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# On the energetics of conformational changes and pH dependent redox behaviour of electron transfer proteins

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Calculations of the electrostatic interaction energies for four metalloproteins that carry out electron transfer are reported. Each protein has a pH dependent redox potential from which the measured electrostatic interaction energy is obtained. The calculations were made using the X-ray structure coordinates and a semimacroscopic model of the interactions. For cytochrome *c*-551 and HIPIP the calculated and observed interaction energies were found to be approximately the same, in agreement with the fact that significant conformational changes do not accompany the ionisations. For cytochrome $c_2$ and azurin, however, major differences were found between the calculated and observed values. These are accounted for primarily by the occurrence of significant conformational changes accompanying the ionisations. The reorganisation energies for these conformational changes are $\sim 7.0$ and $\sim 11.1$ kJ · mol$^{-1}$, respectively.

Electrostatic interaction; Conformational change; Redox potential

## 1. INTRODUCTION

There are many types of redox protein, ranging from the simple electron-transfer proteins such as flavodoxin and ferredoxin to complex enzymes such as catalase and superoxide dismutase. Despite the wide variation in the nature of their redox-active groups and associated biochemical activities, these proteins have a common structural feature: namely, their redox centres are all buried within the protein and are largely inaccessible to bulk solvent. Since all of these centres carry a charge in at least one of their oxidation states, this immediately raises the question: how important are electrostatic interactions in determining their properties? The stability of the buried charge will be greatly affected by the dielectric properties of the surrounding polypeptide and solvent, and it is now apparent that in many proteins this is one of the most important determinants of the redox potential [1–3].

While this view is in accordance with the established electrostatic theory, it is difficult to demonstrate experimentally. For proteins exhibiting redox state dependent ligand binding, experimental information about the strength of an electrostatic interaction can be obtained [4,5]. The most commonly studied ligand binding is the binding of protons and in many redox proteins this takes place for certain groups with a marked redox state dependence to the p$K_a$. The purpose of the present paper is to consider such experimental data for two cytochromes *c*, the blue copper protein azurin, and the iron-sulfur protein HIPIP, and to show how calculations of electrostatic interaction energies together with the measured redox shifts in p$K_a$ can give information about the dielectric properties of proteins and the energetics of changes in protein conformation.

The role of electrostatic interactions in influencing the structure and function of proteins has long been recognised to be important [6,7] but it is only

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recently that reliable theoretical modelling methods have been developed to allow a quantitative estimate of the magnitude of such interactions to be made [8,9]. A variety of methods have been described which range from continuum models, in which macroscopic dielectric constants are used, to microscopic polarisability models, in which dielectric constants are not used. The former models are most often employed, partly because of their ease of calculation, even though the use of macroscopic dielectric constants on a microscopic level is open to serious criticism [10]. The relative merits of different methods are considered in several reviews [7–9]. In the present paper the semimacroscopic method of Warwicker and Watson [11] is used. This method is a refinement of the cavity dielectric model and takes into account the exact size and shape of the protein as well as the precise location of charges buried within it.

## 2. METHODS

### 2.1. *Data bases*

Measurements of redox potentials and redox state dependent ionisation constants were taken from the literature. These data are summarised in table 1. X-ray coordinates were taken from the Brookhaven protein data bank.

The question of whether the ionisations are accompanied by extensive conformational changes is difficult to answer fully in the absence of X-ray diffraction structures at different pH values. NMR studies of *Pseudomonas aeruginosa* cytochrome *c*-551 [12,13] and *Chromatium vinosum* HIPIP [18] showing that only the groups whose resonances are significantly shifted by the ionisation of interest are those of the redox centre and the ionising group itself. This indicates that there is not a significant conformational change of these proteins. However, NMR studies of azurin show that there is an extensive conformational change on ionisation of His 35 that affects the methionine ligand to the copper [17]. *Rhodospirillum rubrum* cytochrome $c_2$ has not been studied by NMR to the same extent as the other proteins but a related protein, *Rhodomicrobium vannielii* cytochrome $c_2$, has and in this case there is an accompanying conformational change that affects many groups in the protein [19]. Therefore, it seems probable that the homologous *R.rubrum* cytochrome $c_2$ will also undergo a conformational change.

### 2.2. *Calculations*

Calculations of interaction potentials were carried out using the algorithm developed by Warwicker and Watson [11]. This uses a three-dimensional Cartesian grid on which grid cubes are assigned as being inside or outside the protein and charges are allocated to grid points according to the algorithm of Edmonds et al. [20].

The longest dimensions of the protein in each direction are derived from the coordinates and multiplied by three. A box of this size is constructed and the protein is placed on the centre such that it occupies most of the central 1/27th of the box. The other 26/27 of the box is assigned as solvent. This box is then divided into uniform cubes such that the total number of cubic elements is close to 216 000 (i.e. $60 \times 60 \times 60$). Thus in the case of azurin we used a box 69 cubes by 53 cubes by 61 cubes, cytochrome $c_2$ (51,68,64), HIPIP (63,61,58) and cytochrome *c*-551 (62,61,58). The size of the cubic elements ranged between 0.5 and 1.5 Å in these calculations. It should be noted that in the case of cytochrome $c_2$, azurin and HIPIP, there are a few residues missing from the coordinates deposited in the databank. These have been accounted for in an approximate manner by the use of dummy side chains.

The programme calculated the potential from Poisson's equation, which relates electrostatic potential to the distribution of charges and dielectric media, by an iterative finite difference procedure in which infinitesimal changes in potential and space were replaced by finite differences on the grid (see [9] for details). The intrinsic dielectric constant of the protein was taken to be 3.5 and that of the solvent water 80.

## 3. RESULTS

### 3.1. *Cytochrome* c-*551*

The calculated interaction potential ($\Delta E_m^c$) for cytochrome *c*-551 assuming the charges are located at the iron and equally on the two oxygens of the propionate, is 90 mV compared to the experimental value of 65 mV. This is in reasonable agreement, especially when compared to the results of other commonly used theoretical models, namely 200–500 mV [21]. However, the calculated values of table 1 ignore the fact that the haem propionic acid group is close to an arginine residue [22]. Fig.1 illustrates the relationship of these groups and shows that if the indicated charge-relay system operates, $\Delta E_m^c$ for the iron-propionate interaction is reduced to 70 mV. There are no other charged groups close to the propionate which could further reduce significantly the interaction potential.

### 3.2. *Cytochrome* $c_2$

As indicated in table 1, the electrostatic interaction for cytochrome $c_2$ is more complex than those of the other proteins. From work with other cytochromes in which the buried propionate is associated with a partly buried histidine [13,23] we propose that $pK_o$ and $pK_r$ are composite $pK_a$ values as illustrated in fig.2. The effect of the involvement of the histidine is to reduce the iron-propionate interaction by about 30 mV (table 2). Even so, the reduced $\Delta E_m^c$ of 119 mV is still substantially higher than the observed value of 47 mV.

Table 1

pH dependent redox behaviour of one-electron transfer proteins

| Protein | $pK_o$[a] | $pK_r$[a] | $\Delta E_m$ (mV) | Conformational change[b] | Ionising group | Ref. |
|---|---|---|---|---|---|---|
| *P.aeruginosa* cytochrome *c*-551 | 6.2 | 7.3 | 65 | no | HP-7[c] | 12,13 |
| *R.rubrum* cytochrome $c_2$ | 6.2 | 7.0 | 47 | probably | HP-7...His | 14 |
| *P.aeruginosa* azurin | 6.1 | 7.4 | 77 | yes | His 35 | 15–17 |
| *C.vinosum* HIPIP | 6.7 | 7.3 | 36 | no | His 42 | 18 |

[a] $pK_o$ and $pK_r$ were determined spectroscopically or from the pH dependence of the midpoint redox potential. (Note: $nF\Delta E_m = RT\ln\Delta pK$)

[b] The occurrence of conformational changes was determined spectroscopically (see the text)

[c] HP, haem propionate

There are no other charged groups which could reduce significantly the interaction potential by a charge-relay mechanism.

### 3.3. *Azurin*

The $\Delta E_m^c$ for azurin of 191 mV is significantly higher than the observed value of 77 mV. The X-ray structure [25] has not been refined and is at a lower level of resolution than the other structures used in this work [22,24,26], thus some of the difference between observed and calculated values may reflect errors in interatomic distances. However, it is unlikely that the structure is so wrong that the entire difference is due to errors.

### 3.4. *High potential iron protein*

The difficulty in obtaining a reliable calculated value for HIPIP is in determining how to place the charge on the [$Fe_4S_4$ - $Cys_4$] cluster. The values in table 2 show that $\Delta E_m^c$ varies from 42 to 23 mV for His 42 ND1 and from 31 to 18 mV for His 42 NE2 depending on which iron the charge is located.

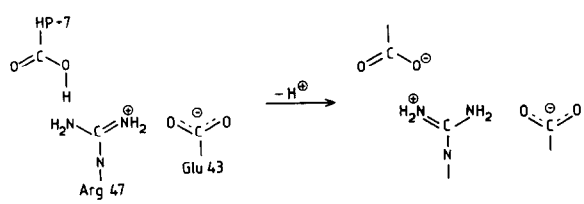


Fig.1. Haem propionate-arginine charge-relay of *P.aeruginosa* cytochrome *c*-551. $\Delta E_m^c$ for the ionisation of HP-7 disregarding Arg 47 and Glu 43 is 90 mV. Taking into account the presence of Arg 47 by assuming that its charge is redistributed as indicated reduces $\Delta E_m^c$ to 70 mV. $\Delta E_m$ is 65 mV.

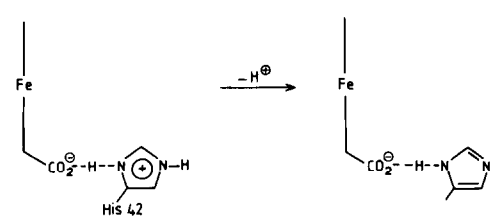


Fig.2. The composite redox-state dependent ionisation of *R.rubrum* cytochrome $c_2$. HP-7 is the ionising carboxylic acid.

Table 2

Calculated pH dependence of the redox potentials of one-electron transfer proteins

| Protein | Charge interaction | | $\Delta E_m^c$ (mV) | Average |
|---|---|---|---|---|
| *P.aeruginosa* cytochrome *c*-551 | Fe --- HP7 | 01A | 104 | 90 |
| | Fe --- HP7 | 02A | 77 | |
| *R.rubrum* cytochrome $c_2$ | Fe --- HP7 | 01A | 155 | 119 |
| | Fe --- HP7 | 02A | 141 | |
| | Fe --- His 42 | ND1 | 98 | |
| | Fe --- His 42 | NE2 | 80 | |
| *P.aeruginosa* azurin | Cu --- His 35 | ND1 | 209 | 191 |
| | Cu --- His 35 | NE2 | 173 | |
| *C.vinosum* HIPIP | Fe1 --- His 42 | ND1 | 42 | 27 |
| | | NE2 | 31 | |
| | Fe2 --- His 42 | ND1 | 32 | |
| | | NE2 | 26 | |
| | Fe3 --- His 42 | ND1 | 23 | |
| | | NE2 | 18 | |
| | Fe4 --- His 42 | ND1 | 25 | |
| | | NE2 | 19 | |

Spectroscopic measurements [27] and structural data [28] indicate that the irons are equivalent and so we have taken the charge to be delocalised over the cluster to give an average value of 30.5 mV for the His 42 ND1 interaction and 23.5 mV for the His 42 NE2 interaction. Since we do not know the charge distribution within His 42 we have given the averaged value of 27 mV in table 2. Assuming that the charge is delocalised onto the sulphides as well as onto the iron atoms, does not affect the average value of $\Delta E_m^c$.

Although there are uncertainties in the calculations for HIPIP, the agreement between the observed value of 36 mV and the various calculated values (table 2) is good. In the worst case, $\Delta E_m^c$ is only 18 mV different from $\Delta E_m$.

## 4. DISCUSSION

### 4.1. *Calculations of electrostatic interactions*

The major uncertainties with the calculations reported herein are the use of macroscopic dielectric constants and the precise location of the charges. The former uncertainty can be removed by the use of microscopic polarisability models [8], although there are other problems with these [9]. The latter uncertainty cannot be removed by these models. For the charge-relay systems described in this work, that involving a buried propionate-histidine pair has experimental support [13,23] in agreement with the calculations (table 2) and this gives us confidence in the proposed propionate-arginine charge-relay (fig.1).

Experimental determinations of charge locations are difficult and as electrostatic calculations are improved, so that confidence in their results is strengthened, they will become a major method for determining such locations.

### 4.2. *Comparison of calculated and observed interaction potentials*

A central problem in comparing calculated and observed values of interaction potentials is correcting for ionic strength and ion-binding effects. The calculation assumes an ionic strength of zero whereas the measured values are usually at an ionic strength of 0.01–0.2 M. Work with cytochrome *c*-551 over a wide range of ionic strength has shown that the ionic strength dependence of $\Delta E_m$ is negligible [12,13] and this is also likely to be the case for *R.rubrum* cytochrome $c_2$ judged on the basis of work done with related proteins [19,23].

Analogous ionic strength studies have not been reported for azurin or HIPIP but the indication from related studies is that there will be, at most, a minor ionic strength effect for these proteins. For azurin, a spectroscopic measurement of $pK_r$ at a significantly higher ionic strength than that used for the measurement in table 1 found a value of about 7 [30]. For HIPIP, the related study is an ionic strength dependence of the rate of electron transfer measured as a function of pH [31]. The reported $\Delta E_m$ from data extrapolated to infinite ionic strength was 25 mV.

### 4.3. *Energetics of conformational changes in electron transfer proteins*

Despite the caveats over the differences between $\Delta E_m$ and $\Delta E_m^c$ given above, observation of significant differences for two proteins that undergo associated conformational changes and negligible differences for two proteins that do not undergo associated conformational changes (cf. tables 1 and 2) is important. The natural conclusion is that $\Delta E_m^c - \Delta E_m$ for cytochrome $c_2$ and azurin is an indication of the energy required for their conformational changes; 72 mV and 114 mV (7.0 $kJ \cdot mol^{-1}$ and 11.1 $kJ \cdot mol^{-1}$), respectively.

Measurements of the energetics of conformational changes associated with changes in the net charge and charge distribution of proteins are difficult to obtain. However, they are important data, especially for electron-transfer proteins whose action may be described by the classical theory of Marcus [32]. This shows that the reorganisation energy of the protein accompanying electron transfer may be a rate determining factor. In the absence of other data it seems reasonable to assume that the reorganisation energy associated with the change in ionisation state of a buried group will be comparable to that for the change in oxidation state of the metal ion. Therefore, the values we have given for the conformational changes in cytochrome $c_2$ and azurin may be applicable to the calculation of electron transfer rates. These values compare reasonably well with the oxidation state reorganisation energy of mitochondrial cytochrome *c*, 5 $kJ \cdot mol^{-1}$, calculated by Warshel and Churg [33].

Proteins containing more than one redox centre

can also be analysed by the approach used in the present paper. Instead of interaction potentials between the charges on a metal ion and an ionisable group, the interaction potential for two redox centres can be considered. The tetrahaem cytochromes $c_3$ appear to be an excellent case for such an analysis. The microscopic redox potentials of the four haems show that there is cooperativity between them. Significantly, some of the interaction potentials are positive, i.e. the oxidation of one haem favours the oxidation of another. Simple electrostatic considerations argue for negative potentials and therefore these data indicate major conformational changes accompanying electron transfer [34].

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