The Redox Potentials of the Two-Iron Plant and Algal Ferredoxins

AN ELECTROSTATIC MODEL

By RICHARD J. KASSNER and WILLIAM YANG

Department of Chemistry, University of Illinois, Chicago Circle, Chicago, Ill. 60680, U.S.A.

(Received 25 October 1972)

The two-iron-sulphur co-ordination centre in plant and algal ferredoxins is considered as a collection of charged ions whose net negative charge is twice that of the one-iron-sulphur protein rubredoxin. Calculation of the electrostatic free-energy changes for reduction of the two types of proteins indicates that the redox potential of the two-iron-sulphur proteins should be more negative than that of the one-iron-sulphur protein and that in biological systems the ferredoxins should function as one-electron transfer proteins.

Two-iron proteins characterized by acid-labile sulphide and redox potentials, E'_0 values at pH 7, between -0.405 V (Arnon, 1965) and -0.420 V (Fee *et al.*, 1971; Tagawa & Arnon, 1968) have been isolated from a large number of plants. Such proteins constitute part of a much larger class of iron proteins characterized by a variable number of iron atoms, inorganic acid-labile sulphide and a wide range of redox potentials, which participate in electron-transfer reactions. In each of these reactions, the proteins function as one-electron redox components and the valence state of one of the two-iron atoms changes from +3 to +2 (Fry *et al.*, 1963). Several models (Blumberg & Peisach, 1965; Van Voorst & Hemmerich, 1966; Beinert *et al.*, 1965) have been proposed to account for various chemical and spectroscopic properties of these proteins.

The present paper considers an electrostatic model for plant and algal ferredoxins as a theoretical basis for correlating the structures of plant and algal ferredoxins with their redox properties.

Formulation of the Model and Computational Results

A proposed structural model (Rao *et al.*, 1971) for plant and algal ferredoxins corresponding to a cluster complex with the two iron atoms each tetrahedrally co-ordinated to mercaptide ligands and sulphide ions is represented in Fig. 1. We suggest that the reduced form of the cluster may have a similar structure except for the change in valence of one of the iron atoms from +3 to +2. Such a cluster may be compared with the iron-sulphur complex of the one-iron atom protein, rubredoxin (Fig. 2), for which the iron is also tetrahedrally co-ordinated to four cysteinyl mercaptide ligands (Herriot *et al.*, 1970). We suggest that the cluster complex in these ferredoxins cor-

responds to two 'fused' single-iron rubredoxin complexes with the sulphide-bridging ligands each corresponding to two cysteinyl mercaptide ligands. To a first approximation the difference in redox potentials of these proteins may be attributed to the difference in electrostatic free-energy changes accompanying the one-electron reduction of these systems. To emphasize the significance of these effects we may superficially characterize the iron complex in rubredoxin as a complex of net charge -1 in its oxidized state. Correspondingly, the oxidized two-iron complex illustrated in Fig. 1 for ferredoxin corresponds to a complex with net charge -2 . Reduction of each of these systems corresponds to the addition of an electron to a Fe^{III} ion. Such a reduction decreases the charge on the iron ion from +3 to +2 and increases the negative charge on the rubredoxin-type complex to -2 . Correspondingly, the addition of an electron to the cluster complex in ferredoxin decreases the charge on one of the iron atoms and increases the negative charge on the cluster to -3 . Thus it should be electrostatically more difficult to add an electron to the cluster complex in ferredoxin than it should be to reduce the complex in rubredoxin. The actual extent of the difference in redox potentials will depend on the charge distributions in the complexes and the resultant sum of all electrostatic interactions. As an approximation point charges are used to characterize the average distribution of charge in the co-ordination centres. The charges on the iron and sulphur atoms may be much lower than the formal charges indicated as a result of the effects of covalency. The use of formal charges on the iron and sulphur atoms in the present model maximizes the electrostatic potential energy of each of the systems in both their oxidized and reduced states. As a result, the calculated differences between the electrostatic potential-energy changes for reduction of the

Fig. 1. Model for the one-electron reduction of the oxidized metal–ligand co-ordination site in the two-iron plant and algal ferredoxins

For details see the text.

Fig. 2. Pictorial representation of the one-electron reduction of the metal–ligand co-ordination site in rubredoxin

For details see the text.

complexes represent minimum values. The electrostatic potential-energy changes for the reduction of such complexes correspond to electrostatic contributions to the free-energy changes. The difference between these changes may be related to the difference in redox potentials in volts between the cluster and the single-iron atom complex. The electrostatic potential energy (U) corresponding to the interaction of two charged species is:

$$U = \frac{kqq'}{4\pi\epsilon_0 Dr}$$

where k is a proportionality constant, q and q' are the charges on the two species, ϵ_0 is the permittivity of the free space, D is the microscopic dielectric constant and r is the distance between point charges. The electrostatic potential energy ($U_{\text{ox.}}^{\text{Fd.}}$) for the oxidized (ox.) cluster model of ferredoxin in Fig. 1 is given by:

$$\frac{4\pi\epsilon_0 D}{k} U_{\text{ox.}}^{\text{Fd.}} = -\frac{12q^2}{r_{23}} - \frac{6q^2}{r_{13}} - \frac{6q^2}{r_{34}} + \frac{9q^2}{r_{35}} + u$$

where, e.g. r_{23} is the distance between atoms 2 and 3 as labelled in the figures, u is the sum of the potential

energy terms involving Fe_5^{III} , sulphide and cysteinyl mercaptide ligands (atoms with subscripts are as labelled in the figures), and Fd. represents ferredoxin. We shall consider the $\text{Fe}^{\text{III}}\text{--S}^{2-}$ and $\text{Fe}^{\text{III}}\text{--S}^-$ bond lengths to be equivalent such that:

$$\frac{4\pi\epsilon_0 D}{k} U_{\text{ox.}}^{\text{Fd.}} = -\frac{18q^2}{r_{13}} - \frac{6q^2}{r_{34}} + \frac{9q^2}{r_{35}} + u$$

The electrostatic potential energy ($U_{\text{s-red.}}^{\text{Fd.}}$) of the one-electron reduction complex (Fig. 1), which we will refer to as the semi-reduced (s-red.) state, is given by:

$$\frac{4\pi\epsilon_0 D}{k} U_{\text{s-red.}}^{\text{Fd.}} = -\frac{12q^2}{r_{13}} - \frac{4q^2}{r_{34}} + \frac{6q^2}{r_{35}} + u$$

where the distances separating metal ions and ligands are considered to be the same as those in the oxidized state. The change in electrostatic potential energy ($\Delta U_{\text{ox.} \rightarrow \text{s-red.}}$) corresponding to the one-electron reduction of the cluster complex is thus given by:

$$\begin{aligned} \frac{4\pi\epsilon_0 D}{k} \Delta U_{\text{ox.} \rightarrow \text{s-red.}} &= \frac{4\pi\epsilon_0 D}{k} (U_{\text{s-red.}}^{\text{Fd.}} - U_{\text{ox.}}^{\text{Fd.}}) \\ &= \frac{6q^2}{r_{13}} + \frac{2q^2}{r_{34}} - \frac{3q^2}{r_{35}} \end{aligned}$$

The reported bond lengths and bond angles in rubredoxin are distorted from that of normal tetrahedral symmetry (Herriot *et al.*, 1970). Likewise, the symmetry about the iron atoms in the ferredoxins appears to be distorted from that of a normal tetrahedron (Dunham *et al.*, 1971; Rao *et al.*, 1971). In the absence of detailed structural data we have chosen to consider the iron co-ordination centres as regular tetrahedra for the purpose of estimating the distances separating iron atoms and non-adjacent mercaptide ligands. Such distances may be readily related to the Fe-S⁻ or Fe-S²⁻ bond length. Thus:

$$\frac{4\pi\epsilon_0 D}{k} \Delta U_{\text{ox.} \rightarrow \text{s-red.}}^{\text{Fd.}} = \frac{6q^2}{r} + \frac{2q^2}{1.9144r} - \frac{3q^2}{1.1543r}$$

where r is the Fe^{III}-S⁻ bond length. Likewise, the difference in electrostatic potential energy ($\Delta U_{\text{ox.} \rightarrow \text{red.}}^{\text{Rd.}}$) for the reduction of the iron complex in rubredoxin (Rd.) is:

$$\frac{4\pi\epsilon_0 D}{k} \Delta U_{\text{ox.} \rightarrow \text{red.}}^{\text{Rd.}} = \frac{4\pi\epsilon_0 D}{k} (U_{\text{red.}}^{\text{Rd.}} - U_{\text{ox.}}^{\text{Rd.}}) = \frac{4q^2}{r}$$

The electrostatic potential-energy change $\Delta(\Delta U)$ per ion for the two-iron cluster model of ferredoxin relative to the one-iron atom complex in rubredoxin is:

$$\begin{aligned} \Delta(\Delta U) &= \Delta U_{\text{ox.} \rightarrow \text{s-red.}}^{\text{Fd.}} - \Delta U_{\text{ox.} \rightarrow \text{red.}}^{\text{Rd.}} \\ &= \frac{k}{4\pi\epsilon_0 D} \cdot \frac{0.4457q^2}{r} \end{aligned}$$

The relative molar potential-energy change $[\Delta(\Delta U)_M]$ is thus:

$$\Delta(\Delta U)_M = \frac{kNq^2}{4\pi\epsilon_0 D} \cdot \frac{0.4457}{r}$$

where N is Avogadro's number. The relative electrostatic potential-energy change is equivalent to the difference in electrostatic free energy (ΔG_e) for the reduction of the cluster complex relative to the single-iron atom complex, i.e.:

$$\Delta(\Delta G_e)_M = \Delta(\Delta U)_M$$

The relative electrostatic free-energy change may be related to the difference in redox potentials by the expression $\Delta G = -nFE$, where n is the number of electrons transferred. Thus the difference between the redox potentials ($\Delta E'_0$) of the cluster model of ferredoxin and rubredoxin for the one-electron addition reactions is

$$\begin{aligned} \Delta E'_0 &= E'_{0(\text{Fd.})} - E'_{0(\text{Rd.})} \\ &= \frac{-kNq^2}{4\pi\epsilon_0 DF} \cdot \frac{0.4457}{r} \end{aligned}$$

or

$$\Delta E'_0 = \frac{14.41}{D} \cdot \frac{0.4457}{b} = \frac{6.421}{Db}$$

where b is the Fe-S bond length in Å.

The value for $\Delta E'_0$ is dependent on the bond length and the microscopic dielectric constant (D) characteristic of the medium separating the charges.

An average Fe-S bond length of 2.305 Å (0.2305 nm) in rubredoxin may be calculated from measured Fe-S bond lengths. This value compares with an average Fe-S bond length of 2.360 Å (0.2360 nm) for iron tetrahedrally co-ordinated to four sulphur ligands in bis(imidotetramethyldithiophosphino-SS)-iron(II) (Churchill & Wormald, 1971). Utilizing the average Fe-S bond length in rubredoxin as an estimate of the Fe-S bond lengths in the ferredoxins:

$$\Delta E'_0 = -\frac{6.421}{D(2.305)} = -\frac{2.786}{D}$$

An estimate of the difference in redox potentials would thus be uniquely determined by the microscopic dielectric constant, a quantity which is, however, not subject to direct experimental determination. A dielectric constant of 1 has been implicit in calculations of the lattice energies of salts such as NaCl whose properties approach those predicted for ideal ionic crystals. By comparison the present cluster and single-iron atom complexes correspond less well to an ideal ionic lattice. Thus we may expect microscopic dielectric constants for these complexes to be significantly greater than 1. It is apparent from the above equation that large differences in redox potentials between these two types of proteins may be expected for values of D much greater than 1. The difference between the experimentally determined redox potentials of ferredoxin ($E'_0 = -0.420$ V) (Tagawa & Arnon, 1968) and rubredoxin ($E'_0 = -0.057$ V) (Lovenberg & Sobel, 1965) $\Delta E'_0$ is -0.363 V. Thus we may observe that a value of $D = 7.67$ would provide an effective correlation between the proposed model and the difference between the redox potentials of the two proteins. Such a microscopic dielectric constant for the metal-ligand complex corresponds to an internal molecular dielectric constant, D_1 (Kirkwood & Westheimer, 1938), which for the present system describes the polarization of metal and ligand orbitals in the formation of metal-ligand bonds. The above value for D is significantly greater than 1 and may thus be interpreted as a measure of the extent to which the metal-ligand co-ordination centres deviate from pure ionic systems.

The present model may be further evaluated by considering the second one-electron reduction of these ferredoxins as indicated in Fig. 3. The electrostatic potential-energy changes for this reaction may also be compared with those for the one-electron reduction of the single-iron ferric complex in rubredoxin. The potential-energy difference may similarly be related to the difference between the second redox potential of ferredoxin and the redox potential of rubredoxin. The value of D noted above may be used to estimate

Fig. 3. Model for the one-electron reduction of the semi-reduced metal–ligand co-ordination site in the two-iron plant and algal ferredoxins

For details see the text.

the redox potential for the reduction of the second ferric site in the cluster complex.

On this basis the difference between the second redox potential of ferredoxin and the redox potential of rubredoxin

$$\Delta E'_0 = E'_{0(\text{Fd.})} - E'_{0(\text{Rd.})}$$

is -1.07 V . The oxidation–reduction potential, $E'_{0(\text{Fd.})}$, for the second one electron reduction of the two-iron plant and algal ferredoxins would then be

$$E'_{0(\text{Fd.})} = \Delta E'_0 + E'_{0(\text{Rd.})} = -1.12\text{ V}$$

Discussion

The present electrostatic model for the two-iron plant and algal ferredoxins has been considered to account for the very low redox potentials of these proteins relative to the redox potentials of other iron–sulphur proteins also co-ordinated to sulphide and/or cysteinyl mercaptide ligands.

The structural model considered in this report appears consistent with most recent electron–nucleus double resonance (ENDOR) (Fritz *et al.*, 1971), Mössbauer (Dunham *et al.*, 1971; Rao *et al.*, 1971), magnetic susceptibility (Palmer *et al.*, 1971) and spectroscopic studies (Eaton *et al.*, 1971). It has been noted (Arnon, 1965; Rao *et al.*, 1971; Fee *et al.*, 1971), however, that studies of these proteins and their proposed models have not yielded a satisfactory rationalization of their redox potentials. It may further be observed that such studies have not provided a satisfactory explanation for the one-electron acceptor properties of these proteins in biological electron-transfer reactions. Based on the proximity of the two-iron atoms, Gibson *et al.* (1966) suggested that the extra electron of the ferrous complex in the semi-reduced state would be on a molecular orbital which would take it on to the ferric

complex, reducing the electron affinity of the ferric complex, thus preventing it from also accepting an electron. However, such a delocalization of the charge density about both of the iron atoms would on the average have the effect of only partially decreasing the electron affinity of each of the ferric sites, such that both sites should together have a combined residual affinity for accepting an additional electron.

An alternative view presented here is that in the oxidized state the complex with each iron in its ferric state surrounded by four cysteinyl mercaptide and two sulphide ligands is already 'electron rich' (corresponding to a net charge of -2) by comparison with the single-iron, ferric complex in rubredoxin having a net charge of -1 . The electrostatic model predicts that the iron cluster in ferredoxin should have less electron affinity than that of rubredoxin. Further, this viewpoint accounts for the fact that these iron–sulphur proteins do not normally act as two-electron acceptors, since the addition of a second electron corresponds to an increase in the net charge on the cluster from -3 to -4 , which may be expected to involve a correspondingly much higher free-energy change than the first electron reduction. The second one-electron redox potential calculated above is much less than that of the low-potential photosystem in plants. This observation is therefore consistent with the fact that these proteins function as one-electron transfer carriers.

We may conclude that the present electrostatic description of the two-iron sulphur co-ordination site in the plant and algal proteins may be sufficient to account for their redox potentials relative to that for rubredoxin and for the one-electron acceptor property of these proteins. The electrostatic model considered here should however not be identified with a simple ionic model for the iron–sulphur co-ordination centre in these proteins. On the contrary, the thorough spectroscopic studies of these two types

of proteins indicate that a pure ionic description of the iron co-ordination centre is not justified. The consideration of a microscopic dielectric constant greater than 1 is consistent with the covalent character of the metal-ligand bonds. Yet the electrostatic model is not inconsistent with previously described molecular orbital models of these proteins (Gibson *et al.*, 1966; Thornley *et al.*, 1966) which provide an interpretation of their magnetic and spectroscopic properties. The present model, however, emphasizes the importance of considering the effect of the net charge on each of the iron centres in explaining their relative redox properties.

References

- Arnon, D. I. (1965) *Non-Heme Iron Proteins: Role in Energy Conversion, Symp.* 137-173
- Beinert, H., Der Vartanian, D. V., Hemmerich, P., Veege, C. & Van Voorst, J. D. W. (1965) *Biochim. Biophys. Acta* **96**, 530-533
- Blumberg, W. E. & Peisach, J. (1965) *Non-Heme Iron Proteins: Role in Energy Conversion, Symp.* 101-107
- Churchill, M. R. & Wormald, J. (1971) *Inorg. Chem.* **10**, 1778-1782
- Dunham, W. R., Bearden, A. J., Salmeen, I. T., Palmer, G., Sands, R. H., Orme-Johnson, W. H. & Beinert, H. (1971) *Biochim. Biophys. Acta* **253**, 134-152
- Eaton, W., Palmer, G., Fee, J. A., Kimura, T. & Lovenberg, W. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 3015-3020
- Fee, J. A., Mayhew, S. G. & Palmer, G. (1971) *Biochim. Biophys. Acta* **245**, 196-200
- Fritz, J., Anderson, R., Fee, J. A., Palmer, G., Sands, R. H., Tsibris, J. C. M., Gunsalus, I. C., Orme-Johnson, W. H. & Beinert, H. (1971) *Biochim. Biophys. Acta* **253**, 110-133
- Fry, K. T., Lazzarini, R. A. & San Pietro, A. (1963) *Proc. Nat. Acad. Sci. U.S.* **50**, 652-657
- Gibson, J. F., Hall, D. O., Thornley, J. H. M. & Whatley, F. R. (1966) *Proc. Nat. Acad. Sci. U.S.* **56**, 987-990
- Herriot, J. R., Sieker, L. C., Jensen, L. H. & Lovenberg, W. (1970) *J. Mol. Biol.* **50**, 391-406
- Kirkwood, J. G. & Westheimer, F. H. (1938) *J. Chem. Phys.* **6**, 506-512
- Lovenberg, W. & Sobel, B. E. (1965) *Proc. Nat. Acad. Sci. U.S.* **54**, 193-199
- Palmer, G., Dunham, W. R., Fee, J. A., Sands, R. H., Iizuka, T. & Yonetani, T. (1971) *Biochim. Biophys. Acta* **245**, 201-207
- Rao, K., Commack, K. R., Hall, D. O. & Johnson, C. E. (1971) *Biochem. J.* **122**, 257-265
- Tagawa, K. & Arnon, D. I. (1968) *Biochim. Biophys. Acta* **153**, 602-613
- Thornley, J. H. M., Gibson, J. F., Whatley, F. R. & Hall, D. O. (1966) *Biochem. Biophys. Res. Commun.* **24**, 877-879
- Van Voorst, J. D. W. & Hemmerich, P. (1966) *J. Chem. Phys.* **45**, 3914-3918