strate indicating a template- and a substrate- induced conformational change of the enzyme.

P2

Estimation of the Distance between the Two Binding Sites of Ovotransferrin

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Ovotransferrin (conabulmin, M.wt. 76,000) has been found in large quantities in egg white. Its physiological function is not known with certainty. Because of its large affinity for iron, it is an effective antimicrobial agent which could be important in the protection of the developing chick embryo [1]. In the presence of carbonate, or a difunctional anion, metal ions bind transferrin at two specific sites [2]. The binding of metal ions to the second specific site takes place after the first site is filled, *i.e.* a sequential rather than a random binding process takes place [3].

In this communication the results of the addition of Gd(III) ion in the presence of malonate, using high resolution nuclear magnetic resonance spectroscopy, are reported.

To 1.23 m*M* solution of protein containing 12.3 m*M* malonate, kept at pH 6.00 ± 0.02 by the presence of 50 m*M* trideuteroacetate buffer, one and two equivalents of Gd(III) per protein, dissolved in D_2O, were added. In each case, the spectrum was recorded using a Bruker-270 MHz spectrometer (Fig. 1). The spectrum of the protein solution containing one equivalent of Gd(III) shows that all histidine C2-H resonances have been broadened. In fact the signals of the six histidines which were assigned to be involved in the binding sites [3] have nearly disappeared, indicating that these resonances broaden by at least 80 Hz which could make them undistinguishable from noise. On addition of the two equivalents, all the histidine C2-H resonances have disappeared, Fig. 1.

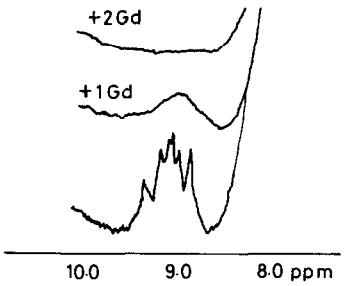

Fig. 1. 270 MHz spectra of the histidine region of ovotransferrin after addition of one and two equivalents of Gd(III) ion.

Theoretical consideration of the line broadening by Gd(III):

The line-width of the NMR signals is given by,

$$\pi\Delta\nu = \frac{1}{T_{2M}} \tag{1}$$

where $\Delta\nu$ is the line-width (in Hz) at the half height and T_{2M} is the transverse relaxation time of the protein. In the presence of Gd(III) ions T_{2M} is given by the Solomon–Bloembergen equation [4]:

$$\frac{1}{T_{2M}} = \frac{1}{15}\frac{\gamma_I^2 g^2 J(J+1)\beta^2}{r^6} f_2(\tau_c) \tag{2}$$

$$f_2(\tau_c) = 4\tau_c + \frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{13}{1+\omega_S^2\tau_c^2}$$

where τ_c is the correlation time which modulates the interaction, and r is the distance between the proton and the paramagnetic centre.

Assuming τ_c is the rotational tumbling time of the macromolecule (τ_R), then from Stockes law,

$$\tau_R = \frac{M\bar{V}\eta}{RT}$$

where M is the molecular weight $\bar{V}$ is the partial specific volume, η is the viscosity of the solvent, T is the temperature and R is the gas constant. Using the above equation, a value of $\tau_R = 2.2 \times 10^{-8}$ s^{-1} could be obtained for ovotransferrin. At 270 MHz eqn. (1) reduces to

$$\pi\Delta\nu = \frac{1.03 \times 10^{18}}{r^6}$$

giving r = 21 Å for an increase of 80 Hz in the line-width due to the presence of Gd(III). This is the upper limit of the distance between the first binding site of ovotransferrin and the C2-H protons in the second binding site. Thus we conclude from the broadening of the spectrum of the whole protein, when it contains two equivalents of Gd(III), that all the C2-H protons of histidines in ovotransferrin lie in a sphere of radius not exceeding 31.5 Å.

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