Discussion Letter

Control of redox properties of cytochrome *c* by special electrostatic interactions

Geoffrey R. Moore

The Inorganic Chemistry Laboratory, South Parks Road, Oxford OX1 3QR, England

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An assessment is made of the proposal: electrostatic interactions between the ferric ion of oxidised cytochrome *c* and its haem propionate sidechains assists in determining the value of the redox potential and plays an important role in the redox state conformation change. Differences between the properties of homologous cytochromes are proposed to be due to differences associated with the charge on their haem propionates.

Cytochrome *Conformation change* *Potential* *Electrostatic interaction*

1. INTRODUCTION

Monohaem cytochromes *c* of the mitochondrial-type function as electron carriers in a variety of eukaryotic and prokaryotic electron-transport chains [1,2]. These proteins have markedly homologous structures [2–4] but in spite of this, whereas some undergo a conformation change with the change in redox state [5–7], others apparently do not [8,9]. In addition to this major difference, monohaem cytochromes *c* exhibit midpoint redox potentials (E_M) at pH 7 from 100–450 mV [1,2,10,11]. No satisfactory explanation has previously been offered for these differences in redox properties. Here, I propose that electrostatic interactions between a propionate sidechain of the haem and the positive charge on the ferric ion assists in determining the value of E_M and plays an important role in the redox state conformation change of some cytochromes. Differences between the properties of homologous cytochromes are proposed to be due to differences associated with the charge on their haem propionates.

2. CYTOCHROMES *c*

Mitochondrial-type cytochromes *c* can be classified into large and small cytochromes [3,4] (fig.1). Large cytochromes, such as mitochondrial cytochrome *c* itself and *Rhodospirillum rubrum* cytochrome c_2, consist of 100–130 amino acids wrapped around the haem in such a way as to completely enfold it except for one hydrophobic edge [7,8]. Small cytochromes, such as *Pseudomonas aeruginosa* cytochrome *c*-551, consist of ~80 amino acids that enfold the haem in a similar way to the large cytochromes but whose fold leaves more of the haem exposed. In the small cytochromes, as well as one hydrophobic edge of the haem a part of the edge that carries the propionate groups is exposed, with the result that propionate-6 is partly exposed to solvent. Propionate-7 is buried within the protein [9]. Neither of the buried haem propionates of mitochondrial cytochrome *c* ionises over pH 4.5–9.0 [12,13] and this has generally been taken as an indication that their pK_a-values are >9 [14]. However, propionate-7 interacts with the

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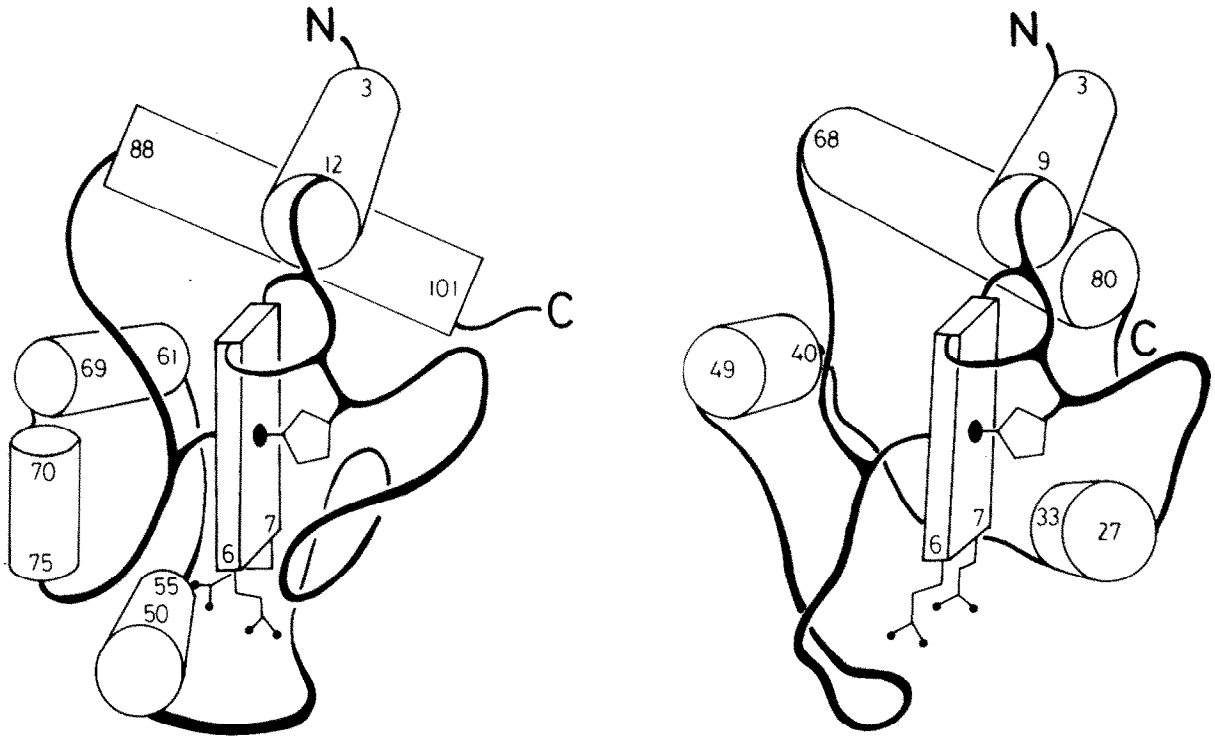

Fig.1. Schematic drawing comparing the polypeptide folding in tuna cytochrome *c* (left) and *Ps. aeruginosa* cytochrome *c*-551 (right). The slab in the centre of each illustration represents the haem group with the methionine and histidine ligands to the left and right, respectively. The haem propionate sidechains are shown at the base of the haem with the number above them referring to their substituent position. The cylinders represent α-helices and the numbers on them are the terminal residue numbers. *Ps. aeruginosa* cytochrome *c*-551 is 21 residues shorter than tuna cytochrome *c*. The main deletion from the *c* structure to make the *c*-551 structure is residues 40–55 (tuna numbering). This deletion leads to the removal of two α-helices on the left of the molecule, though there is an additional α-helix formed on the right, and to the partial exposure of haem propionate-6. Haem propionate-7 is buried in both molecules and haem propionate-6 is also buried in tuna cytochrome *c*.

sidechains of Arg-38, Tyr-48 and Trp-59, and it is clearly possible that these interactions, especially that with Arg-38, reduce the pK_a of propionate-7 to <4.5. Studies of the homologous protein *Rhodomicrobium vanniellii* cytochrome c_2 supports this proposal. In this protein, which contains [2] both Tyr-48 and Trp-59 but has Gln-38 in place of Arg-38, propionate-7 ionises with pK_a 6.3 in the ferric form and pK_a 7.4 in the ferrous form [15]. Thus at pH 7 the internally located haem propionate-7 of mitochondrial cytochromes *c* (all of which have Arg-38) and most prokaryotic cytochromes c_2 must bear a negative charge. The relevance of this buried negative charge to the E_M of the proteins will be examined, followed by a discussion of its relevance to the redox state conformation change.

3. REDOX POTENTIALS

The effect of the negative charge upon the E_M is best demonstrated by the E_M-values of cytochromes in which the propionate-7 pK_a-values are 6–8. Whereas the E_M-values of most mitochondrial and many prokaryotic cytochromes *c* are independent of pH over 4.5–9.0, the E_M-values of some cytochromes [10,11,15–17] vary with pH in the manner shown in fig.2. The separation of pK_a values and the consequent decrease in E_M result from electrostatic interactions between the ionising group and the positive charge on the ferric ion rather than by large conformational changes. NMR measurements for the proteins in table 1 identify the ionising group to be haem propionate-7 and they show that a large conformational

change does not accompany the ionisation. Some mitochondrial cytochromes *c* have a small pH dependence to their E_M [17] ($\Delta E_M = 23$ mV) over pH 6–8 but NMR investigations [19] show this is caused by the ionisation of a surface His rather than by the ionisation of haem propionate-7. The calculated equivalent electrostatic interaction free energy ($\Delta G'_{el}$), obtained by equating ΔE_M to $\Delta G'_{el}$, for cytochromes exhibiting the behaviour of fig.2 is given in table 1.

A theoretical ΔG_{el} can be calculated [20], assuming no conformational changes, in kJ.mol^{-1} at 298 K from:

$$\Delta G_{el} = 1347\ (q_1 . q_2)/\epsilon.D$$

where q_1 is the charge on the iron, which changes from +2 to +3 as a result of the redox change (the nett charge at the iron is 0 and +1 because the porphyrin is a dianion), q_2 is the charge on the propionic acid, which changes from 0 to −1 as a result of the ionisation, D is the distance between the charges in Å and ϵ is the effective dielectric constant. D can vary from 10–15 Å for the haem iron–propionate distance: in tuna ferrocytochrome *c* [7] the distance between the iron and the carboxyl carbons of propionates-6 and -7, respectively, is 11.8 Å and 10.1 Å. In fig.3, ΔG_{el} is plotted as a function of $\epsilon.D$ for a charge product of −1.0.

The main problem with calculations of the kind shown in fig.3 is assigning a value to ϵ. The value

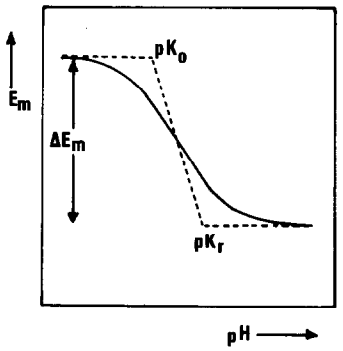

Fig.2. pH dependence of E_M exhibited by some cytochromes. The analysis of Pettigrew [10,11] shows that the general appearance of the curve is due to a pK_a on the ferricytochrome *c* (pK_o) and a pK_a on the ferrocytochrome *c* (pK_r).

Table 1

pH dependence of midpoint redox potentials of cytochromes *c*

	pK_o	pK_r	ΔE_M (mV)	$\Delta G'_{el}$ (kJ.mol^{-1})
Rhodomicrobium vannielii c_2	6.3	7.4	65	−6.27
Pseudomonas aeruginosa c-551	6.2	7.3	65	−6.27
Rhodospirillum rubrum c_2	6.2	7.0	47	−4.53

The values of pK_o and pK_r and ΔE_M were taken from [10,11,15]. $\Delta G'_{el}$, the empirical electrostatic interaction free energy, was obtained from $\Delta G'_{el} = -nF\Delta E_M$ by assuming that the decrease in E_M results from electrostatic interactions

usually quoted [20] for the interior of a globular protein is in the range 1–5, and with a charge product of −1.0 that leads to large values of ΔG_{el}. However, Rees [21] has shown that ϵ for the electrostatic interaction between certain lysines and the iron of cytochrome *c* is about 50. Because the propionate is buried rather than exposed on the protein surface the ϵ for the iron–propionate interaction is probably less than that for the iron–lysine interaction. A value of 10–40 seems likely leading to ΔG_{el} in the range −13.5 to −2.2 kJ.mol^{-1} (fig.3). Despite the uncertainty concerning ϵ it is clear that ΔG_{el} corresponds reasonably well with $\Delta G'_{el}$ (table 1). Therefore, for cytochromes *c* in which propionate-7 has a low pK_a (<4.5) and in which the range of E_M is 100–450 mV, much of the difference in E_M at pH 7 could be due to a variation in the electrostatic interaction between the propionate and ferric ion. (A difference in E_M of 350 mV corresponds to a $\Delta G'_{el}$ of 34 kJ.mol^{-1} at 298 K.) Electrostatic interactions between the ferric ion and charged surface groups also contributes slightly to the difference in E_M [18,21]. Other factors which may be important are the degree of haem exposure to solvent [22] and the apolarity of the haem environment [23], although since changes in these factors will probably change ϵ, they will also change ΔG_{el}.

4. CONFORMATION CHANGE

The importance of the ferric ion–propionate in-

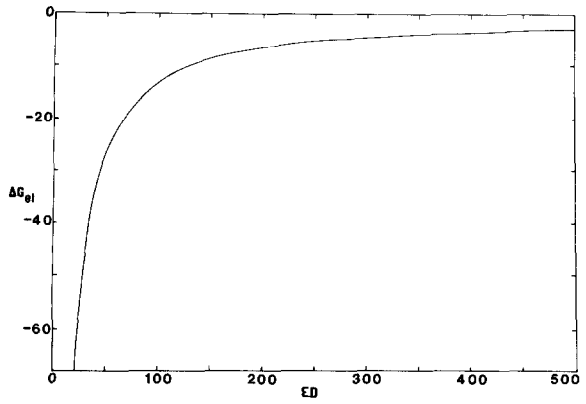

Fig.3. The calculated change in electrostatic interaction free energy (ΔG_{el}) as a function of the product of the effective dielectric constant (ϵ) and the distance between the charges (D).

teraction to the small redox state conformation change can be seen from a comparison of the X-ray structures of tuna cytochrome *c* [7], *Rsp. rubrum* cytochrome c_2 [8] and *Ps. aeruginosa* cytochrome *c*-551 [9]. Mitochondrial cytochromes *c* undergo a redox state conformational change that is greatest at the base of the haem around the propionate groups [6,7,19]. It seems likely that the trigger for the conformation change is the difference in ΔG_{el} between the propionate and iron in its two oxidation states which results in changes in the atomic positions of the groups interacting with propionate-7. These changes are then transmitted throughout the protein, probably by movements of some of the α-helices (fig.1) since it is amino acids in the α-helices from residue 50–55 and from residue 61–69 that are most affected by the redox change [7].

Although the conformation change of mitochondrial cytochrome *c* is a relatively minor one, the attempt to completely reduce crystals of tuna ferricytochrome *c* or to completely oxidise crystals of tuna ferrocytochrome *c* result in the crystals breaking [7]. In contrast to this both *Rsp. rubrum* cytochrome c_2 [8] and *Ps. aeruginosa* cytochrome *c*-551 [9] can be completely oxidised and reduced in the crystalline state without damaging the crystals, and in addition their X-ray structures show that there are no redox state conformational differences. The important point to note is that both the cytochrome c_2 and cytochrome *c*-551 X-ray structure analyses were carried out between pH 5.7–6.0 [8,9,24], which is below their propionate-7 pK_a-values (table 1), and thus these groups were largely unionised. Since the 112 amino acid *Rsp. rubrum* cytochrome c_2 (which contains Asn-38 in place of Arg-38) has a similar packing of α-helices to that of tuna cytochrome *c* [8], it probably undergoes a redox state conformation change at higher pH. However, the 82 amino acid *Ps. aeruginosa* cytochrome *c*-551 has a different distribution of α-helices (fig.1) and so even at higher pH it may not undergo a conformation change. Certainly NMR studies of *Ps. aeruginosa* cytochrome *c*-551 indicate that the ionisation of propionate-7 is not accompanied by any more than small perturbations of the side chains immediately surrounding it [16,17].

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