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Haemoglobin-$^{17}O_2$ Revisited

HAEMOGLOBIN derives its ability to bind oxygen reversibly from the particular type of bond formed between the haem-iron and molecular oxygen. So far X-ray crystallography[1] has not resolved conclusively the structure of this bond.

Maričić and co-workers[2] reported that they had determined the structure of the haemoglobin–oxygen bond by studying the nuclear magnetic resonance (NMR) signal of $^{17}O_2$ bound to haemoglobin. Because the only signal which they detected was a singlet, they concluded that both oxygen nuclei reside in identical electronic environments. This supported Griffith's proposal[3] that the haem-iron oxygen bond is a three centre bond. Maričić and co-workers[4] reported that the sample on which their NMR measurements were made contained neither oxygen nor haemoglobin—bacteria had metabolized $^{17}O_2$ into $H_2{}^{17}O$, the observed signal, and deoxyhaemoglobin had oxidized to methaemoglobin. No attempt was made to examine authentic oxyhaemoglobin.

In view of the considerable interest that this problem has generated, we report here the results of our investigation of the ^{17}O NMR spectrum of oxyhaemoglobin.

As will soon become obvious, the method by which oxy-haemoglobin solutions are prepared and the conditions in which their spectra are measured are of crucial importance in this problem. Therefore, experimental details are given below.

Solutions of haemoglobin (rabbit) were prepared by the method of Drabkin[5]. To avoid contamination by bacteria, erythrocytes were lysed in a toluene–water mixture. After dialysis the haemoglobin solution was concentrated to 30% (w/v) by the method of Kohn[6]. Natural abundance water ($^{17}O = 0.037\%$) in the concentrated haemoglobin solutions was replaced with depleted water ($^{17}O = 0.009\%$, from the separation plant of this institute) by successive dialysis of the haemoglobin solution against saturated depleted water solutions of $(NH_4)_2SO_4$ and freshly lyophilized depleted water. The resulting solution displayed an absorption maximum at 415 nm (ref. 7), indicating that it was free of methaemoglobin.

A sample of 2 ml. of 30% haemoglobin in depleted water was degassed in a tube (outer diameter 1.5 cm) by three freeze–thaw cycles. Labelled oxygen (from the separation plant of this institute) (95% ^{17}O, 10 ml. (STP), 4.24×10^{-4} mol) was added to the sample and the resulting pressure of $^{17}O_2$ above the solution was 1 atm.

The ^{17}O NMR spectra of fresh oxyhaemoglobin solutions were measured at room temperature on a Varian DA-60 spectrometer with a V4210 variable frequency unit set at 8.0 MHz. Spectra were recorded for a range of 20,000 p.p.m. up and down field with combinations of audio frequency modulation amplitudes and r.f. powers, ranging from 0.5 to 5.0 and 0.1 to 1 gauss, respectively. To obtain maximum sensitivity, the spectra were measured as the first derivative of the dispersion mode.

No increase in the amplitude of $H_2{}^{17}O$ signal was detected in the samples when $^{16}O_2$ was replaced with $^{17}O_2$. Furthermore, the $H_2{}^{17}O$ signal did not increase in amplitude while the NMR measurements were being made, which took several days for each sample. The visible absorption spectrum of each sample taken at the completion of NMR measurements showed no change in the absorption maximum at 415 nm. These data indicate that neither incorporation of ^{17}O from $^{17}O_2$ into H_2O nor decomposition of oxyhaemoglobin to methaemoglobin had occurred in our samples.

Scanning 20,000 p.p.m. up and down field from the $H_2{}^{17}O$ signal, no signal other than the weak signal of depleted water was observed. The fact that when $^{17}O_2$ (3.74×10^{-5} g atom ^{17}O) was bound to deoxyhaemoglobin no increase in the $H_2{}^{17}O$ signal (4.9×10^{-6} g atom ^{17}O) was observed rules out the possibility that a narrow oxyhaemoglobin signal is buried under the signal of $H_2{}^{17}O$.

Because the electric quadrupole moment of the ^{17}O nucleus renders the band-width of the ^{17}O NMR signal quite sensitive to the rate of tumbling of the nucleus, we suspect that the slow rate of tumbling of the haemoglobin molecule broadened the $^{17}O_2$ signal beyond recognition. We are now studying the ^{17}O NMR signal of $^{17}O_2$ bound to relatively smaller synthetic oxygen carriers, to develop techniques which are sensitive enough to detect the oxyhaemoglobin signal.

CHARLES S. IRVING
AVIVA LAPIDOT

Isotope Department,
Weizmann Institute of Science,
Rehovot

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Erratum

IN the article "Role of Different Sponge Cell Types in Species Specific Cell Aggregation" by H. A. John, M. S. Campo, A. M. Mackenzie and R. B. Kemp (*Nature New Biology*, **230**, 127; 1971), on page 128, "and the aggregates were the colour of most of the mucoid cells" should have read, "and the aggregates were the colour of the majority, that is, the mucoid cells".

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