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Electrostatic control of midpoint potentials in the cytochrome subunit of the *Rhodopseudomonas viridis* reaction center

(electrochemistry/photosynthesis)

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ABSTRACT **The photosynthetic reaction center of *Rhodopseudomonas viridis* has four hemes in a linear arrangement with alternating high- and low-potential sites. Their midpoints are −60, 20, 310, and 380 mV [Dracheva, S. M., Drachev, L. A., Konstantinov, A. A., Semenov, A. Y., Skulachev, V. P., Arutjunjan, A. M., Shuvalov, V. A. & Zaberezhnaya, S. M. (1988) *Eur. J. Biochem.* 171, 253–264]. Electrostatic calculations reproduce the 440-mV midpoint spread and assignments of high- and low-potential hemes. When calculations on model compounds to connect the theoretical midpoints to the standard hydrogen electrode are used, the absolute electrochemical midpoints for the reaction center hemes are also in good agreement with experiment. The free energy of oxidation is found to be dependent on pairwise interactions with charged amino acids, heme propionic acids, previously oxidized hemes, and axial ligands and on the reaction field induced by heme oxidation.**

Cytochromes are an important family of electron transfer proteins that use heme as an electron donor. Cofactor electrochemistry is modified by the protein, and electrochemical midpoints (E_m values) in the cytochrome family extend over more than 600 mV (1). The photosynthetic reaction center from the bacterium *Rhodopseudomonas viridis* has four *c*-type cytochrome hemes. Two, with midpoints near 350 mV, are electron donors to the bacteriochlorophyll dimer that is oxidized in the first step in light-initiated charge separation. The others, with midpoints near 0 mV, are preoxidized under physiological conditions (for reviews, see refs. 2 and 3).

The reaction center is the first intrinsic membrane protein with a structure known at atomic resolution (4, 5). It consists of four subunits (L, M, H, and C). L and M span the membrane, H is predominantly on the cytoplasmic side of the membrane, and the heme-containing C subunit extends into the periplasm. The hemes are 14–16 Å apart from center to center and 7–8 Å apart from edge to edge. Their midpoints were determined from the dichroism of optical or EPR spectra as a function of the redox potential in protein oriented in chromatophore multilayers (6, 7), Langmuir–Blodgett films (8, 9), or crystals (10) and from the pattern of transient heme oxidation in light-activated electron transfer (7, 11, 12). Their order, moving from the bacteriochlorophyll dimer out into the periplasm, is the highest-potential heme (heme 1 is *c*-559 at 380 mV), a low-potential heme (heme 2 is *c*-552 at 20 mV), a high-potential heme (heme 3 is *c*-556 at 310 mV), and the lowest-potential heme (heme 4 is *c*-554 at −60 mV) (average experimental values are given). The E_m of isolated hemes with His-Met axial ligands (such as hemes 1, 3, and 4) is −70 mV, whereas the E_m is −220 mV for bis-His hemes (such as heme 2) (13, 14). In this report we consider how the protein produces the observed *in situ* electrochemistry.

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There have been various suggestions for how cofactor midpoints can be modulated by electrostatic interactions (15). The importance of placing the heme in the low dielectric protein (16–20), the charges on the protein (21) and on heme propionic acids (22–24), and the electron-donating ability of the axial ligands (25) have been considered. Recent advances in the application of continuum electrostatics allow these contributions to the free energy of oxidation to be calculated for proteins of known structure (26). Related methods have been used to predict E_m values of reaction-center cofactors that are not experimentally accessible (27, 28). It will be shown here that the midpoints of each of the hemes in the *Rps. viridis* reaction center can be largely understood in terms of electrostatic interactions with the protein.

METHODS

The starting point for the calculations is the x-ray crystal structure coordinates of the *Rps. viridis* reaction center (5). Protons were added to the protein and subjected to energy minimization with heavy atoms fixed, minimizing van der Waals overlaps while maximizing hydrogen bonding using the program XPLOR (29). Protons were added to cofactors with the program DISCOVER (30). The four C-terminal amino acids of the C subunit (Ala-Ala-Ala-Lys), not localized in the crystal structure, were omitted from the analysis. The net charge of the missing segment is zero. The 200 crystallographic waters and seven sulfates were removed. The nearest water is 7 Å from the iron of heme 1 and all others are >11 Å from any heme center. All sulfates are >50 Å from the hemes. The carotenoid dihydroneurosporene and two lauryl dimethylamine oxide detergent molecules, >20 Å from the hemes, were retained.

The electrostatic potential (Φ) throughout the reaction center was obtained with the program DelPhi, which solves Poisson's equation for Φ when given a distribution of charges and dielectric constants (31–33). DelPhi assigns a radius to the 19,987 atoms of the reaction center (34, 35) and maps them onto a 65^3 point grid. A probe with a 1.4-Å radius defines water-accessible regions, including cavities within the protein. Once all grid points are assigned a charge and dielectric constant, Poisson's equation is solved with a finite difference algorithm (31, 32). Successive overrelaxation yields convergence within 30 sec on a Convex C2 (33). The protein interior was assigned a dielectric constant of 4, and 80 was used for water (36). Results were obtained with and without a layer of dielectric constant of 4 at the position of the membrane. Since the cytochrome subunit is in the aqueous phase, inclusion of the membrane has little effect on the calculated E_m values. The values reported do not include the membrane. To ensure that results are not sensitive to grid dimensions, a series of focusing calculations were made, centered on each site of interest (37). All reported values are the result of a series of six calculations with a final grid spacing of 0.17 Å.

The electrostatic free energy of oxidation (ΔG_{ox}) is described as the sum of (*i*) pairwise interactions of the heme charge with charges in the protein, screened by the polarizability of water and protein (35) plus (*ii*) the reaction field energy that results from polarization of electrons and dipoles in the solvent and protein induced by oxidation of the heme (38). The solvation energy, which is due to polarization of the high dielectric water, provides the bulk of the reaction field energy. Both contributors to ΔG_{ox} are dependent on the dielectric response of protein and solvent and are obtained directly through solution of the Poisson equation. The reaction field energy was obtained as described (38).

The contribution of pairwise interactions on ΔG_{ox} was obtained from

$$\Delta G_{ox} = \sum_i \Phi(\Delta q_{ox}, i) q_i, \quad [1]$$

where i is the number of atoms in the protein and $\Phi(\Delta q_{ox}, i)$ is the electrostatic potential at atom i induced by changes in heme electron density upon oxidation (Δq_{ox}). The contribution of a specific residue to ΔG_{ox} was obtained by considering only its atoms in the sum. Unless otherwise stated, partial charges on the protein atoms q_i were taken from the DISCOVER partial charge set (30) and Δq_{ox} was +1.0 at the iron.

Ionized acidic and basic residues influence the midpoints. However, at a given pH, the distribution of charged and neutral forms need not be the same in the protein and solution. In analogy to heme oxidation, the free energy of protonation is influenced by pairwise interactions with other charges in the protein and by the reaction field induced by each protonation state. Residue pK_a values in the protein were estimated using (26, 39):

$$pK_{protein}(m) = pK_{soln}(m) - \frac{c_m}{2.303 k_b T} \times \left[\Delta\Delta G_{desolv}(m) + \Delta G_{dipolar}(m) + \sum_{n \neq m}^{n} f(n)\Phi(\Delta q_m, n) q_n \right], \quad [2]$$

where pK_{soln} is the solution pK_a; c_m is +1 for a base and −1 for an acid; $\Delta\Delta G_{desolv}$ is the difference between the reaction field free energy of the charge free in water and within the protein; $\Delta G_{dipolar}$ is evaluated with Eq. 1, with the sum over partial charges on nonionizable side chains and backbone atoms given by $\Phi(\Delta q_m, i)$, where Δq_m is the change in charge distribution on ionization of site m; and the last term is the influence of other ionizable sites (n). The fraction of n ionized [$f(n)$] is obtained from the standard expression

$$\log\{f(n)/[1 - f(n)]\} = c_n \cdot [pK(n) - pH]. \quad [3]$$

Thus, the pK_a at each site depends on the pH, the pK_a of all other ionizable residues, and the redox state of the protein. An estimate of the pK_a values was obtained by iterating Eqs. 2 and 3 to obtain a consistent set of $pK_a(m)$ values and $f(m)$ values for all residues (40). Related calculations that use an improved method of finding a self-consistent set of ionization states have been made of *in situ* pK_a values in lysozyme (39).

RESULTS

Calculations on Isolated Hemes. The standard hydrogen electrode is the reference for measured cytochrome midpoints while the reference state for the calculations is oxidation in the absence of other charges or dielectric boundaries (35, 38). Calculations were performed on heme coordinates isolated from the reaction center, retaining axial ligands and propionic acids, to connect the two free-energy scales. The electrostatic contributions to ΔG_{ox} are shown in Table 1. If Δq_{ox} is +1.0 at the heme iron, solvation of the isolated heme by water favors oxidation by −457 meV (1 eV = 1.602×10^{-19} J). Histidine and methionine axial ligands stabilize the oxidized heme by −236 meV, primarily due to the negative partial charge representing the electron-donating ability of the histidine nitrogen directly involved in binding the ligand. The two negatively charged propionic acids favor oxidation by only a small amount (−50 meV) because the high dielectric water screens the heme. The total difference in electrostatic free energy between the neutral ferrous heme and positively charged ferric heme is −744 meV (−693 meV without the propionic acids). The E_m of a His-Met heme in solution with esterified propionic acids is −70 mV (14). Thus, an additive factor of 623 meV converts the DelPhi ΔG_{ox} for a heme without propionic acids to the experimental E_m. This constant will be used to scale all calculations when Δq_{ox} is assumed to be at the iron. It includes the quantum-mechanical electronic contributions to ΔG_{ox}, assumed to be independent of environment, and the calibration of the two reference scales.

For hemes either in solution (Table 1) or in the protein (data not shown), similar results are obtained for the reaction field free energy and longer-range pairwise interactions (e.g., with the propionic acids) if oxidation is treated as loss of electrons from the heme iron or from its nitrogens. However, short-range interactions with the axial ligands are sensitive to both the location of electron loss and the ligand charge distribution. Several amino acid charge sets were tested for their ability to reproduce the experimental observation that the midpoint of a His-Met heme is 150 meV higher than a bis-His heme (14, 41) (Table 1). The most important contributor to the difference is the pairwise interactions with ligand partial charges, but there is also a small change in reaction field energy because the different shapes of the ligands modify the contact between heme charge and water. With CHARMM partial charges, the E_m of the His-Met heme is 158 meV higher than the bis-His heme, if Δq_{ox} is 1.0 at the iron, and 74 meV, if Δq_{ox} is 0.25 at each heme nitrogen. With iron-centered oxidation (oxidation at the nitrogens is shown in parentheses) the difference with DISCOVER is 207 meV (84 meV) (30) and

Table 1. Electrostatic contributions to heme midpoints in solution

		Electrostatic contribution, meV					Midpoint, mV	
Δq_{ox}	Axial ligand	Propionic acid	Axial ligands	Reaction field energy	ΔG, meV	Scale factor	Calculated	Measured
+1.0 on Fe	His-Met	−50 ± 6	−236 ± 5	−457 ± 7	−744	623	−70	−70
	His-His	−42	−419	−482	−943	623	−278	−220
+0.25 on N	His-Met	−53 ± 8	−71 ± 7	−420 ± 3	−543	421	−70	−70
	His-His	−43	−135	−440	−618	421	−154	−220

Data given ± SD are for the three-reaction center His-Met heme conformers. The standard hydrogen electrode is the midpoint reference state. The electrostatic contributions of the axial ligands were calculated using DISCOVER amino acid charges. The reaction field free energy is the difference between this energy for oxidized and reduced hemes. In the reaction field calculation the reduced heme charges are Fe^{2+} with −0.5 at each N, while the oxidized heme is either Fe^{3+} with −0.5 at each N or Fe^{2+} with −0.25 at each N. The scale factor is defined so that the E_m of a His-Met heme without propionic acids is −70 mV (14).

−20 meV (6 meV) with OPLS (42). OPLS incorrectly orders the midpoints because its methionine sulfur has a negative partial charge similar to the histidine nitrogen, unlikely given the relative electronegativity of these atoms. DISCOVER gives a large ligand dependence primarily because its sulfur has a small positive charge (0.12), whereas CHARMM, with a small negative charge on the sulfur (−0.12), shows the best match with experiment if Δq_{ox} is localized at the iron.

Calculations of the Reaction Center Heme Midpoints. Within the protein, heme midpoints are affected by charges on ionizable amino acids (arginine, lysine, aspartic acid, glutamic acid, propionic acid, and chain termini), polar groups (the protein backbone and neutral side chains), previously oxidized hemes, reaction field energy, and charges on the axial ligands and heme ring substituents. For heme 4, the lowest-potential site, reaction field energy (−172 meV), axial ligands (−254 meV), and polar groups (35 meV) stabilize the oxidized heme by −391 meV (Table 2). The distribution of other charges is dependent on pH and protein redox state. If all acidic and basic groups are charged and all hemes are reduced, ionized groups contribute −337 meV. This includes the propionic acids of heme 4 (−274 meV), propionic acids of the other hemes (−83 meV), and ionized amino acids (21 meV) (Tables 2 and 3). All electrostatic contributors sum to yield a ΔG_{ox} of −728 meV. By using the scale factor of 623 mV, obtained from calculations with isolated hemes, the E_m value is −105 mV. Analogous calculations for the higher-potential hemes must also take into account the pairwise interactions with the previously oxidized hemes. Thus, the E_m value of heme 1, the highest-potential heme, is the result of the reaction field energy (−104 meV), axial ligands (−254 meV), polar groups (71 meV), propionic acids on heme 1 (−333 meV) and other hemes (−70 meV), ionized amino acids, in particular Arg-C264 (307 meV), and previously oxidized hemes (96 meV). The resulting midpoint is 240 mV. Similar calculations provide E_m values of −100 and −153 mV for hemes 2 and 3. As seen in Fig. 1, the midpoints of hemes 1, 2, and 4 agree reasonably well with the experimental values, but heme 3 is much too easy to oxidize.

Table 2. Calculated midpoints of the reaction center hemes

	Heme 1 (highest)	Heme 2 (low)	Heme 3 (high)	Heme 4 (lowest)
Contribution independent of pH and redox potential (E_h), meV				
Axial ligands	−254	−453	−224	−254
Backbone + polar side chains	71	24	34	35
Reaction field free energy	−104	−136	−119	−172
Sum[a]	−287	−565	−309	−391
Contribution of ionized groups,* meV				
All[b]	−96	−158	−467	−337
All except propionic acids on 3d[c]	−86	−133	−187	−308
Using calculated pK_a values (pH 8)[d]	−113	−52	−108	−191
Oxidized hemes†[e]	96	10	114	0
Calculated midpoint, mV				
Factors a + b	240	−100	−153	−105
Factors a + c + e	346	−65	241	−76
Factors a + d + e	319	16	320	41
Experimental midpoint, mV				
Ref. 6	380	20	310	−60
Ref. 7	400	20	320	−80
Ref. 9	370	50	295	−50
Ref. 10	370	10	300	−60

A value of 623 mV was added to calculated and experimental midpoint free energies to compare the results to the standard hydrogen electrode (see Table 1). a–e, Factors that are included in the calculated midpoint values.

*Arginine, lysine, aspartic acid, glutamic acid, propionic acids, and chain termini.

†Oxidation order is hemes 4, 2, 3, and 1.

Table 3. Contributions to the heme E_m values by individual charged sites

Site	E_m, meV Heme 1	Heme 2	Heme 3	Heme 4	Desolvation penalty
Oxidized heme					
Heme 1	—	**77**	14	5	
Heme 2	**77**	—	52	10	
Heme 3	14	52	—	**62**	
Heme 4	5	10	**62**	—	
Propionic acid location					
1a	**−178**	−29	−7	−3	7.6
1d	**−155**	−16	−10	−5	8.0
2a	−22	**−75**	−15	−5	3.0
2d	−49	**−83**	−25	−10	3.4
3a	−7	−10	**−177**	−16	9.1
3d	−12	−16	**−281**	−46	10.5
4a	−4	−5	−35	**−109**	6.8
4d	−4	−6	−29	**−165**	10.2
Ionizable amino acid					
Arg-C202	**92**	24	7	4	
Arg-C264	**364**	48	9	4	
Arg-C272	**71**	55	14	5	
Asp-M182	**−67**	−18	−8	−4	
Glu-C254	**−124**	−17	−6	−3	
Arg-C137	19	**83**	**70**	14	
Asp-C304	−57	**−118**	**−65**	−10	
Arg-C108	5	8	31	**84**	

Interaction energies >60 meV are in boldface type. The desolvation penalty is the loss of reaction field free energy on removal of the charge from water given in units of energy necessary to shift the pK_a by 1 pH unit, equivalent to 60 meV. Ionizable amino acids contributing >60 meV to the E_m of one or more hemes are shown.

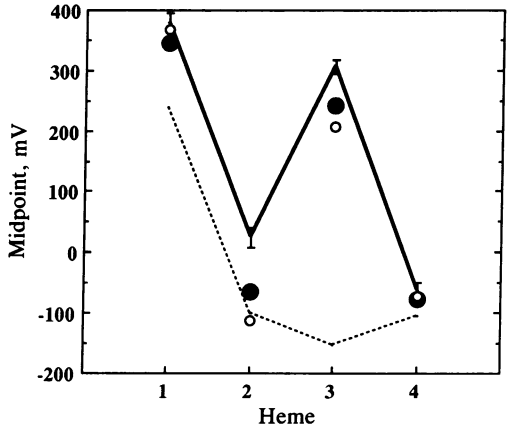

FIG. 1. Comparison of experimental and calculated midpoints for the hemes in *Rps. viridis* reaction centers using the standard hydrogen electrode as the reference. Average experimental midpoints are from refs. 6–10 with error bars showing the standard deviation (solid line). E_m values were calculated assuming all arginines, lysines, chain termini, glutamic and aspartic acids, and propionic acids are ionized (dashed line) (DISCOVER partial charges) and all acids and bases except for propionic acid on 3d are ionized. ●, DISCOVER partial charges; ○, CHARMM partial charges.

One possible source of disagreement between calculated and experimental E_m values is incorrect assignment of the charges on ionizable residues. The pK_a values of the 289 ionizable residues in the protein were calculated. At pH 7–8, where the E_m values were measured (6–8), all tyrosines, cysteines, and histidines are neutral. Of 28 arginines, 20 aspartic acids, 23 glutamic acids, 19 lysines, 8 propionic acids, 2 C termini, and 2 N termini found on the periplasmic side of the membrane, all but 15 are >90% ionized.

In the context of the E_m calculations, the propionic acid on the ring d of heme 3 (3d) is the most important ionizable residue that appears to be uncharged. Examples of proteins shifting pK_a values by large amounts have been found experimentally, including Glu-L212 in the reaction center from *Rhodobacter sphaeroides*, which has a pK_a value of 9.8 (43). The proposal that 3d is neutral is supported by the structure, since the oxygens of propionic acids on 3a and 3d are 2.5 Å apart, making it highly unlikely that both are negatively charged but allowing them to form a hydrogen bond if one of them is neutral. In contrast, the acids of hemes 1, 2, and 4 are oriented so that ionization of one shifts the pK_a value of its neighbor by only 1–2 pH units. If propionic acid 3d is neutral, the E_m values calculated with the DISCOVER charge set (346, −65, 241, and −76 mV) are now in good agreement with the experimental E_m values (380, 25, 306, and −63 mV). The E_m values obtained with CHARMM charges are 368, −112, 206, and −71 mV. Comparison of the CHARMM and DISCOVER results demonstrates the calculated values are only moderately dependent on the exact distribution of partial charges throughout the protein (Fig. 1).

The pK_a calculations suggest other groups have their pK_a values shifted so they are not fully ionized at pH 7. None influences the outcome as significantly as propionic acid 3d.

DISCUSSION

The electrostatics calculations presented here reproduce the effects of axial ligands on heme midpoints in model systems and describe the differences between the E_m values of hemes in solution and in the four reaction center sites. However, the match between calculated and experimental values is not exact. There are a variety of possible contributions to the discrepancies. One is the value of the additive factor, determined from model compounds, used to compare the calculated ΔG_{ox} with the standard hydrogen electrode. Using a scale value of 673 rather than 627 meV decreases the average difference between the calculated and experimental E_m value from 53 meV to 28 meV. The change in scale factor could reflect changes in the heme ligand conformation in solution. Some uncertainty also results from the choice of protein partial charges (Fig. 1). Thus, the overestimate by DISCOVER of the difference between bis-His and His-Met hemes may be responsible for heme 2 (the only bis-His heme) showing the largest difference between experimental and theoretical E_m values. The calculated E_m values also rely on the ionization states of acidic and basic groups. Some error could result from incorrect assignments of the charges on different residues. Calculations of the proton distribution throughout the reaction center at different pH values and in different oxidation states is an area of active interest and further refinements will be tested by their ability to improve the correspondence between calculated and experimental E_m values for the hemes. In addition other factors, including release of protons on heme oxidation, differences in heme electronic distribution, and changes in protein conformation, may make contributions to the small differences between experimental and calculated E_m values in this system. The assumption that the protein conformation does not change significantly upon oxidation is supported by the activity of the *Rps. viridis* cytochromes at 80 K (7).

The calculations reported here provide a general description of how heme midpoints can be modulated in solution and protein. Moving from high-dielectric polarizable water into the rigid low-dielectric protein changes all electrostatic contributors to ΔG_{ox}. This is illustrated by a comparison of the reaction field energy and pairwise interactions with the propionic acids and axial ligands in the two environments (Tables 1–3). As has been described (16, 17), the reaction field of a charge is always smaller in protein. A comparison of Tables 1 and 2 shows that this raises the midpoints of hemes 1–4 by 353, 346, 338, and 285 meV, respectively (heme 2 is compared with the bis-His heme). However, long-range pairwise interactions between charges play a greater role in protein than in water, as shown by the increased interaction with the two propionic acids on each heme (−283, −108, −408, and −224 meV) (see Tables 1 and 3). In contrast, interactions between charges in van der Waals contact are relatively insensitive to their surroundings. This is seen by the small differences in the interactions with the axial ligands in solution and protein (−18, −34, 12, and −18 meV). If only the reaction field energy and interactions with the axial ligands and propionic acids were important, the differences between ΔG_{ox} in solution and the protein could be relatively small (52, 204, −58, and 43 meV).

The analysis of the interaction between heme and axial ligands presented here explores how local electrostatic interactions change heme midpoints. This simple approach, which ignores ligand field effects, gains support from studies showing that ring substituents change the pK_a of a pyridine and the E_m of a heme bound to the pyridine by the same amount (44). Substituents change the pK_a by modifying the charge density at the pyridine nitrogen, affecting the electrostatic potential felt by the proton. The midpoints appear to be affected through the same mechanism. If so, comparison of calculated and experimental E_m values of isolated hemes can test charge distributions on ligand and heme. Dependence of the E_m on axial ligands is consistent with the predominant change in electron density on oxidation (Δq_{ox}) being at the iron. When Δq_{ox} is distributed throughout the macrocycle, as for the oxidation of Fe(I) hemes, substituent effects on E_m values are smaller than on pK_a values (44). This is seen in the smaller calculated dependence of the E_m on the ligand when Δq_{ox} is localized on the heme nitrogens.

The localization of Δq_{ox} to the iron has consequences for how the protein can modulate heme midpoints. Although burying the hemes in the protein raises the $E_m \approx 300$ mV, the reaction field energy for the four sites differs by only 68 meV. This is a consequence of the limited opportunity to change the approach of water to the iron in a six-coordinate heme. In contrast, although none of the propionic acids are exposed to solvent, their reaction field energy varies by 450 meV (Table 3) allowing their pK_a values to be strongly modulated by the carboxylate position.

With Δq_{ox} localized on the iron, the axial ligands are the only part of the protein within 5 Å of the oxidation site. This limits the influence of polar side chain and backbone partial charges since interactions between dipoles and charges fall off rapidly with distance. Thus, only one neutral amino acid other than the axial ligands influences any heme E_m by as much as 50 meV. For redox sites where Δq_{ox} is at the ring periphery or for ionization of acidic and basic residues, specific dipoles are more important. For example, the pK_a values of propionic acids 1a, 4a, and 4d are lowered by hydrogen bonds with backbone amide hydrogens whereas 3a can be stabilized by hydrogen bonds to the hydroxyls of Tyr-C89 or -C102.

In contrast to the effects of dipoles, the interactions with ionized acidic and basic residues or charged cofactors can be important at long range because the coulombic potential falls off more slowly than the dipolar potential. Thus, the net

charge on ionized propionic acids and amino acids and oxidized hemes influence heme midpoints by hundreds of meV (Table 3). Charged sites also affect the ionization of acidic and basic residues. For example, the pK_a values of propionic acids 1a, 1d, 3a, 3d, and 4d are lowered by charges on arginines, whereas close contact with the negatively charged 3a raises the pK_a of 3d by $\approx$10 pH units.

Pairwise interactions between charges depend on how deeply they are buried in the protein as well as the distance between them. This is illustrated by the stabilization of the oxidized heme by the propionic acids (Table 3). Ignoring 3d, their charges lower the E_m by -75 to -178 meV. This variation is not simply a consequence of distance, as the oxygens are in a narrow range of 7.7–8.9 Å from the iron. Rather the interaction is correlated with the propionic acid desolvation penalty, which is the loss of reaction field energy upon removal of the charge from water. Thus, the well-solvated propionic acids on heme 2 provide the least stabilization of the oxidized heme, whereas those acids that are more deeply buried have a more significant influence.

The calculations assume a constant protein dielectric constant of 4 and the surrounding water is at 80. However, neighboring hemes, 14–16 Å apart, have an interaction energy of 50–77 meV, as if responding to an effective dielectric constant (ε_{eff}) of 13–17. The 10- to 14-meV interaction between the two high-potential or two low-potential sites 28 Å apart yields an ε_{eff} of 40–50, and the 5-meV interaction between sites 1 and 4 at 41 Å implies an ε_{eff} of 71. The effects of heme oxidation on the E_m values of the other sites demonstrate the principle that when charges are in a low dielectric surrounded by a high dielectric, the greater their separation the larger the higher dielectric's influence (32).

The use of a protein dielectric constant of 4 assumes that charges not only polarize electrons in the protein ($\varepsilon = 2$) but also cause some rearrangement of protein dipoles. There is theoretical justification for this choice (36) and a number of calculations of experimental quantities support this value (39, 45). Calculations were performed with different protein dielectric constants (data not shown). The overall pattern of alternating high- and low-potential hemes is reproduced in all dielectrics. However, if ε is 2 the E_m values are too sensitive to their location, giving a slope of 1.70 for a comparison of the calculated and experimental midpoints of the four hemes. With an ε of 4, the slope is 0.98, an excellent match to the desired value of 1.0. With an ε of 6, the E_m values vary less than found experimentally (slope = 0.76).

CONCLUSIONS

It has been shown that a classical electrostatic analysis reproduces the midpoints of the four hemes in the *Rps. viridis* reaction center. These results support the experimental assignments of alternating high- and low-potential hemes with the highest-potential site nearest the bacteriochlorophyll dimer (6, 7, 9, 10). The absolute midpoint at each site results from contributions of reaction field energy and both long- and short-range pairwise interactions. Little variation is found in the reaction field energy of the four hemes. Rather the differences between the individual midpoints rely on interactions with specific ionized sites such as the propionic acids and Arg-C264. However, ionized groups yield a monotonic change in ΔG_{ox} with distance from the bacteriochlorophyll dimer, with site 1 the hardest to oxidize. The midpoint of site 2 is lowered, relative to the other hemes, by its two histidine ligands. The last important contributor to the alternation of high- and low-potential sites is the arrangement of the hemes themselves, with intervening oxidized low-potential sites further raising the midpoints of the high-potential hemes.

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