Distance dependence of interactions between charged centres in proteins with common structural features

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Abstract Data collected for interactions among redox centres, and interactions between redox centres and acid–base residues in a family of small multihaem cytochromes are analysed. The distance dependent attenuation of the interactions between non-surface charges, for separations that range from 8 to 23 Å, can be described by a simple function derived from the Debye–Hückel formalism, fit to 9.5 and 7.6 as values for the relative dielectric constant and Debye length, respectively. However, there is considerable scatter in the data despite the structural similarities among the proteins, which is discussed in the framework of using such simple models in predicting properties of novel proteins.

Keywords: Electrostatic interactions; Redox potentials; Dielectric function; Debye–Hückel; Hill factor; Cytochromes

1. Introduction

Electrostatic interactions have a major influence on the properties of biological macromolecules with respect to folding, the reactivity and affinity of active centres, and in the recognition and selectivity of substrates or metabolic partners. Redox proteins present additional challenges in predicting the reduction potential of their active sites because of the variety of contributions. Solvent exposure of the redox co-factors is unarguably a fundamental influence in the reduction potential due to the high polarizability of water [1,2] but may not be dominant [3]: studies based on site directed mutagenesis have identified several other factors that control redox potentials, such as amino acid hydrophobicity, side-chain volume, and charge [4–6].

Redox proteins provide a convenient source of data for the study of interactions between charges in proteins because uptake or release of electrons may occur, in principle, without modification of nuclear coordinates, whereas studies of charge modification based on site-directed mutagenesis require evaluation of the structural alterations in the mutant, and the effects may not be easy to separate. Even the interactions between acid–base and redox centres are likely to be disruptive of the structure since they involve at least movement of the proton to/from the acid–base residue, with the corresponding perturbation of H-bonds or ion-pairs.

The study of interactions between charged centres in metalloproteins may be complicated by redox-linked changes in the preferred coordination geometry of the metal, with the consequent straining of the structure, a well-known effect in copper proteins [7]. However, haem proteins with hexacoordinate iron are convenient subjects because the macrocycle and the axial ligands impose a defined coordination geometry on the metal with only slight modifications in bond length associated with the transition from Fe(II) to Fe(III). Haems *c* are the most convenient since they are covalently attached to the polypeptide chain and, thus, there is no heterogeneity in the haem insertion in the protein, as is observed for haems *b* [8].

Exceptionally large attenuation of electrostatic interactions in proteins has been described in the literature and analysed in the framework of simple Coulomb decay with large effective dielectric constants [9]. Later, it was proposed that the effective epsilon displays an apparent increase with distance, which led to the development of empirical mathematical expressions [10,11]. In this work, the collected data for several homologous multihaem cytochromes *c* is reported. A model that considers coulombic decay enhanced by a Debye–Hückel shielding factor is shown to be able to capture the trend of distance dependence of redox- and redox-Bohr interactions among the various centres.

2. Materials and methods

The interaction energies discussed here were obtained by fitting data from NMR and visible spectroscopy to a model comprising up to 32 microstates, which result from considering up to four haems and one acid/base centre. This model considers that the interactions are pairwise, which implies a degree of averaging as subtle conformational changes have been detected between the fully oxidised and reduced forms [12–14]. Furthermore, even in cases where the principal acid/base group has been identified, there are probably secondary protonation sites. However, this analysis provides for a clear separation between the self-energies of each centre and the interactions among the various charged centres in line with the arguments presented in the literature for the separate treatment of these two factors [15–17]. For consistency, repulsive interactions between charges of equal sign, and attractive interactions between charges of different sign are positive.

Electrostatic interactions taking place in a medium different from vacuum can be considered to be subject to two different influences:

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(1) The medium is polarizable to an extent that depends on its constituent molecules, with the consequent shielding of the interactions. Thus, a dielectric constant larger than in vacuum is used to avoid explicit enumeration of the contribution of each factor involved.

(2) Extra shielding occurs in ionic solutions due to the non-uniform distribution of charges, which modifies the distance dependent decay of the interactions, which appear to be more strongly shielded with distance than is expected from the dielectric constant of the protein medium.

In these circumstances, the interaction (V_i) between two charged particles with unit charge can be described by a model that considers the medium between the interacting particles to have uniform polarizability and the effect of counterions treated in terms of Debye–Hückel shielding [18]. This can be written as

$$V_i = k\frac{1}{\varepsilon r}\exp\left(\frac{-r}{r_D}\right) \quad (1)$$

where k stands for the electron charge divided by the vacuum electric permittivity multiplied by 4π, and r_D is the Debye length which depends on charge density, temperature and ionic strength. This expression can be used taking the distance r between the charged centres as the sole structural information, and can be fit to the experimental data by adjusting two parameters, ε and r_D.

3. Results

Detailed data on the distance dependence of redox interactions among the various centres in multiredox centre proteins are scarce, and current state of the art is limited to proteins with up to four redox centres. Interactions between charged centres were obtained from work on several multihaem cytochromes [19–22] [our manuscript in preparation]. In Fig. 1, the electrostatic interactions are plotted versus distance using the atomic coordinates of the structures of several cytochromes c_3 deposited in the PDB: 1CAO from *Desulfovibrio (D.) africanus*, 2CTH from *D. vulgaris* Hildenborough (DvHc3), 1WAD from *D. gigas* (Dgc3), 2CDV from *D. vulgaris* Miyazaki (DvMc3), and 2CY3 from *Desulfomicrobium norvegicum*. The distances for the tetrahaem cytochrome from *Shewanella frigidimarina* (Sfc3) were taken from [23]. Since there is no apparent correlation between the interaction energy and the relative orientation of the haems, the charge was considered to be localised on the iron. Interactions involving acid–base centres were only considered for the cases of Dgc3, DvHc3 and DvMc3 in which the primary acid–base centre is unambiguously identified as the propionate 13 of haem I [20,24,25] and in this case the average position of the carboxylate oxygen atoms was considered as the location of the charge.

In some cases, specific conformational changes in the protein associated with the redox or acid–base transitions have been identified, that give rise to significant non-electrostatic contributions to the measured interaction [12–14,26–28]. The corresponding data were not considered in the analysis and were plotted in Fig. 1 as open circles. The three remaining negative points closer to the origin were obtained for cytochrome c_3 from *Desulfovibrio gigas* and cytochrome c_7. For both proteins the structures in the reduced and oxidised state do not show redox-linked conformational changes that can be associated with these interactions. Therefore, we consider that these values are a result of the experimental uncertainty of the method used in their determination, and were used in the analysis.

Eq. (1) was fit to the data reported in Fig. 1, to obtain a value for the apparent dielectric constant and Debye length. The values that provide the best least squares fit are 9.5 for the relative dielectric constant and 7.6 Å for the Debye length.

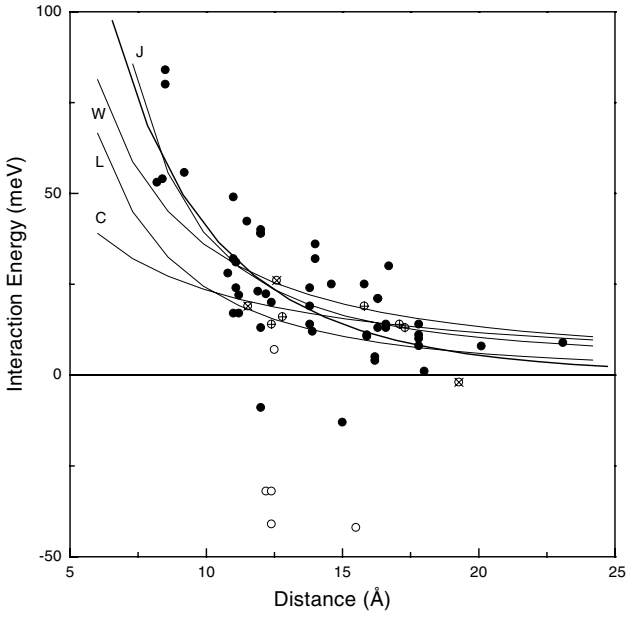

Fig. 1. Distance dependence for electrostatic interactions in multihaem cytochromes reported in units of meV. Hollow data points are believed to be subject to substantial non-electrostatic contributions as discussed in the text. All data were measured at 0.1 M salt except crossed circles, which were measured at 0.36 M (vertical cross) and 0.5 M (diagonal cross). Standard errors on the data range from 1 to 6 meV. The thick line shows the fit of the function in Eq. (1) to the data points while the line labelled C shows the fit of a Coulomb decay to the data with an effective epsilon of 61. W represents the model of Warshel et al. [15], L represents the linear model [11], J represents the model of Johnson et al. [36].

Although the proteins analysed in this work show a span of isoelectric points from 5.3 to 10.5, no trend is observed for the dependence of the Debye length with charge density. This may result from a degree of averaging, in particular over the pH range for which the data was measured for each protein, as mentioned in the introduction, and the fact that most of these proteins have a relatively small overall charge in the experimental pH range, therefore reducing the differences in charge density among different proteins.

4. Discussion

Simple macroscopic models with a limited number of parameters cannot hope to describe accurately the electrostatic properties of all centres in a protein, let alone a set of different proteins. Fig. 1 illustrates the natural heterogeneity of the dielectric properties of the interior of proteins [29] that results from the fact that each centre has a unique environment. As stated in the introduction, exposure to the solvent influences the dielectric environment, but the clear trend for the distance dependence of the electrostatic interactions, shows that this is not a dominant effect in these cases. Furthermore, although the application of the Poisson–Boltzmann equation to these proteins gives good agreement with experimental interactions when conformational effects are not dominant [30,31], the search for simple functions for self-energies or electrostatic

interactions that allow for fast algorithms is a worthwhile objective [32] due to their application in computational structural biology.

The meaning of dielectric constants greater than 1 has been debated extensively in the literature (for a recent review see [16]). The value for the effective dielectric constant in proteins depends on which effects are explicitly considered [16] and relatively large values, ranging between 15 [33,34] and 20 [35,36], are accepted for the dielectric constant of the protein interior. However, the distance dependence of the experimental interactions appears to be different from simple Coulomb decay, shown by the line C in Fig. 1, which was obtained by fitting the data to a Coulomb decay and gave an effective epsilon of 62. Eq. (1) is based on the Debye–Hückel formalism, where the dielectric constant defines the relative dielectric response of the medium, and the Debye length corresponds to the distance where the maximum concentration of counter ions is found. However, when applying this equation to interactions among charged centres buried inside proteins, these parameters become purely empirical. This situation is akin to regarding dielectric constants larger than 1 in other macroscopic models for the interior of proteins as scaling factors to fit the model to the data [16,29,37,38].

4.1. Comparison with other dielectric functions

Over the years, several formulations for a distance dependent dielectric have been proposed, of which representative examples can be found in [11,15,36], mostly developed to fit shifts in pK_as of ionisable groups in proteins upon mutation of other charged residues. For distances below 6 Å, there is evidence that the electrostatic shielding follows a sigmoidal function due to the different dielectric response of the first and second solvation shells surrounding a charge [39]. These effects could not be explored with the data analysed in this work because the closest distance is 8.2 Å. The lines reported in Fig. 1 for the fit of the model presented by Warshel et al. [15], the linear model [11], and the model of Johnson et al. [36] show that various macroscopic functions display similar performance in the distance range of the present data set, with the present model and the model of Johnson et al. showing a better agreement with the data for shorter distances.

4.2. Comparison with other published data

There is a wealth of published work on values of ΔpK_as of amino acid residues in proteins subjected to site directed mutagenesis, measured from the pH dependence of the chemical shift of NMR signals.

Fig. 2 reports typical data for the interaction between surface charged residues for charge reversal and charge neutralisation mutants of Snase [40], barnase and subtilisin [41], for the interaction between charged residues and the haem for iso1-cytochrome *c* [42], and in cytochrome *c* [9], for distances above 6 Å as discussed above. It shows that the magnitude of the electrostatic interactions for surface residues is systematically reduced compared with the expected values from the model presented in this work.

In most cases the titration data were analysed using an adapted version of the Henderson–Hasselbalch equation that includes a Hill factor to improve the fit, which reflects the presence of interactions between titrating groups [43]. A thorough analysis of the factors affecting published data on amino acid pK_as is beyond the scope of this paper, but the

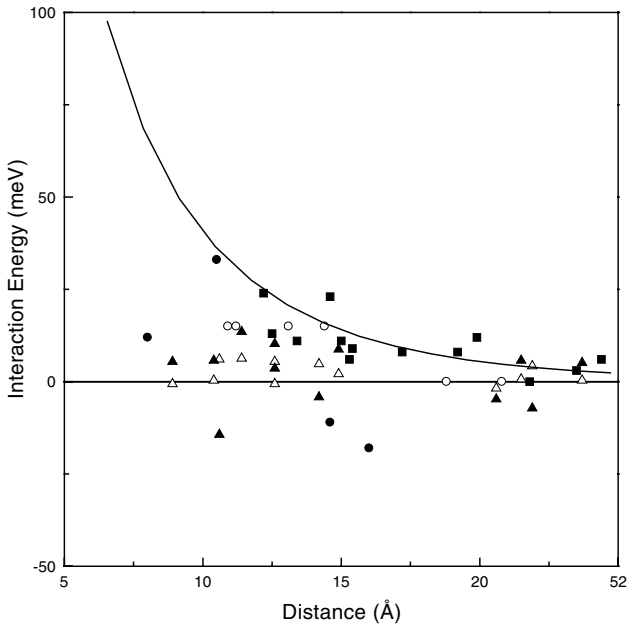

Fig. 2. Comparison of typical published data for interactions between surface charges with the function for distance dependent electrostatic interactions used in this work, and △ data from [40], ■ data from [41], ● data from [42], and ○ data from [9].

observation reported in the literature that the Hill factor can vary up to 20% from unity merits a comment [40,41]. Such variation on the Hill factor corresponds to approximately 25 meV (~0.4 pK units) change on the absolute value of the total interaction felt by each residue. Given the magnitude of the ΔpK_as reported, this value is significant, since the presence of cooperativities may lead to an under- or overestimate of the ΔpK_as, depending on the circumstances. Although the effect of each of the neighbouring titrating residues may be difficult to parse, the major influences on this Hill factor are necessarily caused by residues titrating in the same pH range. Otherwise, instead of affecting the slope of the titration curve, the interaction would affect the position of the curve and thus the ΔpK_as measured.

The modification of the pK_as of ionisable groups at the surface of proteins by the presence of other charges has been shown to diminish as the ionic strength is increased [40,41], as may be expected because ions in solution can approach surface charges. For the data reported in Fig. 1, there is no significant bias towards smaller interactions for the measurements performed at higher salt concentrations. This suggests that the interactions among the various charged centres inside these cytochromes are not affected significantly by ionic strength of the surrounding medium, despite the small size of the proteins.

5. Conclusions

In this work, a large set of experimentally determined electrostatic interactions between charged centres in proteins with common structural features were collected. These data were analysed using a simple electrostatic model in which Coulomb distance decay is enhanced using a function based on the Debye–Hückel formalism. It is clear that, although such

macroscopic electrostatic models are very effective to correctly capture the trend of the distance dependence of electrostatic interactions for non-surface groups with distances in the range from 8 to 23 Å as shown here and by others [16,36,40], there is considerable scatter. This is despite the fact that, with the exception of Sfc3, the arrangement of haems and the structure between specific pairs of charges is similar within each protein. Therefore, users of such functions must accept that substantial deviations may occur between predicted and experimental interactions even when there are no obvious coupled structural rearrangements.

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