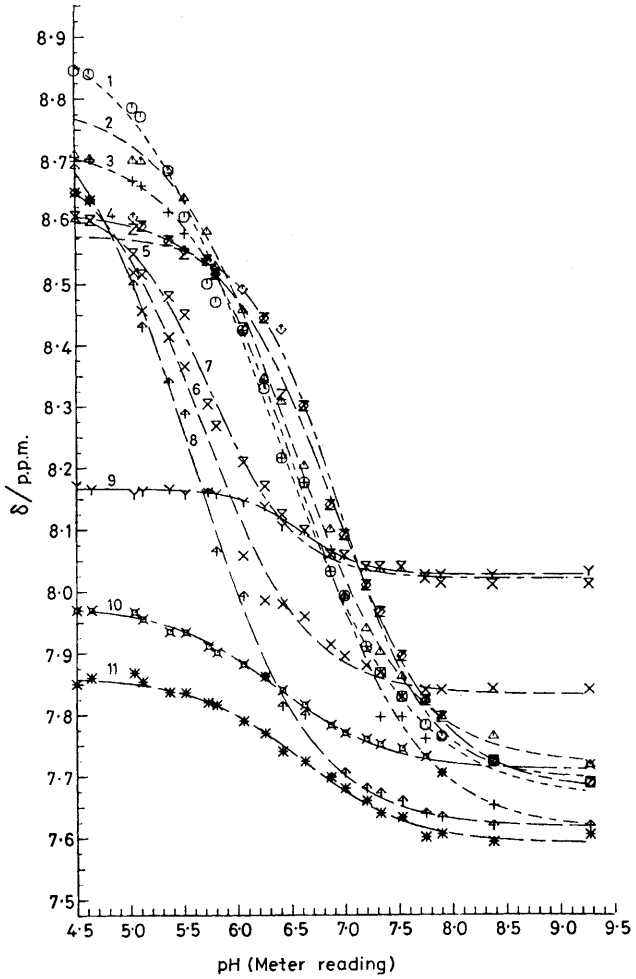

FIGURE 2. ^{1}H N.m.r. titration curves at 270 MHz and 20 °C for H-2 histidine resonances of $Fe^{II}O_2$Mb. At low pH all resonances remain sharp except peak 11 which becomes quite broad, corresponding to greatly decreased stability of the oxy-derivative.

0·1 M NaCl at pH 8 and 40 °C for 22 days, all seven H-2 resonances were removed by deuteriation. The product was converted into Fe^{II}COMb and in the n.m.r. spectra, seven H-2 resonances were absent, corresponding to the six resonances already assigned plus resonance 5 (Figure 1 and Table). Resonance 5 in Fe^{II}COMb and $Fe^{II}O_2$Mb must therefore come from histidine 36. In these derivatives, His-36 is in a normal environment which probably corresponds closely with that observed in the crystal, in which there is a water molecule interposed between His-36 and Glu-38,[1] whereas in metmyoglobin it is proposed that simple rotation about the C^{α}–C^{β} and C^{β}–C^{γ} bonds brings His-36 close to Glu-38.[6]

The remaining titratable histidine residues 24, 64, 82, and 97 cannot be carboxymethylated[10,11] and are not deuteriated (see above). They are therefore probably inaccessible to solvent (although inability to deuteriate the H-2 proton occurs for residues of low pK[12]) which corresponds with the known environments of these residues.[1] There are four titration curves remaining unassigned. Curves 6, 9, and 10 are almost identical to each other in Figures 1 and 2 and have correspondingly similar pK' values. The similarly numbered curves clearly arise from the same histidine residues. The fourth pair of curves (number 11) occur in similar perturbed upfield positions but whereas the pK' is low in Fe^{II}COMb, it is normal in $Fe^{II}O_2$Mb. This curve has been previously assigned to the distal histidine residue (His-64)[3,7] and since this residue would be expected to be the most sensitive to a change of ligand, the normalisation of the pK' in $Fe^{II}O_2$Mb confirms the assignment. Curve 10 is in a perturbed upfield position at low pH, which would be consistent with its assignment to His-97 which is close to the porphyrin ring and agrees with a previous tentative assignment.[7] Curve 6 has a low pK' value and has been tentatively assigned to His-24, since this is hydrogen bonded to His-119, which has a low pK' because of its proximity to Arg-118. Curve 9 represents a perturbed histidine residue which could correspond to His-82 which is doubly hydrogen bonded to Asp-141 and to Gly-80 through a water molecule.[1] The last three assignments are tentative.

The normalisation of the pK' of the distal histidine in going from the carbon monoxide to the oxygen form may be due to a shift of the imidazole ring away from Arg-45, and possibly also the iron atom, in such a way that the ring current shift due to the porphyrin ring is not appreciably changed. This movement, and the possibility of the histidine acting as a 'gate,' has important implications for the binding of ligands. Work is in progress on confirmation of the assignment of the H-2 resonances and an analysis of the H-4 histidine resonances.[2]

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