NATURAL ABUNDANCE ^{13}C SPECTRA OF PROTEINS: CARBOXY-MYOGLOBIN AND HEMOGLOBIN

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1. Introduction

In the past few years the high resolution NMR technique has been successfully applied in studies of biologically important molecules. However, due to the large number of the protons present, the proton NMR spectra of the proteins studied [1–3] are so poorly resolved that a detailed complete interpretation has never been possible. Despite this complexity, the assignments of some resonances to particular groups was possible, when their relative changes furnished some indications of the conformational variations occurring. This is so of the proton resonances of the amino acid residues near to the heme group in hemoglobin and myoglobin (i.e. histidine residues) which are differently affected in the oxy and deoxy forms by the ring current, contact, and pseudocontact shifts [3]. However, no detailed and complete analysis of the spectra has been achieved.

The relative simplicity of the ^{13}C natural abundance spectra resulting from large chemical shifts, absence of any spin-spin coupling, relatively narrow lines not seriously broadened by dipolar interactions, suggests that better resolution and more detailed information can be obtained from ^{13}C NMR spectra. The chief obstacle to date has been the low sensitivity resulting from the character of the ^{13}C nucleus itself and from its low natural abundance (~ 1 per cent) [4, 8]. This limitation has been overcome by the use of the Fourier transform NMR technique [9, 10], In this paper we report the results obtained with this technique for myoglobin and hemoglobin.

2. Methods

The experiments were carried out with a high-resolution carbon-13 Fourier NMR spectrometer (Varian XL100) operating at 25.15 MHz, in 12 mm tubes at room temperature. The apparatus included a noise-modulated proton-decoupler.

The concentrations of the samples were about 10^{-3} M in heme.

3. Results and discussion

Figs. 1, 2 and 3 show the ^{13}C spectra of carboxy-sperm whale myoglobin, horse myoglobin and hemoglobin respectively.

The chemical shift values are in ppm from the $^{13}CS_2$ resonance peak as external reference.

From the data both as observed [11, 14] and as calculated from Grant's rules [15, 16] relative to the amino acids, as well as from those obtained with di-, tri-, and deca-peptides, we have tried to obtain a composite spectrum relative to horse myoglobin which is reported in fig. 1B. Because of the lack of data the resonances relative to the tyrosine, tryptophan, asparagine, and glutamine amino acid residues and to the heme group are not reported in the composite spectrum. Despite the roughness of this approach, some interesting conclusions can be derived.

In principle the experimental spectrum and the composite one, seem to be in good agreement.

In general one may object that the comparison between the ^{13}C resonances of model compounds

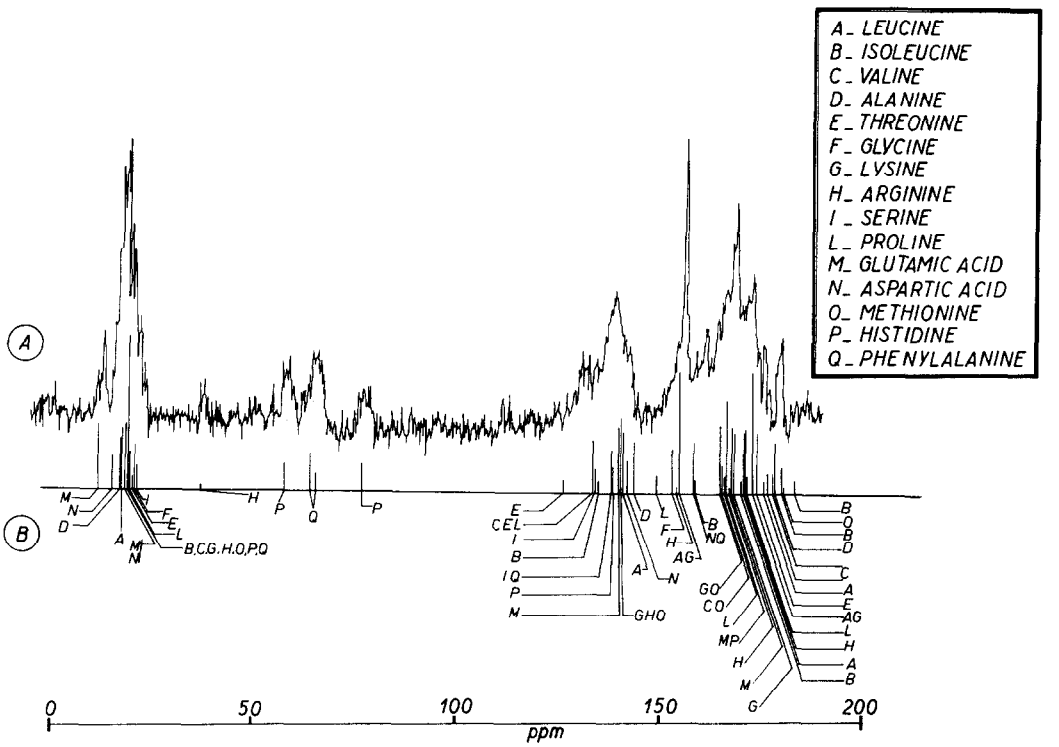

Fig. 1(A). ^{13}C-NMR spectrum of sperm whale myoglobin. (B) Composite spectrum based on predicted chemical shifts of amino acids.

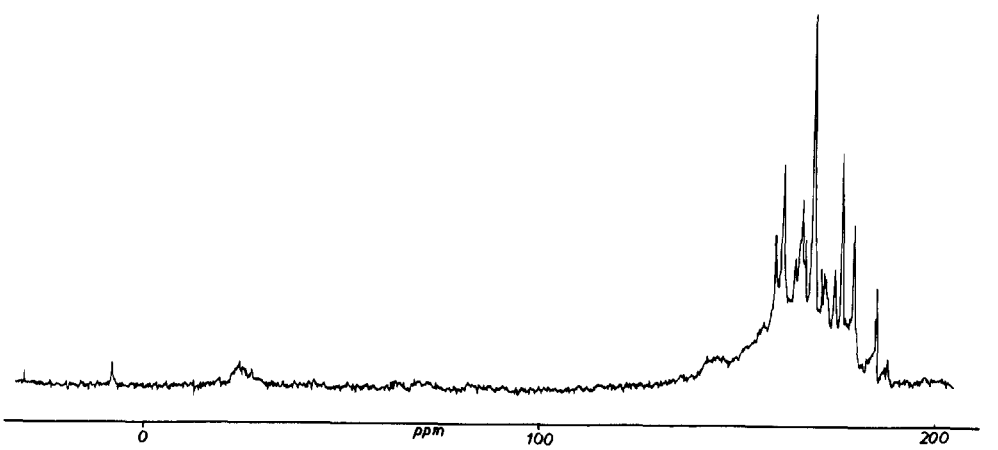

Fig. 2. ^{13}C-NMR spectrum of horse hemoglobin.

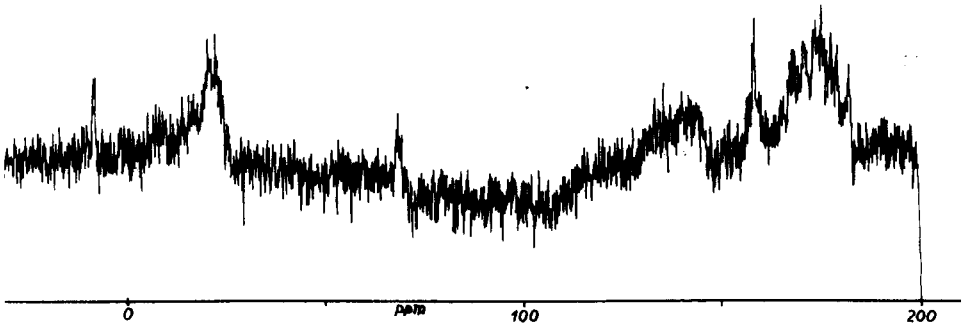

Fig. 3. ^{13}C-NMR spectrum of human hemoglobin.

(amino acids, di- and tri-peptides) and those of residues present in the protein chains themselves is not necessarily valid, as it is not for protons [3, 17, 18]. In this regard it should be pointed out that the paramagnetic term is dominant over conformational effects certainly present but which do not determine to the first approximation the main features of the spectrum. The main support of this conclusion is the general similarity of the computed spectrum with the experimental one.

However in some resonance regions (i.e. relative to C_α, aromatic and to $>C=O$) the experimental spectrum shows some differences compared with the composite one, both in the multiplicity of the resonance lines as well as in their intensity.

By analogy with the results obtained for proton resonances [17, 18], the multiplicity of the ^{13}C resonance lines suggests a dependence of the chemical shift on conformational effects as well as on the presence in the molecules of particular groups like heme, aromatic rings, etc. If this is so, it seems evident that a close inspection of better resolved and detailed spectra can be a very useful tool in conformational studies.

Marked discrepancies are revealed by comparisons between the intensities of different regions of the same experimental spectrum. While there is a good agreement between the predicted and the experimental resonance intensities in the high field region, the intensities of the carbonyl and aromatic resonances are strongly reduced with respect to the predicted ones. The same feature was observed in the ^{13}C spectra of gramicidin-S [13], but never in those of amino acids and dipeptides [11, 14].

A possible explanation of this effect is that it may be due to the quadrupolar relaxation effect of the ^{14}N nucleus as well as to the different correlation times (10^{-6} sec for proteins and 10^{-11} sec for small molecules).

An interesting effect is seen with the ^{13}CO resonances of carbon monoxide. While ^{13}CO in water solution shows a resonance peak at +12 ppm (high field, $^{13}CS_2$ zero) in the compounds examined the resonance peaks are at ~ -12 and -17 ppm from $^{13}CS_2$ for myoglobin and hemoglobin respectively, in a chemical shift range comparable with the $Fe(CO)_5$ carboxyl signals (-19 ppm). Further, with myoglobin only a single sharp resonance is present, with hemoglobin this resonance seems to be split or at least much broadened. This may be due to differences in the binding of the CO to the myoglobin heme and to the α- and β-chain hemes of hemoglobin.

Acknowledgements

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