Protein Control of Iron-Sulfur Cluster Redox Potentials*

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Ralf Langen, Gerard M. Jensen, Uwe Jacob, Philip J. Stephens‡, and Arieh Warshel‡

From the Department of Chemistry, University of Southern California, Los Angeles, California 90089-0482

The relationship between the three-dimensional structures of iron-sulfur proteins and the redox potentials of their iron-sulfur clusters is of fundamental importance. We report calculations of the redox potentials of the [Fe₄S₄(S-cys)₄]²⁻/⁻ clusters in four crystallographically characterized proteins: Azobacter vinelandii ferredoxin I, Peptococcus aerogenes ferredoxin, Bacillus thermoproteolyticus ferredoxin, and Chromatium vinosum high potential iron protein (HiPIP). Our calculations use the "protein dipoles Langevin dipoles" (PDLD) approach. The electrostatic interaction of these proteins presents a major challenge.

The much greater contribution of VQ, in BtFd, PuFd, and CuHiPIP has been determined by x-ray crystallography (5–11). AuFd contains one [Fe₆S₈(S-cys)₈]²⁻/⁻ cluster and one [Fe₆S₈(S-cys)₈]²⁻/⁻ cluster whose midpoint potentials are ~650 mV and ~420 mV (versus SHE), respectively (12). PaFd contains two [Fe₆S₈(S-cys)₈]²⁻/⁻ clusters, which both have midpoint potentials of ~420 mV (13). BtFd contains a single [Fe₆S₈(S-cys)₈]²⁻/⁻ cluster; the midpoint potential of the very nearly identical B. stearothermophilus ferredoxin is ~420 mV (14). HiPIP contains a [Fe₆S₈(S-cys)₈]₄ cluster whose ~2/3–3 couple has not yet been expressed under physiological conditions; instead, the ~1/2–2 couple is active (15, 16).

Proteins containing iron-sulfur ([Fe-S]) clusters occur ubiquitously in nature and play a major role in biological electron transport (1). Thus, the understanding of the control of the redox potential of an [Fe-S] cluster by its protein environment is of fundamental importance. Here, we address this challenge by investigating the origin of the enormous variation in the midpoint potential of the ~2/3–3 couple of the [Fe₆S₈(S-cys)₈] clusters of four small, structurally well-characterized proteins: Azobacter vinelandii ferredoxin I (AuFd), Peptococcus aerogenes ferredoxin (PaFd), Bacillus thermoproteolyticus ferredoxin (BtFd), and Chromatium vinosum high potential iron protein (CuHiPIP). The electrostatic interaction of these [Fe-S] clusters with their protein and water environment is calculated using the "protein dipoles Langevin dipoles" (PDLD) approach (2–4). The predicted variation in the electrostatic contribution of protein and water together to the cluster redox potential is in excellent agreement with experiment. These calculations constitute the first successful modeling of the protein control of [Fe-S] cluster redox potentials.

The structures of AuFd, PaFd, BtFd, and CuHiPIP have been determined by x-ray crystallography (5–11). AuFd contains one [Fe₆S₈(S-cys)₈]²⁻/⁻ cluster and one [Fe₆S₈(S-cys)₈]²⁻/⁻ cluster whose midpoint potentials are ~650 mV and ~420 mV (versus SHE), respectively (12). PaFd contains two [Fe₆S₈(S-cys)₈]²⁻/⁻ clusters, which both have midpoint potentials of ~420 mV (13). BtFd contains a single [Fe₆S₈(S-cys)₈]²⁻/⁻ cluster; the midpoint potential of the very nearly identical B. stearothermophilus ferredoxin is ~420 mV (14). HiPIP contains a [Fe₆S₈(S-cys)₈]₄ cluster whose ~2/3–3 couple has not yet been expressed under physiological conditions; instead, the ~1/2–2 couple is active (15, 16).

The correlation of the structures and redox potentials of these proteins presents a major challenge. In this work we evaluate the effect of the protein on the redox potential of the [Fe₆S₈(S-cys)₈] clusters in these four proteins using the PDLD approach (2–4) as implemented in the program POLARIS (17). The electrostatic interaction of a charged moiety: in this case, the [Fe₆S₈(S-cys)₈]²⁻/⁻ (S = cysteine S) portion of the [Fe-S] cluster, with its surroundings is expressed as the sum of four terms: V₆ₐ, V₆₅, V₆₇, and V₆₈. V₆ₐ and V₆₇ are the interactions of the cluster with the partial charges and induced dipoles of protein atoms, respectively. V₆₅ and V₆₈ are the interactions with solvent water, treated microscopically as a sphere of radius rₙ surrounding the cluster, and macroscopically beyond, respectively. Microscopic water molecules are represented by point dipoles placed on a grid and oriented self-consistently in the combined electrostatic field arising from cluster charges, protein charges and induced dipoles and all other water dipoles.

Calculations have been carried out for the ~2/3–3 couple of the [Fe₆S₈(S-cys)₈] clusters of AuFd, BtFd and CuHiPIP and of one of the two clusters of PaFd (that ligated by cysteines S, 11, 14, and 46); an error in the protein sequence used in the PaFd structure determination in the neighborhood of the second cluster has recently come to light (18), and we have excluded this cluster from consideration. The differential solvation energies of ~2 and ~3 clusters obtained are reported in Table I (which also includes additional details of the calculations) and Fig. 1. Redox potentials obtained thence are compared to experiment in Fig. 2. In principle, the absolute redox potential can be evaluated using the potential of the cluster in aqueous solution as a reference (4). Since this is not available, we here address the trend within the different proteins and we arbitrarily set the calculated and experimental redox potentials of AuFd to be identical. We predict an ordering of potentials: CuHiPIP << AuFd < PaFd < BtFd, in agreement with experiment. The spread for AuFd, PaFd, and BtFd is 447 mV compared to an experimental range of 370 mV.

The contributions of V₆ₐ, V₆₅, V₆₇, and V₆₈ to the total differential solvation energies of BtFd, PaFd, AuFd, and CuHiPIP are given in Table I and are diagrammed in Fig. 1. The much greater contribution of V₆₅ in BtFd, PaFd, and

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‡ To whom correspondence should be addressed.

The abbreviations used are: AuFd, A. vinelandii ferredoxin I; PaFd, P. aerogenes ferredoxin; BtFd, B. thermoproteolyticus ferredoxin; CuHiPIP, C. vinosum high potential iron protein; PDLD, protein dipoles Langvin dipoles.
TABLE I

| Protein | \( \Delta V_{Q_M} \) | \( \Delta V_{Q_W} \) | \( \Delta V_L \) | \( \Delta V_P \) | Total | \( \Delta Q_{oo} \) |
|---------|-----------------|-----------------|---------------|---------------|-------|----------------|
| BuFd | 84.3 | 29.8 | 40.5 | 42.0 | 196.5 | -3.2 |
| PaFd | 80.9 | 23.0 | 44.1 | 42.2 | 190.2 | -3.5 |
| AuFd | 87.4 | 26.6 | 30.3 | 41.9 | 186.2 | -4.0 |
| CuHiPIP | 34.0 | 57.1 | 31.3 | 41.8 | 164.3 | +0.4 |

\( \Delta Q_{oo} \) is the interaction between the cluster and the protein ionized groups (including other clusters). This contribution is evaluated using the microscopic dielectric constant \( \varepsilon(R) \) of Equation 76 of Ref. 2. This approach has been justified and validated elsewhere (2, 3, 29). At the same time the effects of an ionic strength of 0.1 mol/L is also modeled using the approach of Ref. 30. The ionization state of the ionizable protein groups were evaluated by assigning to each group its intrinsic aqueous \( pK_a \) and calculating self-consistently their interactions using the above \( \varepsilon(R) \). A similar trend was obtained by repeating the calculations while estimating the intrinsic \( pK_a \) of groups which are not exposed to water using the PDL method. \( \Delta Q_{oo} \) is relatively small and its addition does not change the trend obtained in its absence.

Although the contribution of the local amide groups accounts for the difference between CuHiPIP and PaFd, it does not explain the variation in redox potential within the BuFd, PaFd, and AuFdI group. This variation originates in changes in the contributions of \( V_{Q_M} \), \( V_{Q_W} \), and \( V_L \) which are comparable in magnitude. The combined contributions of \( V_{Q_M} \) and \( V_{Q_W} \) are PaFd < AuFdI < BuFd, while for \( V_L \), AuFdI < PaFd < BuFd. Thus, the lower potential of AuFdI compared to PaFd is attributable to a substantially smaller \( V_L \) contribution, which outweighs the greater \( V_{Q_M} \) and \( V_{Q_W} \) contribution. In contrast, the lower potential of PaFd relative to BuFd is attributable to the substantially lower \( V_{Q_M} \) and \( V_{Q_W} \) contribution, which outweighs the greater \( V_L \) contribution. The interrelationship of the variations in the protein structure and in \( V_{Q_M} \), \( V_{Q_W} \), and \( V_L \) contributions to the redox potential is complex. Calculations in which protein groups more than 9 Å from the cluster center are discarded exhibit very similar variations, showing that variations in redox potential originate predominantly in changes in protein structure in the neighborhood of the cluster. Of particularly significant interest is the fact that the water contribution \( V_L \) is the dominant

\[ \text{FIG. 1. Contributions to the differential solvation energies of the } [\text{Fe}_{4}\text{S}_{4}(\text{S-cys})_{4}]^{2-3} \text{clusters of BuFd, PaFd, AuFdI, and CuHiPIP.} \]

\[ \text{FIG. 2. Calculated and experimental redox potentials of BuFd, PaFd, AuFdI, and CuHiPIP. The calculated and experimental potentials of AuFdI are arbitrarily set equal (see text). Redox potentials were obtained using the conversion factor 23.06 cal/mV.} \]
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interactions with ionized residues (and other clusters) are expected to be relatively small and can be conveniently estimated using a microscopic model (18, 22–25) with the results given in Table I.

Although the factors governing the redox potentials of [Fe-S] clusters have been widely discussed (18), microscopic calculations of protein tuning of [Fe-S] cluster redox potentials have not been reported previously. Furthermore, the crucial role of the solvent in establishing the trend in redox potentials has not been demonstrated. In view of the agreement between our calculations and experiment, we are extending our studies to encompass other [Fe-S] clusters/redox couples (1) and to examine the results of site-specific mutations in AtdFd (12, 26, 27). More detailed accounts of these studies will be forthcoming.

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Fig. 3. The protein/Langevin dipole system for PaFd (upper panel) and AtdFdI (lower panel). The Langevin dipoles within 6 Å from the clusters are shown as spheres, and the rest are depicted as red/yellow dipoles. The main chain regions which are homologous in both proteins are shown in blue, and the additional C-terminal portion of AtdFdI is shown in gray. Four dipoles are found within 6 Å of the cluster in PaFd; however, there is no space for Langevin dipoles within 6 Å of the cluster in AtdFdI.

factor in causing the difference in redox potential between AtdFdI and PaFd. The environments of the clusters in these two proteins exhibit strong homology (5, 18). Apparently, however, the extent of water penetration to the neighborhood of the clusters is significantly different, as illustrated in Fig. 3. This aspect of the calculations is being studied in more detail.

Our results provide additional support for the PDLG approach in describing electrostatic energetics in aqueous solutions of proteins. We note that in our calculations: (i) there is no change in structure with oxidation state, (ii) all residues (excepting cysteine cluster ligands) are uncharged (see, however, below), and (iii) the second cluster in PaFd and AtdFdI are uncharged. The inclusion of protein reorganization is expected to improve the agreement of calculation and experiment (see, e.g., Ref. 21). The effects of including cluster