CONFORMATIONAL CHANGE OF SPIN LABELLED MYOGLOBIN

Electron paramagnetic resonance in solution and powdered samples

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1. Introduction

Several parameters have been observed to present an anomalous change with temperature in a range below that at which normal denaturation process takes place in some globular proteins [1,2], as myoglobin [3–5]. This change occurs in a temperature range close to the homeotherm regulation temperature and has been associated with a conformational change of the protein.

In the present work EPR spectra of isothiocyanate spin labelled myoglobin (4-isothiocyanate 2,2,6,6 tetramethyl piperdinooxyl) were observed in powdered samples and in solution. This spin label binds to the terminal amino group, since no SH groups are present. The binding is sensitive to pH and to the molecular derivatives [6]. These features make isothiocyanate a convenient spin label to probe the helix A region of myoglobin.

The paramagnetic parameters of the isotyocyanate spin label have been obtained from the powder spectrum. The conformational change of myoglobin was followed by means of measurements of the label's correlation time.

2. Experimental

Lyophilized sperm-whale myoglobin (Sigma Chemical Co) and the 103 spin label of Synvar Associates were utilized. A 10% myoglobin solution, at pH 7.9, has been prepared by addition of 0.05 M phosphate buffer. The spin label has been used at a ratio of two molecules to one of myoglobin. This solution has been mixed gently at a temperature around 0°C. In order to eliminate free spin label the solution was dialyzed against the buffer at a temperature around that used in the mixing. The dialyzed solution was dried to a powder form with nitrogen gas.

The EPR spectra have been obtained with Varian V-4502 spectrometer and V-3400 magnet at X-band, and with a mixed electronic spectrometer and a V-3900 magnet at Q-band. At X-band the frequency has been measured with the Hewlett-Packard hp 5545M counter and the magnetic field has been calibrated with the MgO:Cr g-marker (isotropic spectroscopic factor $g = 1.9797$). At Q-band the frequency and field have been calibrated using simultaneously two g-markers; MgO:Cr and DPPH ($g = 2.0036$).

The Varian V-4540 temperature controller has been used and the temperature has been measured with a chromelconstantant thermocouple immersed in the solution.

3. Results

3.1. *Powder samples*

Figures 1 and 2 show the powder experimental spectra at *X*- and *Q*-bands. These spectra have been simulated with a slightly modified [7] program using the Simpson method of integration with 100 intervals of integration for each angle. Simulated spectra of Lorentzian and Gaussian shapes are also shown in fig.1 and 2. The parameters below give the best fit at each band:

	g_x	g_y	g_z	A_x	A_y	A_z
X-band	2.0092	2.0065	2.0029	8.0 G	8.0 G	34.0 G
Q-band	2.0093	2.0067	2.0030	10.5 G	6.2 G	33.4 G

where g_i and A_i are the principal values of the spectroscopic and hyperfine tensors, respectively.

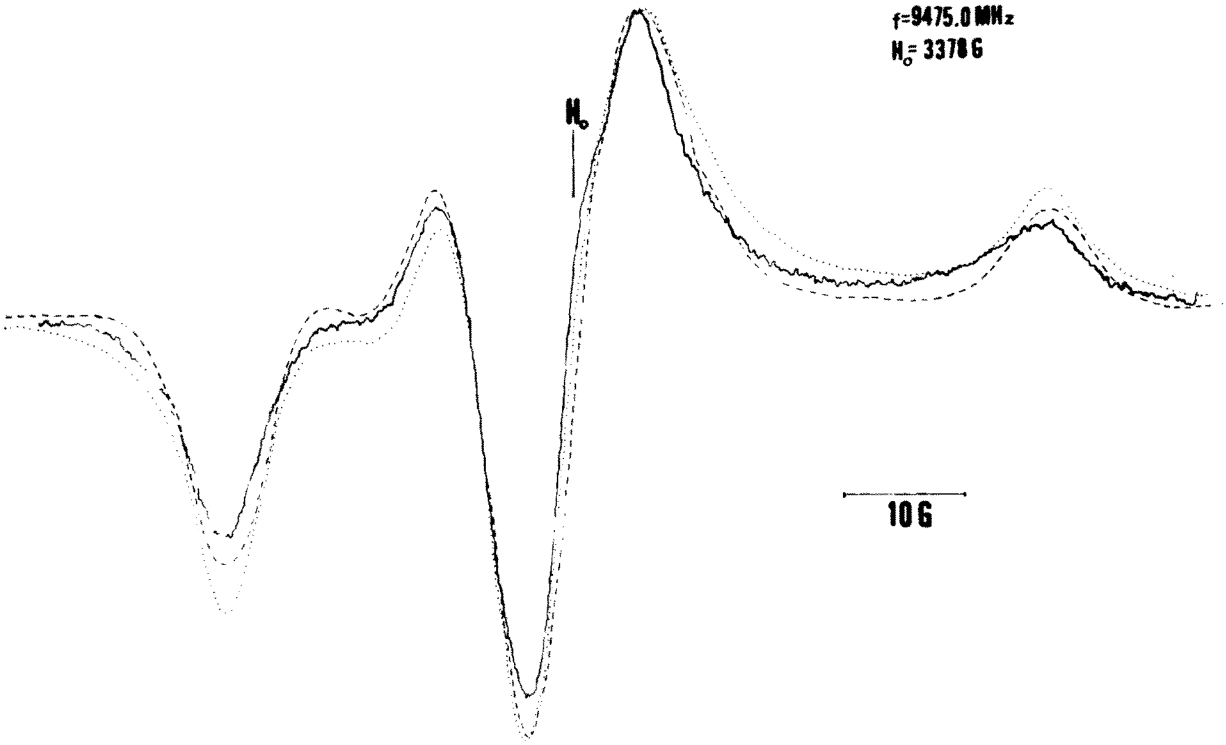

Fig.1. Experimental and simulated rigid spectra of isothiocyanate spin label at X-band (———) experimental, (· · · · ·) simulated with Lorentzian components of 4.6 width and (- - - - - -) simulated with Gaussian components of 6.8 G width. Simulated spectra with g_x = 2.0092, g_y = 2.0065, g_z = 2.0029, A_x = 8.0 G, A_y = 8.0 G, A_z = 34.0 G.

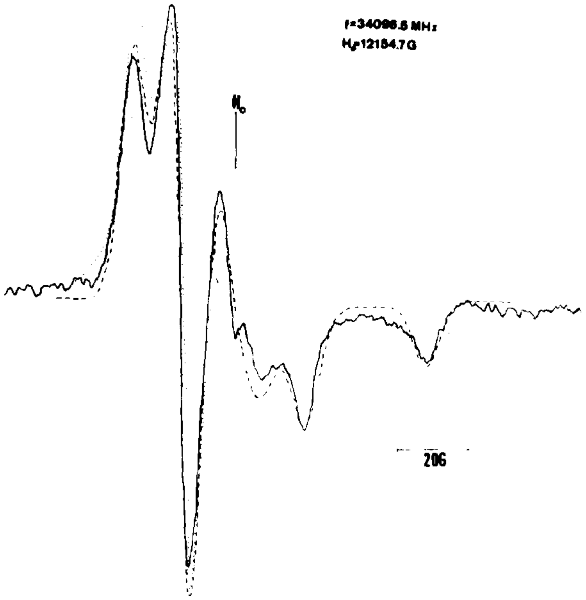

Fig.2. Experimental and simulated rigid spectra of isothiocyanate spin label at Q-band (———) experimental, (· · · · ·) simulated with Lorentzian components with 4.6 G width and (- - - - -) simulated with Gaussian components with 7.1 G linewidth. Simulated spectra with $g_x = 2.0093, g_y = 2.0067, g_z = 2.0030, A_x = 10.5$ G, $A_y = 6.2$ G, $A_z = 33.4$ G.

3.2. *Solution*

The spin label spectra in myoglobin solution are of the weakly immobilized kind. This allows one to obtain the isotropic parameters, g_0 and A_0, by measuring the position of the central line and the distance between two neighbouring lines in the spectra. The values obtained are: $g_0 = 2.0063 \pm 0.0003$ and $A_0 = 17 \pm 0.5$ G.

Kivelson's theory [8] may be used to obtain the correlation time, τ_c. The linewidths are given by:

$$\Delta H(M) = A + BM + CM^2$$

where M is the nuclear spin quantum number of each line in the spectrum. For Lorentzian lines one has:

$$A = \Delta H(0) \quad B = \frac{\Delta H(0)}{2}\left[\sqrt{\frac{I(0)}{I(-1)}} - \sqrt{\frac{I(0)}{I(1)}}\right]$$

$$C = \frac{\Delta H(0)}{2}\left[\sqrt{\frac{I(0)}{I(-1)}} + \sqrt{\frac{I(0)}{I(1)}} - 2\right]$$

where $I(M)$ is the experimentally measured amplitude of the line. The theoretical development for the relaxation time T_2 shows that the parameters B and C are proportional to the correlation time and depend on the x, y and z components of the $\bar{g}$ and $\bar{A}$ tensors. B and C allow for two independent ways of measuring τ_c. As the value of B depends on the microwave power [9] it is more convenient to use the values obtained from C.

In fig.3 we show the experimentally obtained values of B and C as a function of temperature. The parameter C curve shows that the correlation time takes maximum and minimum almost constant values for temperatures below 18°C and above 44°C, respectively. The mean value occurs at 32°C and this temperature was taken as the transition temperature.

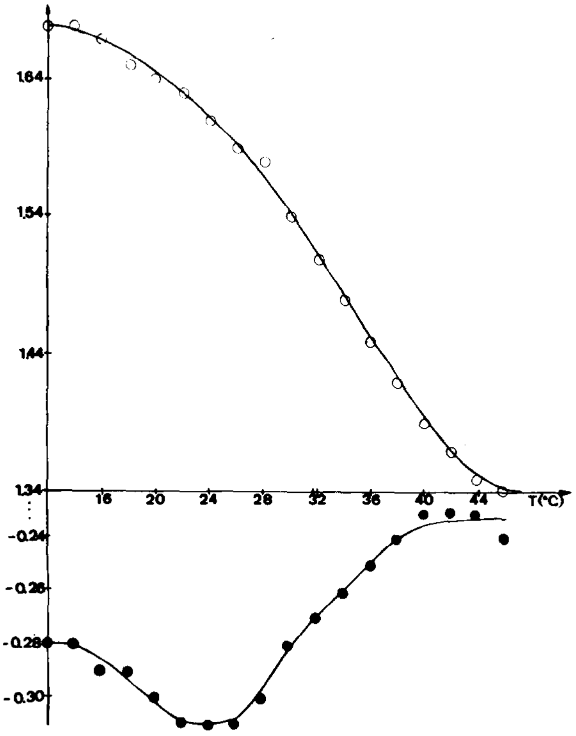

Fig.3. Temperature dependence of the parameters B(O) and C (O).

4. Discussion and conclusion

4.1. *Powder samples*

Figures 1 and 2 make it clear that the theoretical fit of the spectra cannot be made taking into account only one of the line shapes. The experimental line shape can probably be obtained using a mixture of Lorentzian and Gaussian lines. The fit is better using an angular dependence of the form $X = \alpha + \beta \cos^2\theta$ for the components linewidths (α and $\alpha + \beta$ are the minimum and maximum linewidths).

Fitting the data at X- and Q-band we have obtained the magnetic parameters of the 4-isothiocyanate 2,2,6,6-tetramethyl piperdinooxyl bounded to myoglobin. They are:

$$
\begin{aligned}
g_x &= 2.0093 \pm 0.0006 \qquad & A_x &= 9.0 \pm 1.3\ \text{G} \\
g_y &= 2.0066 \pm 0.0006 \qquad & A_y &= 7.1 \pm 0.9\ \text{G} \\
g_z &= 2.0030 \pm 0.0006 \qquad & A_z &= 33.7 \pm 0.5\ \text{G}
\end{aligned}
$$

which correspond to g_0 and A_0 values of 2.0063 ± 0.0006 and 16.7 ± 0.9 gauss in agreement with those obtained from solution spectra.

4.2. *Solution*

Using the parameters B and C and the parameters obtained in the preceeding section, the τ_c values at the transition temperature are 0.78×10^{-9} s and 0.96×10^{-9} s, respectively.

The temperature changes of the parameter C can be taken as corresponding to the temperature dependence of the correlation time τ_c. This kind of curve cannot be associated with the temperature change of the viscosity, since the latter is monotonic. The change in τ_c must be interpreted as being a consequence of a change in the conformation of myoglobin. At temperatures below 18°C the structure of the molecule restricts the label movement while above 44°C it allows for a less restricted movement. This conformational transition of myoglobin is probably related with a structural change near the region corresponding to helix A (where the isothiocyanate is bound) as well as to the CD, DF and GH regions suggested [10]. Figure 4 shows the regions involved in the discussion.

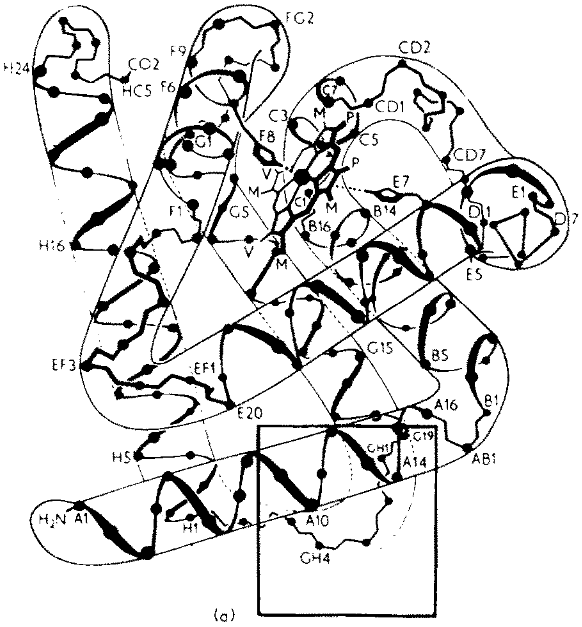

Fig.4. The relative positions of helix A, CD, DF and GH [11].

Acknowledgements

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