Discussion Letter

STRUCTURAL BASIS FOR THE VARIATION IN REDOX POTENTIAL OF CYTOCHROMES

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Received 18 April 1977

1. Introduction

One of the aims of the intensive work undertaken to explore protein structure is to explain variations in the properties of proteins at a structural level. In the case of haem-containing electron transfer proteins one basic property to be understood is the redox potential. The redox potential of cytochromes range from +380 mV to −500 mV [1]. Recently much information has been obtained about the structure of cytochromes [2–5]. Here we correlate this structural information with redox potential data using the known redox potentials of model complexes in aqueous solution [6–8] to guide us to the main factors which are likely to be important. These factors are as follows:

(a) Electrostatic charge on the ligand; the higher the negative charge, the lower the redox potential.

(b) The donor power of the ligand; using pK_a values as a guide to donor power, a higher pK_a gives rise to a lower redox potential.

(c) The acceptor power of the ligand; using unsaturation as a guide, the greater the π-acceptor power of the ligand the higher the redox potential.

(d) Changes of spin-state of the central metal ion which can differentially alter the importance of (b) and (c).

(e) Steric factors which can be chosen so that one or other oxidation state or spin-state is favoured when (b) (c) and (d) could be overridden.

2. Porphyrin substituents and axial ligands

Table 1 classifies cytochromes of known structure according to the nature of their porphyrin substituents and haem ligands. In general proteins containing a given porphyrin type and which have two axial histidine ligands have a lower redox potential than proteins with axial histidine and methionine ligands [1–3]. This is to be expected as nitrogen is a better electron donor than sulphur and sulphur a better π-acceptor ligand than nitrogen (table 2). The different porphyrin substituents (vinyl groups for protohaem and thioether groups for haem *c*) also affect the redox potentials (table 2). Greater unsaturation in protohaem containing proteins leads to higher redox potentials than for haem *c* containing proteins, as is seen in the comparison of group A with group B cytochromes, (table 1). However, the axial ligands provide the

Table 1
Classification of cytochromes

Group	Porphyrin	Axial ligands	E'_0 (mV)	Examples	Refs
A	Protohaem	Histidine, Histidine	+80 to −200	b_5,b_{562}	[1]
B	Haem *c*	Histidine, Histidine	−200 to −500	c_3,c_7	[2,4]
C	Haem *c*	Histidine, Methionine	+400 to ~−60	c,c_2	[1,2]

Table 2
Redox potentials of model systems

	E'_0 (mV)
Mesohaem	−158
Protohaem	−115
Bishistidine mesohaem	−220
Histidine–Methionine mesohaem	−110
Bismethionine mesohaem	+ 20

From ref [9]

dominant effect; compare Group A with Group C cytochromes. A further illustration of this is provided by the redox potentials of *Crithidia oncopelti* cytochrome c_{557} and *Euglena gracilis* cytochrome c_{558} [10] which contain a haem group with one thioether linkage and one vinyl substituent [11], although they both have one histidine and one methionine ligand. At 264 mV and 244 mV respectively, their redox potentials are comparable to other mitochondrial type cytochromes *c*, for example, the redox potential of horse cytochrome *c* is 255 mV. Thus the axial ligands dominate the redox potential.

From these data it is clear that the above deductions based upon model compounds apply, however it is evident from table 1 that there is a fine control upon redox potentials in haemproteins as they vary greatly within a group. We now show how this variation comes about.

3. Group C cytochromes

The early nuclear magnetic resonance (NMR) spectroscopy work of McDonald and Phillips [12,13] and Redfield and Gupta [14] has shown that the resonances of the haem group and axial ligands of Group C cytochromes are readily assignable. Here we are concerned with resonances of the axial histidine and methionine ligands. In the spectrum of the reduced protein resonances of these amino acid residues experience large upfield shifts. For the methionine methyl resonance this shift is of the order of 5 ppm whilst for the histidine C-4 resonance it is about 7.5 ppm from the appropriate primary position [15]. The region +0.5 to −4 ppm of the 270 MHz

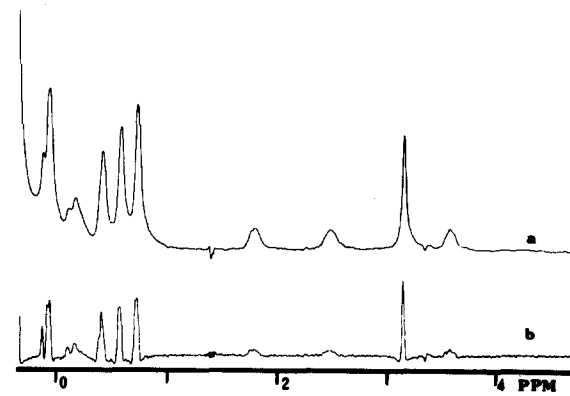

Fig.1. 270 MHz Spectrum of horse ferrocytochrome *c*. (a) Normal spectrum. (b) Convolution difference spectrum [16].

spectrum of horse ferrocytochrome *c* is shown in fig.1. The one proton singlet at 0.15 ppm is from the histidine-18 C-4 proton. The three proton singlet at −3.28 ppm arises from the methionine-80 methyl group whilst the one proton peaks at −0.19 ppm, −1.87 ppm, −2.58 ppm and −3.73 ppm arise from the βCH, β'CH, γCH and γ'CH groups of the methionine. We have observed that there is a correlation between the chemical shifts of these methionine ligand resonances with the redox potential (fig.2). As the redox potential increases the chemical shift of the methionine methyl resonance and γ'CH resonance increases whilst that of the β'CH decreases. The large shifts experienced by the axial ligand resonances arise primarily by ring-current shifts from the haem group. We conclude that the variations in chemical shifts of the methionine resonances for different proteins arise from a difference in the haem ring-current experienced by the methionine ligands as the histidine chemical shifts are only slightly different (table 3). Also the relative chemical shifts for the methionine methyl, γ'CH and β'CH resonances show a continuous variation (fig.3). These straight-line relationships can only be established by a shift mechanism originating at a position relative to the methionine which is very similar in all the proteins. Thus, this indicates that there is a variation in the Fe–S bond length with variation in redox potential. The alternative proposal, a variation in haem plane–Fe–S bond angles, is less likely but we are in the process of determining the geometry of the iron co-ordination centre from NMR data to test this.

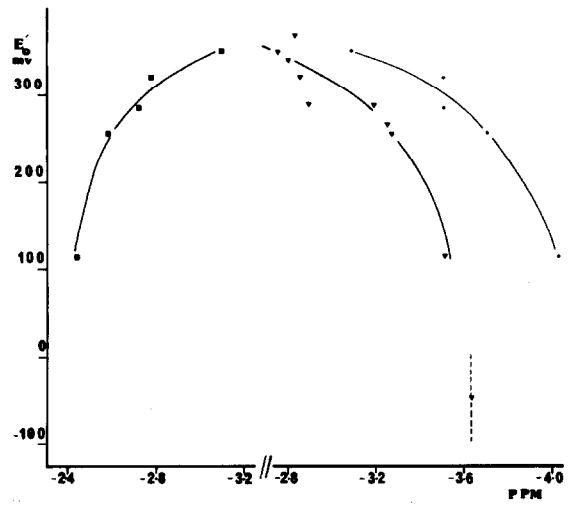

Fig.2. Plot of redox potential against the chemical shift of the methionine ligand methyl (▼), γ'CH (●) and β'CH (■) resonances. Data plotted for horse, tuna, *Rhodopseudomonas capsulata, Rhodospirillum rubrum, Rhodospirillum molischianum*, Halotolerant *Micrococcus, Desulphovibrio vulgaris, Euglena gracilis, Pseudomonas aeruginosa* and *Navicula pellicosa* Group C ferrocytochromes, obtained from refs [1,2,17–23].

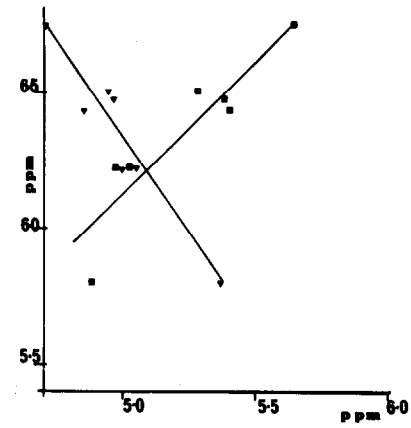

Fig.3. Plots of shifts from primary position for the methionine ligand methyl (■) and β'CH (▼) resonances against the shift from primary position for the γ'CH resonance.

The maximum variation in ring-current shift of 15% corresponds to an approximate 0.1 Å shortening of the Fe–S bond. This is associated with a decrease in redox potential of approximately 400 mV. Such a correlation can be directly related to the donor power of the methionine sulphur. Increase in donor power of a ligand leads to a greater stabilisation of the ferric ion and therefore a decrease in redox potential. The donor power of the methionine sulphur will be governed by the protein conformation and thus the fine control over redox potential in Group C cytochromes could represent an example of the entatic state hypothesis [24].

Acknowledgements

We wish to thank the Medical Research Council for their support. R. J. P. Williams is a member of the Oxford Enzyme Group.

Table 3
Chemical shifts of ligand resonances of cytochromes

Protein	E'_0 (mV)	Methionine Methyl (ppm)	Histidine C-4 (ppm)	Refs
Pseudomonas aeruginosa cytochrome c_{551}	+285	−2.90	+0.15	[1,17,18]
Horse cytochrome *c*	+255	−3.28	+0.13	[1,12–14]
Rhodospirillum fulvum iso-1 cytochrome c_2	n.d.	−3.16	+0.06	[18]
Halotolerant *Micrococcus* Cytochrome c_{554}	+113	−3.52	+0.25	[1,18]

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