NMR STUDIES OF ^{13}CO-HEMOGLOBIN. α AND β CHAIN IDENTIFICATION

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1. Introduction

In a preceding paper [1] it was shown that the resonance signal of ^{13}CO bound to the hemes of human hemoglobin was split while only a single resonance line was present in the case of ^{13}CO myoglobin. It was suggested that the two resonance lines of hemoglobin might correspond to ^{13}CO bound to the α and to the β chains of the molecule. These results have been confirmed and more accurate values of chemical shift have been assigned [2] to the two resonance signals. The measurement of ^{13}CO resonance might then offer the possibility to measure directly the distribution of the CO bound to hemoglobin between the α and the β chains provided that it is known which resonance signal corresponds to which chain. A tentative assignment has been made on the basis of kinetic data [2]. With this aim we have determined the NMR spectrum of the ^{13}CO derivatives of the isolated hemoglobin α and β chains. The data obtained are reported in the present note.

2. Materials and methods

Isolated α and β chains were prepared by the method of Bucci and Fronticelli [3] as modified by Geraci et al. [4]. The chains were electrophoretically homogeneous, free of *p*-chloromercuribenzoate and their equimolar mixture migrated as a single band with the same electrophoretic mobility of hemoglobin. The sample solutions in 50 mM potassium phoshate pH 7.0 were concentrated in dialysis bags covered with dry Sephadex G-100 (Pharmacia). The concentrated solutions were used within three days after the preparation.

The ^{13}CO derivatives were prepared slowly flushing 90% enriched ^{13}CO (Stohler Isotope Chemicals, Switzerland) over the solutions gently stirred into stoppered test tubes. The ligand state of the proteins were checked spectrophotometrically.

NMR spectra were measured using 1.2 ml of sample solutions covered with a layer of paraffin oil in 12 ml tubes, at room temperature. The instrument used was a high resolution carbon-13 Fourier NMR spectrophotometer (Varian XL—100) operating at 25.15 MHz. The apparatus included a noise-modulated proton-decoupler. $^{13}CS_2$ in a capillary inserted within the NMR tubes was used as reference compound.

3. Results and discussion

The ^{13}C spectra of the ^{13}CO derivatives of hemoglobin and isolated α and β chains are shown in fig. 1 and the values of the chemical shifts relative to $^{13}CS_2$ in ppm are listed in table 1. The hemoglobin spectrum shows two resonance lines, relative to the ^{13}CO at −13.71 and −13.25 ppm respectively, in agreement with the values reported by other authors [2] *.

The spectra of the isolated chains, in contrast to hemoglobin, and similarly to myoglobin [1, 2] show

* The two resonance lines were incorrectly reported at approximately −17 ppm in a previous paper. This inaccuracy was essentially due to the much larger sweep width (6000 Hz) used in order to monitor the spectrum of ^{13}C resonance of the amino acids.

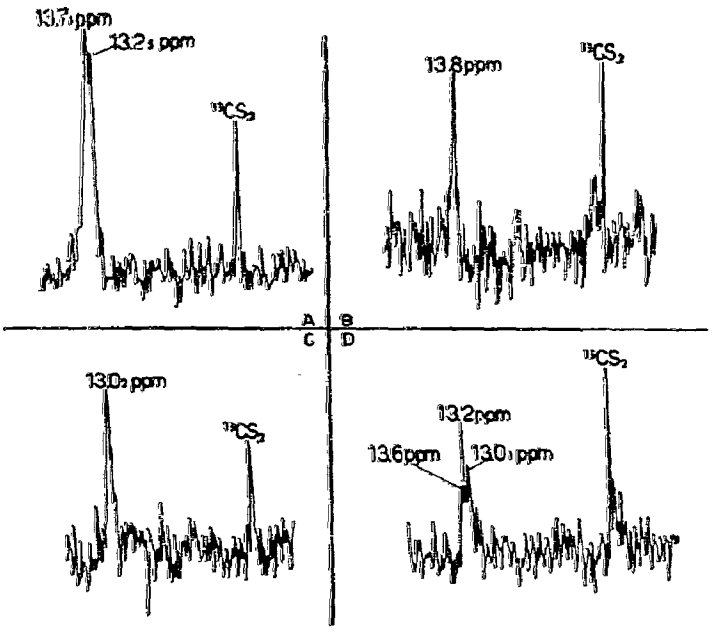

Fig. 1.: A) ^{13}C – NMR spectrum of [^{13}CO]hemoglobin, 15 mM; b) ^{13}C – NMR spectrum of [^{13}CO]α chains, 15 mM; C) ^{13}C – NMR spectrum of [^{13}CO]β chains, 15 mM; D) 13C – NMR spectrum of 5 mM α + 10 mM β chains obtained by mixing 0.5 ml of B plus 1 ml of C.

a single resonance line relative to ^{13}CO woth chemical shift values −13.8 ppm for the α chains and −13.02 ppm for the β chains (table 1 and fig. 1a, b). Thus, the lower field resonance at −13.71 ppm, which is present in the hemoglobin, compares directly with the resonance of isolated α chains. The other resonance of hemoglobin instead appears shifted down-field (about 0.25 ppm) with respect to that of the isolated β chains. Although this difference is small, it suggests that the ^{13}CO resonances of the chains are affected by the specific chain–chain interaction in the hemoglobin tetramer.

In order to check this result, we measured the ^{13}C NMR spectrum of ^{13}CO in mixtures containing different amounts of α and β chains. As it is shown in fig. 1 and table 1, in the presence of an excess of β chains, three different resonance lines are observed with chemical shift values in agreement with those of hemoglobin and β chains respectively.

Changes in heme properties as a result of α–β interaction have been observed before by optical spectroscopy both in the case of the deoxygenated and ligand bound derivatives [5, 6]. However, it was impossible

Table 1
^{13}CO chemical shifts resonances in ppm from $^{13}CS_2$.

Hemoglobin	−13.25	−13.71	–
α Chains	–	−13.8	–
β Chains	−13.02	–	–
(α + β) Chains	−13.2	−13.6	−13.02

to distinguish between the contribution of α and β chains to the hemoglobin spectrum. In contrast the resonances of the heme bound ^{13}CO are still sufficiently distinctive to allow the identification of the contribution of α and β chain to the NMR spectrum of the assembled hemoglobin tetramer.

In passing from the isolated simple chain molecules, α and β_4 to the hemoglobin tetramer $\alpha_2\beta_2$, the β chain ^{13}CO resonances exhibit the larger shift. This may indicate that, at least in the ligand bound derivatives, the effect of chain–chain interaction is greater on the β rather than on the α chains.

The identification of the ^{13}CO resonances gives the possibility to measure directly the equilibrium distribution of ^{13}CO between the α and β chains of hemoglobin at different CO saturation levels. The high concentration required for the measurements of ^{13}CO resonance appears particularly convenient since it permits working even with erythrocyte suspensions.

Acknowledgement

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