Protein Electric Field Effects on the CO Stretch Frequency of Carbonmonoxycytochromes c as a Function of Carbonyl Tilting and Bending Investigated with a Continuum Electrostatic Ap...

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · SEPTEMBER 1997

Impact Factor: 3.3 · DOI: 10.1021/jp9706846

3 AUTHORS, INCLUDING:

Kim A Sharp
University of Pennsylvania
176 PUBLICATIONS **18,551** CITATIONS
SEE PROFILE

Jane M Vanderkooi
University of Pennsylvania
108 PUBLICATIONS **2,861** CITATIONS
SEE PROFILE

Protein Electric Field Effects on the CO Stretch Frequency of Carbonmonoxycytochromes *c* as a Function of Carbonyl Tilting and Bending Investigated with a Continuum Electrostatic Approach

Monique Laberge,* Kim A. Sharp, and Jane M. Vanderkooi

Johnson Research Foundation, Department of Biochemistry & Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received: February 25, 1997; In Final Form: August 4, 1997[⊗]

The effect of the orientation of CO bound to the heme of two *c*-type cytochromes on the CO-stretch frequency ν_{CO} has been investigated using molecular mechanics and finite difference Poisson−Boltzmann calculations. Our approach treats the charge distribution of the protein as an external electric field capable of inducing Stark frequency shifts. Results show that modifying the Fe−C−O bending angle (β) does not change the CO stretch frequency within a range of 100−175°, equivalent to a bending motion away from the propionic acid chains. The calculated Stark shifts range from 5.4 to 8.6 cm^{-1} and are in good agreement with the experimentally observed shift (6 cm^{-1}). However, motion of the CO toward the propionics exerts significant influence on the calculated shifts (−4.5 to −1.8 cm^{-1}), which are then in total disagreement with experiment, not only in magnitude but also in predicting the wrong direction for the shift. The Stark shifts calculated for the tilt angle (τ) show that it has no significant effect on ν_{CO} within a 15° distortion range. With respect to the proximal histidine displacement angle (ω), only a complete (and unlikely) 90° rotation about the Fe−N bond was effective in significantly affecting the Stark shift, and we accordingly rule out a major contribution to the CO distortion from the coordination geometry of the proximal histidine. Overall, the calculations show that the CO ligand could thus enjoy a significant amount of flexibility in the heme pocket—as required to approach and leave the heme group.

Introduction

Externally applied electric fields represent one of the best known perturbations that can affect electronic and vibrational spectroscopic properties.[28] In biological systems, these Stark effects are being investigated both in the visible[24,25−29,37] and the IR regions[8,27,20] of the spectrum. We recently reported electrostatic potential and electric field calculations performed on *c*-type carbonmonoxycytochromes, using a finite difference solution to the Poisson−Boltzmann equation to account for the observed CO frequency shift between horse heart and yeast carbonmonoxycytochromes *c* (HHcCO, YcCO) in terms of the field exerted—not by an externally applied field—but by their respective protein matrices.[19,20] The two cytochromes share 63 invariant amino acid residues, and their respective sequences thus differ mainly in the number of polar residues present. As such, they are ideal candidates for investigating the protein charge effects on the heme moiety.

This study had been undertaken to show that the electric field at the CO ligand arising from the total charge distribution of the protein matrix could be accurately computed and shown to modulate the stretch frequency. As such, the work directly followed the *ab initio* calculations performed earlier by Augspurger *et al.*,[3] who calculated the effect of a range of electric potentials—due to point dipoles and quadrupoles—on the CO stretch and on chemical shielding tensors which led to a model for conformational substates in heme proteins.[31,32] Our calculations supported these findings and were consistent with other recent experimental evidence suggesting that polar interactions can significantly affect the vibrational frequency of the ligand.[15,22,34] We were able to calculate actual electrostatic contributions from the protein matrix and show that they could shift the CO stretch frequency of the carbonmonoxy cytochromes. The CO stretches were observed at 1965.9 and 1960.1 cm^{-1} for horse and yeast cytochrome *c*, respectively. This experimental shift (6 cm^{-1}) was found to be in good agreement with a calculated value of 8 cm^{-1}. In this work, we wish to report on calculations performed on both carbonmonoxy cytochrome *c*'s aimed at assessing the importance of (i) the Fe−C−O angle bending and tilting and (ii) proximal histidine displacements on the accurate prediction of the CO stretch frequency.

The question of the Fe−C−O geometry in carbonmonoxy heme proteins generates ongoing controversy in the literature. For MbCO—recently fully reviewed[36]—the traditional view, based on early neutron diffraction and crystallographic data,[4] held that the heme preferred to bind O_2 rather than CO because CO was bound in a "bent" fashion.[9,7] Very recent spectroscopic work has, however, challenged this interpretation by consistently providing evidence for a nearly linear CO ligand. Lim *et al.*[23] have shown conclusively that a nearly perpendicular CO ligand fits very well in the MbCO heme pocket with a deviation of less than 7° from the heme normal. This confirms IR polarization results[16] reporting deviations from linearity inferior to 10° for the same protein, in agreement with a recent MbCO X-ray structure showing a nearly linear 169° angle for the ligand.[33]

The role of the proximal histidine is also the object of controversy. In an *ab initio* study of a heme prosthetic unit, Jewsbury et al. recently proposed that the nonequilibrium orientation of the proximal histidine exerted major influence on the Fe−C−O distortion and electron distribution.[17] But local density functional calculations performed on a porphyrin−imidazole−CO model structure showed that displacements of the deformation angles of the Fe−His unit had next to no effect on the orientation of the CO.[10]

It is to pursue this line of enquiry that we performed a new

* To whom correspondance should be addressed.

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1997.

S1089-5647(97)00684-6 CCC: $14.00

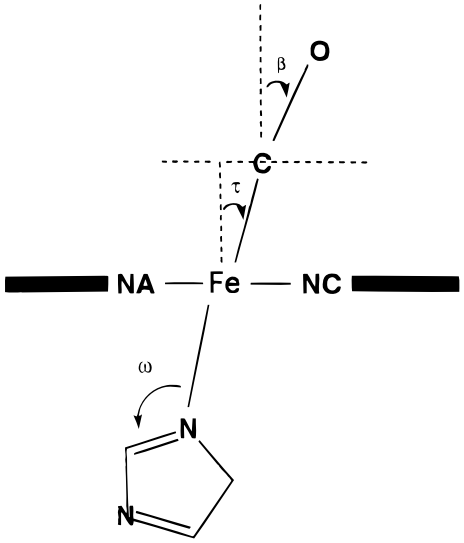

Figure 1. Schematic view of the heme−CO unit in cytochrome *c* showing the various angle modifications for the FDPB calculations. Angles are labeled as per ref 10.

series of FDPB calculations on the carbonmonoxycytochromes *c*, varying both τ and β as well as the geometry of the histidine ligand.

Computational Methods

The starting coordinates for horse heart cytochrome *c*[6] and yeast cytochrome *c*[26] were obtained from the Brookhaven Protein Data Bank,[5] pdb1hrc.ent and pdb1ycc.ent, respectively. The CO ligand was added using the *Builder* module of the *Insight II* software package, and the geometry was optimized using the *Discover* module (Biosym Technologies, San Diego, CA) on a Silicon Graphics IRIS Indigo workstation as described previously.[20] In the original calculations, the Fe−C−O angle had been set (but not constrained) at 180°. Both structures were aligned with the heme in the *x*−*y* plane and the CO ligand along *z*, and their energies were minimized using the CVFF-heme force field[20] using a Newton−Raphson algorithm for which a residual maximum gradient of 0.1 kcal mol^{-1} was set as convergence criterion. Missing hydrogens were added using *Discover*, subject to van der Waals constraints, and X-ray waters were retained. The energy-minimized structures yielded Fe−C−O angles that deviated from the normal by 5.4° (horse) and 9.7° (yeast). In the series of calculations reported in this work, the geometry of the CO bending (β) and tilting (τ) angles was modified (Figure 1) as well as the displacement angle of the proximal histidine (ω). For the β modifications, the NA−Fe−C−O dihedral angle was constrained as per Table 1. This angle (θ)—allowing motion toward or away from the propionics—is defined as

$$\theta = \text{sign}\cos^{-1}(-[(d_{\text{NA-Fe}}d_{\text{Fe-C}})(d_{\text{O-C}}d_{\text{C-Fe}})];\quad \pi \le \theta \le \pi \tag{1}$$

where the sign is

$$[-(d_{\text{NA-Fe}}d_{\text{Fe-C}})(d_{\text{O-C}}d_{\text{C-Fe}})]d_{\text{Fe-C}} \tag{2}$$

After each geometry modification, the structures were minimized as described elsewhere[20] until the derivatives reached <0.1 kcal mol^{-1}. Additionally, the histidine ligand was also subjected to a full 90° rotation around the Fe−N bond.

For each geometry modification, the electrostatic potential and field of the cytochromes were calculated using the finite difference solutions to the Poisson−Boltzmann equation as implemented in the *Delphi* software package.[12,30,35] The calculations were performed using a dielectric constant of 80 for the solvent[11] and replicated using three different dielectric constants, namely 2, 3, and 4 for the protein interior. Our approach does not calculate ν_{CO} directly to investigate how the CO stretch frequency varies as a function of β or τ but rather makes use of the "Stark shift" concept. This approach is fully described elsewhere[20] and calculates the Stark shifts ($\Delta\nu$) using

$$\Delta\nu = \delta\nu \sum_i q_i \Phi_i(\hat{\mu}_{\text{CO}}) \tag{3}$$

This expression assumes that the dot product of a unit dipole vector with the field calculated for a collection of point charges

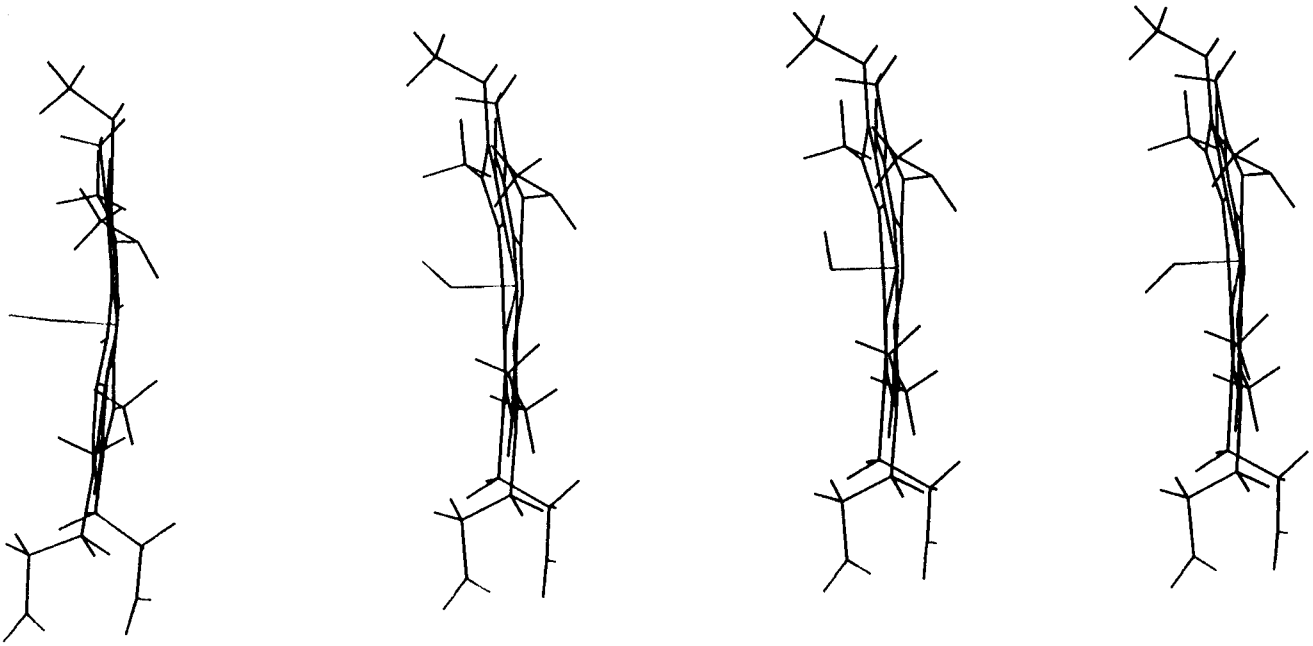

Figure 2. Stereoview of the horse heart cytochrome *c*−CO structure near the heme with different β angles. From left to right, Fe−C−O = 174.83°, 135°, and 100° with $\theta = -151.81$ and 135° with $\theta = 27.81$.

TABLE 1: CO Frequency Shifts in Carbonmonoxy Horse Heart and Yeast Cytochrome *c* as a Function of the Fe−C−O Bending Angle (β) and Protein Dielectric Constant

cyt	θ^a	Fe−C−O angle (deg)	$\sum q\cdot\Phi(\kappa T)$	$\Delta\nu$ (cm^{-1})
		$\epsilon = 2$		
HH	−151.81	174.6	−3.156	−3.9
	−152.19	135.0	−2.089	−2.5
	−152.19	100.0	1.173	1.4
	27.81	135.0	−4.378	−5.3
	27.81	100.0	−2.369	−2.9
Y	51.02	170.3	−9.823	−11.9
	48.91	135.0	−10.741	−13.1
	48.91	100.0	−9.650	−11.8
	−131.09	135.0	0.075	1.3
	−131.09	100.0	−0.103	−0.1
		$\epsilon = 3$		
HH	−151.81	174.6	−2.142	−2.61
	−152.19	135.0	−1.420	−1.73
	−152.19	100.0	0.731	0.89
	27.81	135.0	−2.963	−3.61
	27.81	100.0	−1.562	−1.91
Y	51.02	170.3	−6.586	−8.03
	48.91	135.0	−7.161	−8.74
	48.91	100.0	−6.391	−7.67
	−131.09	135.0	0.751	0.92
	−131.09	100.0	−0.129	−0.16
		$\epsilon = 4$		
HH	−151.81	174.6	−1.6267	−1.98
	−152.19	135.0	−1.116	−1.36
	−152.19	100.0	0.5137	0.63
	27.81	135.0	−2.251	−2.75
	27.81	100.0	−1.161	−1.42
Y	51.02	170.3	−4.955	−6.04
	48.91	135.0	−5.369	−6.55
	48.91	100.0	−4.763	−5.81
	−131.09	135.0	0.5234	0.64
	−131.09	100.0	−0.1381	−0.17

[a] NA−Fe−C−O dihedral angle.

is equivalent to the sum over the product of those charges (q_i) and the potential at each charge from a unit dipole placed on the CO group [$\Phi_i(\mu_{CO})$]. The Stark shift ($\Delta\nu$) is then obtained as $1.22 q_i\Phi_i$ cm^{-1} summed over the protein residues multiplied by the empirical Stark tuning rate, $\delta\nu$, defined as

$$\delta\nu = \delta h\nu_{01}/\mathrm{dE} \tag{4}$$

where $h\nu_{01}$ is the energy difference between the $\upsilon = 0$ and $\upsilon = 1$ states of the Hamiltonian and h is Planck's constant. For the purposes of these calculations, $\delta\nu$ is equal to 4.9×10^{-7} cm^{-1} per V/cm.[19] Since we have no frequency value for the CO ligand in the absence of the external (i.e protein matrix) field, we use the Stark shift difference ($\Delta\nu_s$) between the $\Delta\nu$ values obtained for the horse heart and yeast cytochromes to compare with the experimentally observed shift.

Results and Discussion

Choice of an adequate protein dielectric constant for continuum electrostatic calculations continues to generate interest and controversy. Even if both experiment and theory are in agreement for a low protein dielectric as far as the intrinsic polarizability in the presence of an externally applied electric field is concerned, the literature quotes values ranging between 1 and 20, and this has to do with the screening of charge−charge interactions and the effect of burying ("solvating") charges in the protein matrix.[14] If these effects are to be taken into account, than a higher dielectric value is in better agreement with experiment. For instance, Antosiewicz et al.[2] have used $\epsilon = 15$ in their electrostatic studies of hemoglobins and shown that, in the case of small soluble proteins, better agreement was possible with experimental data when ϵ was set to 20, thus accounting for phenomena not specifically included in the model such as conformational relaxation and ion binding.[1] In FDPB calculations performed on photosynthetic reaction centers, the use of $\epsilon = 4$ has yielded very good correspondence with experimetal data.[5,13,21] Gilson and Honig[11] have discussed how a dielectric constant of 2 implies that charges only polarize electrons in the protein while 4 assumes that the protein dipoles also undergo rearrangement. The boundary is thin however, and any number ranging between 3 and 5 is known to be appropriate for the response of a protein that does not undergo drastic conformational change. Table 1 shows the potentials and Stark shifts calculated using different dielectric constants for the protein. Considering that our experimentally observed shift difference is 6 cm^{-1}, it would seem that using a permittivity of 3 for the protein yields closer agreement with experiment. Tables 2 and 3 accordingly present some of the Stark shift differences calculated using the FDPB results obtained with $\epsilon = 3$.

TABLE 2: Stark Shift Difference (from Horse Heart to Yeast Cytochrome *c*) Due to Protein-Induced Electric Field as a Function of the β Angle and Protein Dielectric Constant ($\epsilon_{protein} = 3$)

	$\Delta\nu_s$ (cm^{-1})		
β angle (deg) [θ]	$\epsilon = 2$	$\epsilon = 3$	$\epsilon = 4$
172.4 [−151.8]	8.0[a]	5.42	4.06
135.0 [−152.2]	10.6	7.01	5.19
100.0 [−152.2]	13.2	8.56	6.44
135.0 [27.8]	−6.6	−4.53	−3.39
100.0 [27.8]	−2.8	−1.75	−1.25

[a] Laberge et al., 1996.

TABLE 3: Stark Shift Difference (from Horse Heart to Yeast Cytochrome *c*) Due to Protein-Induced Electric Field as a Function of the CO β and τ Angles and the Proximal His ω Angle ($\epsilon_{protein} = 3$)

β angle (deg) [θ]	$\Delta\nu_s$ (cm^{-1})
172.4 [−151.8]	5.42
135.0 [−152.2]	7.01
100.0 [−152.2]	8.56
135.0 [27.8]	−4.53
100.0 [27.8]	−1.75
τ angle (deg)	
80.0	2.77
85.5	5.42
90.0	4.61
95.0	6.89
100.0	5.92
ω angle (deg)	
132.0	5.42
100.0	4.66
145.0	4.47
115.0	4.79
152.0	4.04

The FDPB calculations performed while modifying the three angles of interest, i.e. the Fe−C−O bending (β) and tilting (τ) angles and the displacement angle of the proximal histidine (ω) (Figure 1) can be summarized as follows: (i) in the case of (β), distortions ranging between 100° and 175° ($\theta = -151.8$ for HHcCo and $\theta = 51.02$ for YcCO), equivalent to a bending motion away from the propionic acid chains), yield calculated $\Delta\nu_s$ values between 5.4 and 8.6 cm^{-1}, in good agreement with the experimentally observed shift (6 cm^{-1}); however, motion of the CO toward the propionics ($\theta = 25-50$) yields $\Delta\nu_s$ values (−4.5 to −1.8 cm^{-1}) in total disagreement with experiment, not only in magnitude but also in predicting the wrong direction for the shift (cf. Table 2); (ii) in the case of the tilt angle τ, the Stark shifts calculated for τ ranging between 85° and 100° are also close to the experimental shift; (iii) varying the proximal

histidine displacement angle ω between 100° and 152° also yields values in agreement with experiment (cf. Table 3). This correspondence with the experimentally observed shift (6 cm^{-1}) suggests that the protein allows the ligand mobility in the heme pocket within a 15° τ-angle variation, a 50° ω-angle variation, and a 75° β-angle variation when the ligand moves away from the propionics; within those ranges, the effect of angle distortions on ν_{CO} are not significant, and we conclude that the τ and ω angles are not major determinants of $\nu_{CO.}$ In the case of β, motion toward the propionics effectively affects the Stark shift and β should accordingly be considered a major determinant of ν_{CO} when the bending motion occurs in that direction. As far as ω is concerned, we calculate a significant influence on ν_{CO} only with a complete 90° rotation of the proximal histidine about the Fe−N bond, which can only be achieved with the application of unrealistic tethering constraints. Our calculations yield $\Delta\nu_s = 1.43$ cm^{-1} for this type of (unreasonable) geometry. The MbCO Ab initio study[17] concluded that the strains implied by the X-ray structures for the large Fe−C−O distortion was provided by the tertiary structure of the protein via the proximal histidine, concluding that the influence of the distal histidine was not as significant. In the case of carbonmonoxy-cytochromes—heme proteins that lack a distal histidine—our results clearly show that the Fe−C−O bending angle β is capable of delivering a major contribution to the observed ν_{CO}; thus, the presence of a distal histidine does not seem required to significantly distort the Fe−C−O unit.

Our findings also corroborate recent local density calculations[10] that showed that in-phase τ-β CO distortions of the order of 25° are energetically possible and major determinants of the CO orientation. Unlike these authors, we were not able to couple the τ-β deformations, but our results (72° of possible distortion for the β angle) also point to a major contribution from β when the bending motion occurs in the direction of the propionics and rule out a significant contribution from the coordination geometry of the proximal histidine. The overall emerging picture is in good agreement with the speculations offered by Ghosh et al.[10] in which we can visualize a CO ligand that has to be capable of some significant flexibility in order to rotate and exit the heme pocket. Our work is also very consistent with a recently published theoretical study of the effect of distal steric and electrostatic factors on ν_{CO}.[18] In this work, the CO frequency stretch of a model CO-porphyrin was calculated as a function of the presence of nearby point charges. Three geometries were used for the CO ligand: linear, bent, and tilted, and the authors concluded that charged groups and FeCO geometry can strongly affect ν_{CO} (cf. 1969, 1949, 1976 cm^{-1}, respectively, for linear, tilted, and bent FeCO).[18] Our approach now illustrates these effects in a naturally occuring protein system.

Acknowledgment. This work was supported by National Institute of Health Grant PO1 GM55004-01.

References and Notes

(1) Antosiewicz, J.; McCammon, J. A.; Gilson, M. K. *J. Mol. Biol.* **1994**, *238*, 415−436.
(2) Antosiewicz, J.; Porschke, D. *Biophys. J.* **1995**, *68*, 655−664.
(3) Augspurger, J. D.; Dykstra, C. E.; Oldfield, E. *J. Am. Chem. Soc.* **1991**, *113*, 2447−2451.
(4) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer Jr, E. F.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. *Eur. J. Biochem.* **1977**, *80*, 319−324.
(5) Beroza, P.; Fredkin, D. R.; Okamura, M. Y.; Feher, G. *Biophys. J.* **1995**, *68*, 2233−2250.
(6) Bushnell, G. W.; Louie, G. V.; Brayer, G. D. *J. Mol. Biol.* **1990**, *214*, 585−595.
(7) Case, D. A.; Karplus, M. *J. Mol. Biol.* **1978**, *213*, 697−701.
(8) Chattopadyay, A.; Boxer, S. G. *J. Am. Chem. Soc.* **1995**, *117*, 1449−1450.
(9) Collman, J. P.; Brauman, J. I.; Halbert, T. R.; Suslick, K. S. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3333−3337.
(10) Ghosh, A.; Bocian, D. F. *J. Phys. Chem.* **1996**, *100*, 6363−6367.
(11) Gilson, M. K.; Honig, B. *Biopolymers* **1986**, *25*, 2097−2119.
(12) Gilson, M. K.; Sharp, K. A.; Honig, B. *J. Comput. Chem.* **1987**, *9*, 327−335.
(13) Gunner, M. R.; Honig, B. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 9151−9155.
(14) Harvey, S. C. *Proteins: Struct., Funct., Genet.* **1989**, *5*, 78−92.
(15) Hu, S.; Vogel, K. M.; Spiro, T. G. *J. Am. Chem. Soc.* **1994**, *116*, 11187−11188.
(16) Ivanov, D.; Sage, J. T.; Keim, M.; Powell, J. R.; Asher, S. A.; Champion, P. M. *J. Am. Chem. Soc.* **1994**, *116*, 4139−4140.
(17) Jewsbury, P.; Yamamoto, S.; Minato, T.; Saito, M.; Kitagawa, T. *J. Phys. Chem.* **1995**, *99*, 12677−12685.
(18) Kushkuley, B.; Stavrov, S. S. *Biophys. J.* **1996**, *70*, 1214−1229.
(19) Laberge, M.; Sharp, K.; Vanderkooi, J. M. *Biophys. J.* **1996**, *70*, A223.
(20) Laberge, M.; Vanderkooi, J. M.; Sharp, K. A. *J. Phys. Chem.* **1996**, *100*, 10793−10801.
(21) Lancaster, C. R.; Michel, H.; Honig, B.; Gunner, M. R. *Biophys. J.* **1996**, *70*, 2469−2492.
(22) Li, T.; Quillin, M. L.; Phillips, G. N. J.; Olson, J. S. *Biochemistry* **1994**, *33*, 1433−1446.
(23) Lim, M.; Jackson, J. A.; Anfinrud, P. A. *Science* **1995**, *269*, 962−966.
(24) Lockhart, D. J.; Boxer, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 107−111.
(25) Loesche, M.; Feher, G.; Okamura, M. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7537−7541.
(26) Louie, G. V.; Brayer, G. D. *J. Mol. Biol.* **1990**, *214*, 527−555.
(27) Maiti, S.; Walker, B. R.; Cowen, R.; Pippenger, R.; Moser, C. C.; Dutton, P. L.; Hochstrasser, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 10360−10364.
(28) Merzbacher, E. *Quantum Mechanics*; Wiley: New York, 1961.
(29) Middendorf, T. R.; Mazzola, L. T.; Lao, K.; Steffen, A.; Boxer, S. G. *Biochim. Biophys. Acta* **1993**, *1143*, 223−234.
(30) Nicholls, A.; Honig, B. *J. Comput. Chem.* **1991**, *12*, 435−445.
(31) Oldfield, E.; Guo, K.; Augspurger, J. D.; Dykstra, C. E. *J. Am. Chem. Soc.* **1991**, *113*, 7537−7541.
(32) Park, K. D.; Guo, K.; Adebodun, F.; Chiu, M. L.; Sligar, S. G.; Oldfield, E. *Biochemistry* **1991**, *30*, 2333−2347.
(33) Quillin, M. L.; Arduini, R. M.; Olson, J. S.; Phillips Jr., G. N. *J. Mol. Biol.* **1993**, *234*, 140−155.
(34) Ray, G. B.; Li, X.-Y.; Ibers, J. A.; Sessler, J. L.; Spiro, T. G. *J. Am. Chem. Soc.* **1994**, *116*, 162−176.
(35) Sharp, K.; Honig, B. *Annu. Rev. Biophys. Chem.* **1990**, *19*, 301−332.
(36) Springer, B. S.; Sligar, S. G.; Olson, J. S.; Phillips, G. N., Jr. *Chem. Rev.* **1994**, *94*, 699−714.
(37) Steffen, M. A.; Lao, K.; Boxer, S. G. *Science* **1994**, *264*, 810−816.