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Citation for published version:

Moore, GR, Pettigrew, G & Rogers, NK 1986, 'Factors influencing redox potentials of electron transfer proteins', *Proceedings of the National Academy of Sciences (PNAS)*, vol. 83, no. 14, pp. 4998-9.

Link:

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Document Version:

Publisher's PDF, also known as Version of record

Published In:

Proceedings of the National Academy of Sciences (PNAS)

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Factors influencing redox potentials of electron transfer proteins

(electrostatic interactions/metalloproteins/protein complexes)

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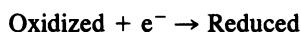
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Communicated by Martin D. Kamen, March 13, 1986

ABSTRACT The redox potentials of electron transfer proteins vary over a wide range, even when the type of redox center is the same. Rees [Proc. Natl. Acad. Sci. USA (1985) 82, 3082–3085] proposed that this variation of redox potential partly reflects the different net charges of the proteins, and he presented a linear correlation between these two properties for 36 proteins. A review of the factors that influence protein redox potentials makes it clear that this linear correlation is fortuitous. The key factors influencing redox potentials are the contributions to the Gibbs energy difference between the two redox states, resulting from bonding interactions at the redox center, electrostatic interactions between the redox-center charge and polar groups within the protein and solvent, and redox-state conformational changes. The relative importance of these terms is likely to vary from protein to protein.

The factors that determine the magnitude of a redox protein's reduction potential (E°) have been explored for many years, and it is now clear that no single factor is dominant. In the present paper, we discuss various key factors with particular reference to a recent paper of Rees (1) concerning the influence of the charge of a protein on its redox potential.

The Gibbs energy change (ΔG°) for the redox reaction



is composed of three major terms. The first is the Gibbs energy difference between the two redox states resulting from bonding interactions at the redox center (ΔG_{cen}), and the second is the Gibbs energy difference resulting from electrostatic interactions between the redox-center charge and polar groups within both the protein and the solvent (ΔG_{el}). Both of these terms are likely to be substantial. A third term arises from Gibbs energy changes due to redox-state conformational differences (ΔG_{conf}), but these are often negligible for simple electron transfer proteins.

In order to analyze either ΔG_{el} or ΔG_{cen} , the measured ΔG° must be separated into its different components, a process that is generally not possible. An empirical approach to the problem is to consider one class of related proteins in which the redox center is the same and to assume that differences in ΔG° reflect differences in only one of the contributing factors. An example of this kind of approach is provided by studies of histidine-methionine ligated cytochromes *c* (2, 3), in which it was assumed that the ΔG_{cen} term was constant and ΔG_{el} variable. However, the situation is further complicated because there are a number of factors contributing to the ΔG_{el} term. This can be written as

$$\Delta G_{\text{el}} = \Delta G_{\text{ion}} + \Delta G_{\text{H}_2\text{O}} + \Delta G_{\text{int}} + \Delta G_{\text{surf}}$$

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ΔG_{ion} is a term describing the effect of ions in solution upon the redox energy. It includes contributions from the nonspecific Debye-Hückel screening effect and, in some cases, from specific ion-protein association. It is the existence of this term that has led some workers to advocate extrapolating ΔG° to an ionic strength of zero (i.e., $\mu = 0$) when $\Delta G_{\text{ion}} = 0$ (3–5).

$\Delta G_{\text{H}_2\text{O}}$ is the energy of a charge inside a protein of low dielectric surrounded by H_2O , a solvent of high dielectric. It is the Gibbs energy difference between the charge in the protein and the charge in the solvent (6). Kassner (7) considered this factor with regard to protein redox potentials, and his analysis indicates that it is significant for proteins with buried redox centers. This has recently been confirmed for mitochondrial cytochrome *c* in a theoretical modeling study of the difference between its redox potential and that of the heme octapeptide (8).

ΔG_{int} is the Gibbs energy of interaction between the redox-center charge and other charges that are not exposed to solvent. In cases where the redox center is buried, ΔG_{int} may be large, since both charges will then be situated in a relatively low-dielectric medium. For example, in *Pseudomonas aeruginosa* cytochrome c_{551} , the buried charge on the ferriheme macrocycle and the charge on the buried heme propionate have a ΔG_{int} of approximately $-6.3 \text{ kJ}\cdot\text{mol}^{-1}$ (equivalent to $\Delta E^\circ \approx 65 \text{ mV}$ at 25°C) (9, 10).

ΔG_{surf} is the Gibbs energy of interaction between the redox-center charge and the charges that are exposed to solvent. This is the term that Rees (1) suggests plays a key role in influencing the magnitude of the reduction potential, and a graph of E° against molecular charge estimated from the amino acid sequence (Q) for 36 electron transfer proteins covering an E° range of 400 to -400 mV and a Q range of 8 to -17 is consistent with this. A linear least-squares fit to these data gave a correlation coefficient of 0.64 and a slope (dE°/dQ) of 23 mV (1). We suggest that this apparent linear correlation is fortuitous for the following reasons.

(i) ΔG_{cen} and ΔG_{conf} are unlikely to be constant over the range of proteins selected, since it includes representatives of four classes of hemoproteins and four classes of non-heme iron proteins, as well as two blue copper proteins and a flavoprotein.

(ii) The E° values were not corrected for ionic-strength effects, and therefore ΔG_{ion} is unlikely to be constant over the range of proteins chosen.

(iii) No allowance was made for the fact that ΔG_{int} is known to be significant for many of the cytochromes *c* (2, 11) and two of the high-potential iron proteins (HiPIPs) (12).

(iv) The analysis ignores $\Delta G_{\text{H}_2\text{O}}$ and ΔG_{conf} .

It is true that, in the data set of ref. 1, low-potential proteins are generally negatively charged and high-potential proteins tend to be positively charged, but whether this is a general phenomenon is questionable. For example, the tetraheme cytochromes *c*₃, all of which have $E^\circ < -200 \text{ mV}$, have pI values ranging from ≈ 5 to between 10 and 11 (13–15), charlock cytochrome *f* has a pI of 5 and an E° of 365 mV (16),

Euglena gracilis cytochrome c_{552} has a net charge of -8 in the ferric state at pH 7 and an E° of 325 mV (17), and *Tetrahymena pyriformis* cytochrome c has a pI of 6.5, instead of the more usual pI ≈ 10 possessed by other mitochondrial cytochromes c , but a normal E° in the range 240–260 mV (18). None of these proteins were considered by Rees (1).

Another reason for questioning the generality of a direct relationship between Q and E° is that reaction usually takes place between electron transfer proteins in complexes held together predominantly by complementary electrostatic interactions. Thus it might be anticipated that two physiological partners would have opposite net charges or be oppositely charged in the regions encompassing the reaction sites (i.e., where ΔG_{surf} might be expected to be largest). If the surface charge were a controlling factor in determining redox potentials, it is difficult to envisage how multiprotein electron transfer chains could be constructed on the bases of consistently increasing E° yet alternating Q .

The above comments should not be taken as an indication that ΔG_{surf} is always negligible. Schejter *et al.* (3) estimate that ΔG_{surf} is $10 \text{ kJ}\cdot\text{mol}^{-1}$ (i.e., $E^{\circ} = -105 \text{ mV}$), compared to the ΔG° of $-26.4 \text{ kJ}\cdot\text{mol}^{-1}$ ($E^{\circ} = 275 \text{ mV}$) obtained by them for *E. gracilis* cytochrome c_{552} at pH 7 and $\mu = 0$. However, in the only comparable study known to us, that of horse cytochrome c at pH 7 and $\mu = 0$ (3), ΔG_{surf} is estimated to be only $-4.6 \text{ kJ}\cdot\text{mol}^{-1}$ ($E^{\circ} = 48 \text{ mV}$) compared to the ΔG° of $-26.4 \text{ kJ}\cdot\text{mol}^{-1}$ ($E^{\circ} = 275 \text{ mV}$).

Similar criticisms to those given above can be leveled at other proposed correlations of E° with just one particular factor. Thus, the proposed correlations for cytochromes c (19), blue copper proteins (20) and iron-sulfur proteins (21, 22), which ascribe difference in ΔG° to variations in ΔG_{cen} , neglect the ΔG_{el} and ΔG_{conf} terms, and the proposed correlation of E° with the heme solvent accessibility for a wide range of heme proteins (23) neglects both the ΔG_{cen} and ΔG_{conf} terms and components of the ΔG_{el} term.

We also wish to comment on the role of electrostatic interactions in modulating the redox potentials of complexed electron transfer proteins. Classical considerations indicate that electron transfer takes place in an activated complex in which the donor and acceptor centers have the same redox potential (24). We have previously suggested (25) that the ΔG_{el} terms for the individual proteins may be changed in the activated complex so as to equalize their respective activation-state redox potentials. This modification to ΔG_{el} could result from a variation of any of the individual terms, though probably changes in $\Delta G_{\text{H}_2\text{O}}$ and ΔG_{int} will be the largest, and, of course, there may be protein conformational changes that modify ΔG° . Rees (1) has also suggested that electrostatic interactions in a protein complex will modulate the relative redox potentials in both the collision complex and the activated electron transfer complex. However, experimental studies indicate that redox potentials are not usually greatly perturbed by the formation of collision complexes with other proteins (26). Since redox potentials in the activated state have not been measured, the proposal that ΔG_{el} is modified cannot be assessed at present. What is required is an estimate of ΔG_{el} , using the recent developments in theoretical modeling (27–29), for cytochrome c in its free and hypothetical activated-state complex with cytochrome c peroxidase (30).

In conclusion, ΔG° is composed of three major terms, ΔG_{cen} , ΔG_{el} , and ΔG_{conf} . ΔG_{el} results from the interaction of the redox-center charge with all other charges and dipoles of the protein and solvent. In cases where there are no buried charges other than the redox-center charge, ΔG_{el} is dominated by the interaction between the charge and the protein and solvent dipoles. Where another charge is buried, interaction between this charge and the redox-center charge may be significant. In general, interactions between the redox-center charge and charges on the protein surface are relatively small.

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