

$A^{13}\text{C}$ -NMR study of mutant hemoglobins with altered oxygen affinity

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The ^{13}CO -NMR spectra of carbonylhemoglobins Saint Mandé (β 102Asn $\rightarrow$ Tyr), Malmö (β 97His $\rightarrow$ Gln), Hôtel Dieu (β 99Asp $\rightarrow$ Gly) and A_0 have been determined. The positions of the ^{13}CO resonances for hemoglobins A_0 , Malmö and Hôtel Dieu were similar indicating similar ligand environments for all. The ^{13}CO resonance for the β -subunit of Saint Mandé was upfield-shifted compared to the others. This is evidence that structural changes at the β 102 position directly affect iron-ligand bonding as well as quaternary structure.

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1. INTRODUCTION

The ^{13}CO -NMR spectrum of heme-bound CO reflects the ligand environment. In HbACO and MbCO reconstituted with 2,4-disubstituted deuterohemes, ^{13}CO feels electronic effects at the porphyrin periphery in a manner predictable from the Hammett constants σ , ρ [1]. The ^{13}CO resonance of Hb Zürich CO (β 63His $\rightarrow$ Arg) also depends upon the nature of the distal residue [2]. The solvent was found to influence the ^{13}CO resonance position of a carbonyl heme protein model, *N*-methylimidazolecarbonylprotoheme [3].

Here we report the ^{13}C -NMR spectra of three carbonylhemoglobins with mutations at the $\alpha_1\beta_2$ interface and a short interpretation of the results. HbMö and HbHD are high-affinity hemoglobins

with reduced cooperativity [4,5]. Both mutations are adjacent to β 98Val, which is positioned close to heme pyrrole II (Fischer nomenclature) (fig.1). The poor functions of HbHD, Hb Kempsey (β 99Asp $\rightarrow$ Asn) and Hb Yakima (β 99Asp $\rightarrow$ His) are due to loss of the hydrogen bond, in the deoxy form, between the β 99Asp and the β 42Tyr-94Asp residues across the $\alpha_1\beta_2$ interface. HbSM is a low-affinity mutant with reduced cooperativity, and the mutation is located close to the heme pyrrole 3-methyl group. This mutation is similar to HbK (β 102Asn $\rightarrow$ Thr) in which the hydrogen bond β 102Asn/ α 94Asp, of importance to the ligated state in HbA, is lost [6]. Two previous reports have concluded that the ^{13}CO resonances of HbCO do not respond to mutations at the $\alpha_1\beta_2$ interface [7,8].

2. MATERIALS AND METHODS

Hemoglobins were isolated by chromatography in potassium phosphate, 1 mM EDTA and stored in liquid nitrogen as in [9]. The metHB contents

Abbreviations: Hb A, normal hemoglobin A_0 ; HbHD, hemoglobin Hôtel Dieu; HbK, hemoglobin Kansas; HbMö, hemoglobin Malmö; HbSM, hemoglobin Saint Mandé; Mb, myoglobin; δ , chemical shift in ppm from internal DSS

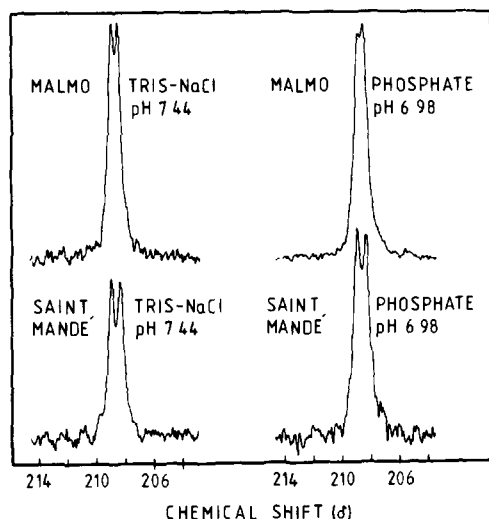


Fig.1. The ^{13}CO -NMR spectrum of mutant hemoglobins Malmö and Saint Mandé. From 60000 to 220000 transients were accumulated for each sample. The 'pH' of the gently stirred samples was determined immediately after each experiment.

were measured by the cyanide method using $\epsilon = -3.0 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ and were found to be HbA, 4.5; HbMö, 1.3; HbHD, 9.5; HbSM, 5.1% metHb. For experiments in 10 mM phosphate, 1 mM EDTA, pH 7.0 buffer, equal volumes of ^2H buffer were added and the samples concentrated to

2.5–4 mM heme under nitrogen. For experiments in Tris, samples were passed through a column of Sephadex G-25 equilibrated with 0.1 M Tris, 0.1 M NaCl, 1 mM EDTA, pH 7.4, and then diluted with $[^2\text{H}]$ Tris buffer and concentrated as above.

Concentrations were determined on the oxygenated Hbs using $\epsilon_{576} = 15.15 \text{ mM}^{-1} \cdot \text{cm}^{-1}$. All ^{13}C -NMR spectra were obtained under identical conditions at 5.85 T using 16K real data points, internal ^2H lock, and a controlled probe temperature of 25°C . The pulse repetition rate was 0.8 s and the digital resolution 0.047 ppm. Spectra were smoothed for display by 5 Hz line-broadening and four examples are shown in fig.2. Because of the adverse effects of DSS on proteins the chemical shifts, well reproducible, are reported using the observed DSS frequency of an HbACO + 1% DSS sample as reference. ^{13}C -NMR measurements were made on a Bruker WM-250 instrument operating at 62.89 MHz. Electronic spectra were recorded on a Beckman DU-7 spectrophotometer. pH values in $^2\text{H}_2\text{O}$ are given as read on a Radiometer instrument and electrode and are denoted 'pH'.

3. RESULTS

The resonance positions of CO bound to the various Hbs are given in table 1. The chemical shift

Table 1
 ^{13}C chemical shifts of ^{13}CO bound to normal and mutant human hemoglobins

Hemoglobin	Subunit	Tris-NaCl, 'pH' 7.4 δ	Phosphate, 'pH' 7.0 δ	$\Delta\delta_{\alpha-\beta}$	$\Delta\delta_{\alpha-\beta}$
A ₀	α	208.91	208.87	0.43	0.35
	β	208.48	208.52		
Malmö β 97His-Gln	α	208.89	208.81	0.43	0.35
	β	208.46	208.46		
Hôtel Dieu β 99Asp-Gly	α	—	208.78	—	0.35
	β	—	208.43		
Saint Mandé β 102Asn-Tyr	α	208.97	208.93	0.62	0.59
	β	208.35	208.34		

differences ($\Delta\delta$) between the α - and β -chains for HbA (≈ 0.4 ppm) are in excellent agreement with other studies [2]. For HbMöCO in Tris, the positions of the ^{13}CO resonances are very close to and $\Delta\delta$ exactly equal to that observed for HbACO. For HbMöCO and HbHDCO in phosphate they are again precisely as observed for HbACO though both α and β resonances are shifted about 0.1 ppm upfield compared to HbACO. The $\Delta\delta$ values for HbSMCO in both Tris and phosphate buffers (≈ 0.6 ppm) are larger than that observed for any other HbCO. Most of this is due to the upfield shift of the ^{13}CO resonance of HbSMCO, which suffers increasing shielding of at least 0.1 ppm more than any other β resonance.

These resonance positions are uniformly 1.5 ppm to low field compared to earlier reports [7,8]. This effect is probably not due to inclusion of $^2\text{H}_2\text{O}$ in the samples, since the oxygenation isotherm has been shown to be unaffected by $^2\text{H}_2\text{O}$ [10]. The major cause of this difference is probably bulk susceptibility because an internal standard (DSS) was used here.

4. DISCUSSION

Since the resonance positions of ^{13}CO bound to the subunits of the high-affinity HbMö and HbHD and to HbACO are very similar, the electronic and steric environments must be similar. The ^{13}CO bound to the low-affinity β -subunit of HbSM is shifted to higher field than with other Hbs. Two earlier studies failed to find any difference in the resonance positions between HbACO and the related low-affinity HbK, probably because of the low field strengths then available [7,8]. Our observed shift is consistent with a reduction of bond strength between β -subunit iron and ligand according to previous work [1]. A plausible reason for this would be that changes in conformation at the $\beta 102$ residue, due to loss of the $\beta 102\text{Asn}/\alpha 94\text{Asp}$ hydrogen bond, are communicated to the β iron-ligand bond. This finding is consistent with a previously proposed mechanism of Hb

cooperativity which points to the indirect coupling between iron and hydrogen bonding at the $\alpha_1\beta_2$ interface [11]. This proposed mechanism also predicted strong hydrogen bonding between the $\beta 99\text{Asp}/\alpha 42\text{Tyr}-94\text{Asp}$ pairs, which has been recently confirmed by resonance Raman spectroscopy [12].

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REFERENCES

- [1] Moon, R.B., Dill, K. and Richards, J.H. (1977) *Biochemistry* 16, 221–228.
- [2] Choc, M.G. and Caughey, W.S. (1981) *J. Biol. Chem.* 256, 1831–1838.
- [3] Perkins, T., Satterlee, J.D. and Richards, J.H. (1983) *J. Am. Chem. Soc.* 105, 1350–1354.
- [4] Wiechelman, K.J., Fairbanks, V.F. and Ho, C. (1976) *Biochemistry* 15, 1414–1420.
- [5] Blouquit, Y., Branconnier, F., Galacteros, F., Arous, N., Saria, J., Zittous, R. and Rosa, J. (1981) *Hemoglobin* 5, 19–31.
- [6] Atha, D.H., Johnson, M.L. and Riggs, A.F. (1979) *J. Biol. Chem.* 254, 12390–12398.
- [7] Moon, R.B. and Richards, J.H. (1974) *Biochemistry* 13, 3437–3443.
- [8] Shulman, R.G., Ogawa, S., Mayer, A. and Castillo, C.L. (1973) *Ann. NY Acad. Sci.* 222, 9–39.
- [9] Smith, M.L., Hjortsberg, K., Romeo, P.H., Rosa, J. and Paul, K.G. (1984) *FEBS Lett.* 169, 147–150.
- [10] Viggiano, G., Ho, N.T. and Ho, C. (1979) *Biochemistry* 18, 5238–5247.
- [11] Smith, M.L. and Paul, K.G. (1983) *Inorg. Chim. Acta, Bioinorg. Chem.* 79, 178–179.
- [12] Copeland, R.A., Dasgupta, S. and Spiro, T.G. (1985) *J. Am. Chem. Soc.* 107, 3370–3371.