J|A|C|S COMMUNICATIONS

Published on Web 10/21/2010

QM/MM Study of Dehydro and Dihydro β-Ionone Retinal Analogues in Squid and Bovine Rhodopsins: Implications for Vision in Salamander Rhodopsin

Sivakumar Sekharan,[†] Ahmet Altun,[†] and Keiji Morokuma*[,†,‡]

Cherry L. Emerson Center for Scientific Computation and Department of Chemistry, Emory University, Atlanta, Georgia 30322, and Fukui Institute for Fundamental Chemistry, Kyoto University, 34-4 Takano Nishihiraki-cho, Sakyo, Kyoto 606-8103, Japan

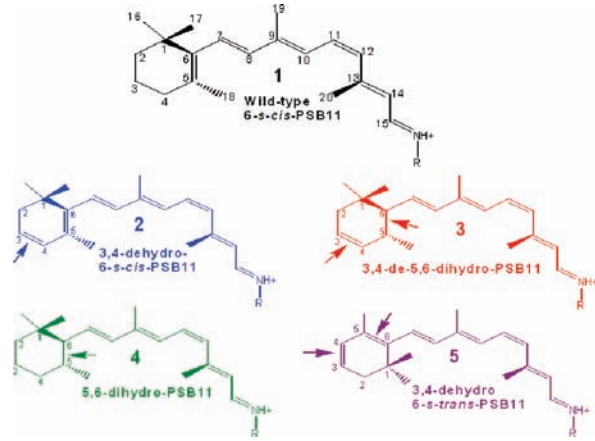
Received June 10, 2010; E-mail: morokuma@emory.edu

Abstract: Visual pigment rhodopsin provides a decisive crossing point for interaction between organisms and environment. Naturally occurring visual pigments contain only PSB11 and 3,4-dehydro-PSB11 as chromophores. Therefore, the ability of visual opsin to discriminate between the retinal geometries is investigated by means of QM/MM incorporation of PSB11, 6-*s*-*cis* and 6-*s*-*trans* forms of 3,4-dehydro-PSB11, and 3,4-dehydro-5,6-dihydro-PSB11 and 5,6-dihydro-PSB11 analogues into squid and bovine rhodopsin environments. The analogue−protein interaction reveals the binding site of squid rhodopsin to be *malleable* and *ductile*, while that of bovine rhodopsin is *rigid* and *stiff*. On the basis of these studies, a tentative model of the salamander rhodopsin binding site is also proposed.

Visual pigment rhodopsin is a G-protein coupled receptor (GPCR) responsible for black/white vision. Invertebrate (squid) and vertebrate (bovine) rhodopsins contain a light absorbing 6-*s*-*cis*-11-*cis*-retinal chromophore covalently bound to the opsin apoprotein via a protonated Schiff base (PSB11) linkage to the ε-amino group of K305 (squid) and K296 (bovine). The positive charge on the Schiff base nitrogen is counterbalanced with a negatively charged counterion (E180 in squid and E113 in bovine).[1−3] E180 of squid is ∼4 Å from the Schiff base nitrogen while E113 of bovine is H-bonded to it. We have recently shown theoretically that E180 and E113 counterions exert almost the same effect of an ∼100 nm blue shift on the vertical excitation energy of PSB11 that is almost fully responsible for the spectral shift going from retinal in *vacuo* (610 nm) to those in protein environments: 490 nm (squid)[4a,b] and 495 nm (bovine).[4c]

One of the most intriguing and less studied facets of visual pigments is their ability to discriminate between the retinal geometries. In particular, only PSB11 and 3,4-dehydro-PSB11 have been found to act as chromophores in naturally occurring visual pigments.[5] Also an analogous molecule, 5,6-dihydro-PSB11, which is similar in all other respects to PSB11 except that the ring is saturated, has been found to bind to opsin but has not been isolated from retina so far.[6] Accumulated evidence from spectral tuning studies on visual pigment using retinal analogues indicate that (a) tiger salamander selects the 6-*s*-*cis* form of 3,4-dehydro-PSB11 and absorbs at ∼520 nm,[7] (b) chicken cone iodopsin selects the 6-*s*-*trans* form of PSB11 to absorb at 562 nm,[8] (c) deep red cone pigments select the 6-*s*-*trans* geometry that achieves a significant red shift and also enhances its stability within the binding pocket,[9] and (d) 3,4-dehydro-5,6-dihydro-PSB11 and 5,6-dihydro-PSB11 absorb at ∼460 nm and behave as a five-double-bonded chromophore.[6b] Interestingly, dihydro retinals have also been used to characterize the structure of bacterio-,[10] sensory-,[10] and halorhodopsin[11] and have been recently shown to even outperform the native pigment in conferring visual photosensitivity.[12]

Scheme 1. Schematic Representation of the Structures of Wildtype, Dehydro-, and Dihydro- Protonated Schiff Base of 11-*cis*-Retinal Chromophores Incorporated into Squid and Bovine Rhodopsins[a]



[a] R− refers to K305/K296 in their respective protein environments. The arrow points to the location at which the retinal is modified.

In this study, we have attempted to computationally incorporate 3,4-dehydro-6-*s*-*cis*-(**2**), 3,4-dehydro-5,6-dihydro-(**3**), 5,6-dihydro-(**4**), and 3,4-dehydro-6-*s*-*trans*-(**5**) analogues of PSB11 (see Scheme 1) into bovine and squid rhodopsin binding sites not only to compare and contrast the calculated photophysical properties with that of the native pigment (**1**) but also to gain insights into the evolutionary elasticity of visual rhodopsins. In particular, the aim of the study is fivefold: (i) to document the effect of ring modifications on retinal geometry, (ii) to characterize the general architecture of bovine and squid rhodopsin binding sites, (iii) to identify the origin of the spectral tuning mechanism in dehydro and dihydro rhodopsins, (iv) to examine the hypothesis that optical activity of visual pigments can be manipulated by extension or contraction of the polyene chain,[6b] and finally, (v) to predict the structure of salamander rhodopsin that contains 3,4-dehydro-6-*s*-*cis*-PSB11 (**2**). As we have chosen retinal analogues that are known to form bleachable visual pigments,[13] the calculations are susceptible for further experimental verifications.

To begin with, the QM/MM optimized structure of wild-type squid and bovine rhodopsins are taken from ref 4a and 4c. By taking the six-double-bonded wild-type PSB11 (**1**) as a template, an additional double bond is introduced at the C3−C4 position to give the seven-double-bonded 3,4-dehydro PSB11 (**2**). Subsequent saturation at the C5═C6 position gives 3,4-dehydro-5,6-dihydro

[†] Emory University.
[‡] Kyoto University.

10.1021/ja105050p

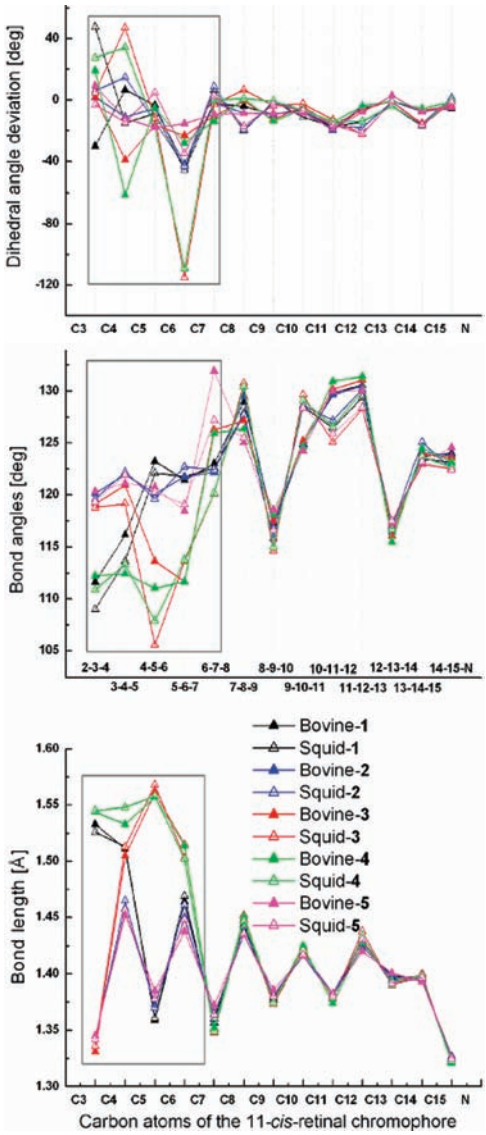


Figure 1. Comparison of the bond length alternation (bottom), bond angles (middle), and dihedral angles (top) along the conjugated carbon chain of the optimized QM/MM geometries of PSB11 and analogues shown in Scheme 1. Unfilled/filled triangles denote the PSB11 models optimized in squid/bovine rhodopsin. The gray colored box indicates the region that has undergone maximum perturbation along the length of the retinal chromophore. The dihedral angle deviations are from either a *cis* (0°) or *trans* (180°) configuration.

PSB11 (**3**) and at C3═C4 position (in **2**) gives the five-double-bonded 5,6-dihydro PSB11 (**4**). In other words, the retinal π-conjugation running from C5—NH^+ is, first, extended to C3—NH^+ and, second, aborted and then truncated to only C7—NH^+. Also, the 6-*s*-*trans* form of model **2** calculated to be 3.6 kcal·mol^{-1} less stable than the 6-*s*-*cis*-isomer in squid rhodopsin is included to complete the study (**5**). In the case of PSB11, the 6-*s*-*cis* isomer is calculated to be ∼10 kcal·mol^{-1} more stable than the 6-*s*-*trans* form in both squid and bovine rhodopsins and also in good agreement with theoretical findings on deep red cone pigments.[9]

The overall charge of the total system is +1, due to the PSB11-K305/K296 linkage. All atoms of the protein containing retinal analogues were fully optimized without any constraints using the hybrid QM/MM (QM = B3LYP/6-31G*; MM = AMBER96) method in ONIOM (Our own *N*-layer Integrated molecular Orbital + molecular Mechanics) electronic embedding scheme implemented in Gaussian03.[14]

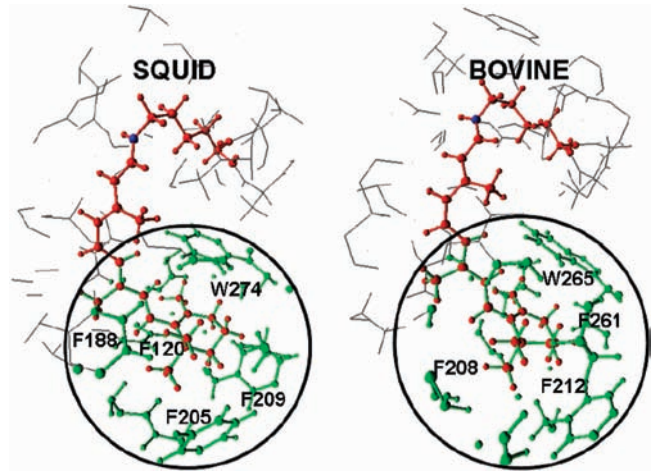


Figure 2. Comparison of the squid (left) and bovine (right) rhodopsin binding pockets. Residues within the 4 Å environment of any atom in PSB11 are shown in gray (lines). Black circle indicate the residues (shown in green colored ball and stick model) within the 4 Å environment of any atom in the β-ionone portion of PSB11.

Ab initio multireference QM/MM calculations on the resulting structures at the spectroscopy oriented configuration interaction (SORCI+Q)/6-31G* level[15] was applied using the ORCA 2.6.19 program package on top of the three-root (6e,6o) complete active space self-consistent field (CASSCF) wave functions to calculate the absorption and circular dichroism (CD) spectra in the gas phase (QM) and in the protein (QM/MM). The vertical excitation energies as well as oscillator and rotatory strengths to first (S_1) and second (S_2) excited states were calculated for all of the structures discussed in this study. We estimate the accuracy of this computational setup to be 15 nm, and we showed previously that the present setup yielded results in excellent agreement with experimental measurements.[4]

As we enter into the protein environment, the most logical place to start the investigation is to look at the geometrical parameters of the chromophore (Figure 1) as a consequence of binding to the opsin. The details of the bond length alternation (BLA) pattern (Figure 1, bottom) of model **1** in both bovine and squid rhodopsins have been documented elsewhere.[4] In the case of models **2** and **5**, extending the π-conjugation by one more double bond at C3═C4 reduces the ensuing C4—C5 single bond by 0.05 Å (1.51→1.46 Å). Aborting the conjugation by saturating the C5═C6 bond (**3**) bestows adequate flexibility to the retinal as evident in the dramatic increase of the C5—C6 bond length to ∼1.57 Å in both protein environments. Truncating the retinal to only five double bonds (**4**) eases the strain on the C4—C5 single bond and increases it by 0.04 Å (1.51→1.55 Å). Therefore, with respect to the BLA it is fair to suggest that changes incorporated into the cyclic portion of the retinal seem to exert significant perturbation only on the neighboring single/double bonds and that the other half of the retinal backbone essentially remains unperturbed. Apparently, both bovine and squid rhodopsin binding pockets consist of at least five aromatic residues within the 4.0 Å environment of the β-ionone ring (Figure 2).[3,4] Therefore, contact between the β-ionone ring and opsin through secondary hydrophobic interactions[16] leading to retinal photoisomerization[17] cannot be ruled out at this stage.

Perusal of the bond (Figure 1, middle) and dihedral (Figure 1, top) angle deviations also reveal the extensive perturbation the retinal has undergone starting from C3 to C8 (Figure 1, gray box). In particular, comparing models **3** and **4** provides indirect evidence

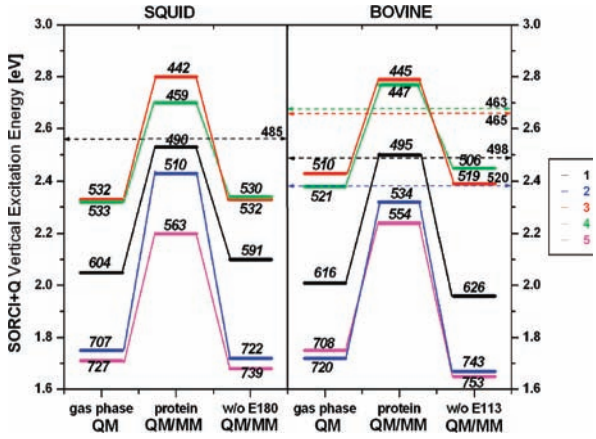


Figure 3. SORCI+Q calculated $S_1 \rightarrow S_0$ vertical excitation energies in eV (wavelength in nm) of the PSB11 analogues in gas phase (QM) and protein (QM/MM) environments of squid and bovine rhodopsin. Calculations involving the protein environment without charges of its counterion (w/o E180, w/o E113) are also plotted. Color code: black, **1**; blue, **2**; red, **3**; green, **4**; magenta, **5** for PSB11 models depicted in Scheme 1. Horizontal dashed lines on the left (squid) and right (bovine) hand sides indicate the corresponding experimental values taken from refs 6, 7, and 23−26. The calculated $S_2 \rightarrow S_0$ values of all the PSB11 analogues discussed in this study are given in the Supporting Information.

for the presence of weak H-bonding interaction at the Schiff base terminal in squid rhodopsin[4] in agreement with FTIR studies.[18] The dramatic change in the C6−C7 dihedral angle (from −45° in model **1** to −120° in models **3** and **4**) in conjunction with a slight displacement of the position of the non-H-bonding counterion (E180) provides evidence for a flexible binding site in squid rhodopsin. On the contrary, little change in the C6−C7 dihedral angle between model **1** and models **3** and **4** coupled with an unaltered position of the H-bonding counterion (E113) throughout the investigation (see Supporting Information) indicates a stiff binding site in the bovine rhodopsin. For models **1**, **2**, and **5**, almost all the calculations yield a distorted C6−C7 single bond with a torsional angle in the vicinity of −45° in good agreement with both the experimental[19] and theoretical findings.[20]

Generally, the spectral tuning of dehydro and dihydro rhodopsins has been known to depend on the length of retinal conjugation.[13] This property is evident in the calculated vertical excitation energy in both gas phase and protein environments which correlates well with the number of double bonds in PSB11 (Figure 3). Compared to the wild-type (**1**) that contains six double bonds and absorbs at 604/616 nm in the gas phase and 490/495 nm in the protein, model **2** contains seven double bonds and absorbs at 707/720 in the gas phase and 510/534 nm in the protein. However, model **3** also contains six double bonds but with an aborted retinal conjugation (from C5 to C7) absorbs at 532/510 nm in the gas phase and 442/445 nm in the protein. Model **4** that contains only five double bonds also absorbs at 533/521 nm in the gas phase and 459/447 nm in the protein, thus supporting the hypothesis that model **3** may essentially behave as a five-double-bonded retinal.[6b]

Model **5**, an isomer of model **2**, also absorbs at 727/708 nm in the gas phase and 563/554 nm in the protein. Therefore, a increase/decrease in the retinal conjugation by one double bond separates the vertical excitation energy by ∼100 nm in the gas phase and ∼60 nm in the protein. As we turn off charges of the counterion (E180 in squid and E113 in bovine), the calculated vertical excitation energy in the protein becomes almost on par with that of the gas phase (see w/o E180, w/o E113 in Figure 3). Therefore, similar to the native visual[4,21] and archaeal[22] rhodopsins, the mechanism of spectral tuning in dehydro and dihydro rhodopsins is also steered by the strong electrostatic interaction between PSBR and the counterion, and the calculated results are also in good agreement with experimental findings on related rod visual pigments.[6−8,13,23,24]

The optical activity of rhodopsin is induced when retinal is bound to opsin. However, squid acid metarhodopsin does not show circular dichroism[25] which suggests there are exceptions. Generally, out-of-plane distortion of the C11═C12 (negative) and C12−C13 (positive) bonds imparts a positive helicity on retinal yielding a positive rotatory strength (R).[26] Surprisingly, the magnitude of the calculated R of model **2** (+0.66 au) is almost double that of model **1** (+0.32 au), while that of model **3** is almost equal (+0.33 au) to that of model **1** in squid rhodopsin. A relatively similar trend is also seen in the case of bovine rhodopsin (see Supporting Information).

In conclusion, dehydration and hydration of the β-ionone ring in both the squid and bovine rhodopsins are computationally studied for the first time. Our findings suggest that (i) modification of the retinal conjugation in β-ionone ring does not seem to affect the other half of the retinal backbone; (ii) of all the retinal analogues considered, 3,4-de-5,6-dihydro and 5,6-dihydro retinals serve as more suitable probes to characterize the protein binding sites (the binding site of invertebrate squid rhodopsin as *malleable and ductile*, and that of vertebrate bovine rhodopsin as *rigid and stiff*); (iii) the mechanism of spectral tuning in both dehydro and dihydro rhodopsins is steered by the strong electrostatic interaction between PSBR and the counterion; (iv) twisting of the β-ionone ring relative to the polyene chain is almost irrelevant to the optical activity of visual pigments;[6b] and finally, (v) as the binding site of salamander rhodopsin containing model **2** remains unknown, our calculations incorporating model **2** into squid and bovine binding sites allows us to predict that the same overall electrostatic and steric properties of the retinal structure are likely to exist for salamander as well. In other words, the red shift of ∼20 nm separating PSB11 in squid and/or bovine rhodopsin from that of 3,4-dehydro-PSB11 in salamander rhodopsin is essentially due to the extension of retinal π-conjugation and not due to any particular residue-based mechanism.

Acknowledgment. The authors would like to thank Dr. Tetsuji Okada at Gakushin University for valuable discussions during the course of this work. The work at Emory is supported in part by a grant from the National Institutes of Health (R01EY016400-04) and at Kyoto by a Core Research for Evolutional Science and Technology (CREST) grant in the Area of High Performance Computing for Multiscale and Multiphysics Phenomena JST.

Supporting Information Available: ONIOM (QM/MM)-optimized Cartesian coordinates, geometric parameters, excited state properties of the models discussed in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Birge, R. R. *Annu. Rev. Phys. Chem.* **1990**, *41*, 683–733. (b) Kandori, K.; Shichida, Y.; Yoshizawa, T. *Biochemistry* **2001**, *66*, 1197–1209.

(2) (a) Terakita, A.; Yamashita, T.; Tachibanaki, S.; Shichida, Y. *FEBS Lett.* **1998**, *439*, 110–114. (b) Yokoyama, S. *Prog. Ret. Eye. Res.* **2000**, *19*, 385.

(3) (a) Shimamura, T.; Hiraki, K.; Takahashi, N.; Hori, T.; Ago, H.; Masuda, K.; Takio, K.; Ishiguro, M.; Miyano, M. *J. Biol. Chem.* **2008**, *283*, 17753–17756. (b) Murakami, M.; Kouyama, T. *Nature* **2008**, *453*, 363–367.

(4) (a) Sekharan, S.; Altun, A.; Morokuma, K. *Chem.—Eur. J.* **2010**, *16*, 1744–1749. (b) Sekharan, S.; Morokuma, K. *J. Phys, Chem. Lett.* **2010**, *1*, 668–672. (c) Altun, A.; Yokoyama, S.; Morokuma, K. *J. Phys. Chem. B* **2008**, *112*, 16883–168890. (d) Altun, A.; Yokoyama, S.; Morokuma, K. *J. Phys. Chem. A* **2009**, *113*, 11685–11692.

(5) Dartnall, H. J.; Lythgoe, J. N. *Vision Res.* **1965**, *5*, 81–100.
(6) (a) Blatz, P. E.; Dewhurst, P. B.; Balasubramaniyan, P.; Balasubramaniyan, V. *Nature* **1968**, *219*, 169–170. (b) Azuma, M.; Azuma, K.; Kito, Y. *Biochim. Biophys. Acta* **1973**, *295*, 520–527.
(7) (a) Cornwall, M.; Macnichol, E. F., Jr.; Fein, A. *Vision Res.* **1984**, *24*, 1651–1659. (b) Makino, C. L.; Kraft, T. W.; Mathies, R. A.; Lugtenburg, J.; Miley, M. E.; van der Steen, R.; Baylor, D. A. *J. Physiol.* **1990**, *424*, 545–560. (c) Makino, C. L.; Groesbeek, M.; Lugtenburg, J.; Baylor, D. A. *Biophys. J.* **1999**, *77*, 1024–1035. (d) Isayama, T.; Alexeev, D.; Makino, C. L.; Washington, I.; Nakanishi, K.; Turro, N. J. *Nature* **2006**, *443*, 649.
(8) Chen, J. G.; Nakamura, T.; Ebrey, T. G.; Ok, H.; Konno, K.; Derguini, F.; Nakanishi, K.; Honig, B. *Biophys. J.* **1989**, *55*, 725–729.
(9) Amora, T. L.; Ramos, L. S.; Galan, J. F.; Birge, R. R. *Biochemistry* **2008**, *47*, 4614–4620.
(10) Spudich, J. L.; McCain, D. A.; Nakanishi, K.; Okabe, M.; Shimizu, N.; Rodman, H.; Honig, B.; Bogomolni, R. A. *Biophys. J.* **1986**, *49*, 479–483.
(11) Lanyi, J. K.; Zimanyi, L.; Nakanishi, K.; Derguini, F.; Okabe, M.; Honig, B. *Biophys. J.* **1988**, *53*, 185–191.
(12) DeGrip, W. J.; Bovee-Geurts, P. H. M.; van der Hoef, I.; Lugtenburg, J. *J. Am. Chem. Soc.* **2007**, *129*, 13265–13269.
(13) Arnaboldi, M.; Motto, M. G.; Tsujimoto, K.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1979**, *101*, 7082–7084.
(14) Vreven, T.; Byun, K. S.; Komaromi, I.; Dapprich, S.; Montgomery, J. A., Jr.; Morokuma, K.; Frisch, M. J. *J. Chem. Theory Comput.* **2006**, *2*, 815.
(15) Neese, F. A. *J. Chem. Phys.* **2003**, *119*, 9428–9443.
(16) Matsumoto, H.; Yoshizawa, T. *Nature* **1975**, *258*, 523–526.
(17) Ahuja, S.; Hornak, V.; Yan, E. C.; Syrett, N.; Goncalves, J. A.; Hirshfeld, A.; Ziliox, M.; Sakmar, T. P.; Sheves, M.; Reeves, P. J.; Smith, S. O.; Eilers, M. *Nat. Struct. Mol. Biol.* **2009**, *16*, 168–175.
(18) Ota, T.; Furutani, Y.; Terakita, A.; Yoshinori, S.; Kandori, H. *Biochemistry* **2006**, *45*, 2845–2851.
(19) Mollevanger, L. C. P. J.; Kentgens, P. M.; Pardoen, J. A.; Weeman, W. S.; Lugtenburg, J.; DeGrip, W. J. *Eur. J. Biochem.* **1987**, *163*, 9–14.
(20) Honig, B.; Hudson, B.; Sykes, B. D.; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1289–1293.
(21) (a) Gascon, J. A.; Sproviero, E. M.; Batista, V. S. *Acc. Chem. Res.* **2006**, *39*, 184–193. (b) Sekharan, S.; Sugihara, M.; Buss, V. *Angew. Chem., Int. Ed.* **2007**, *46*, 269–271. (c) Altun, A.; Yokoyama, S.; Morokuma, S. *J. Phys. Chem. B* **2008**, *112*, 6814–6827.
(22) (a) Wanko, M.; Hoffmann, M.; Strodel, P.; Kosolowski, A.; Thiel, W.; Neese, F.; Frauhenheim, T.; Elstner, M. *J. Phys. Chem. B* **2005**, *109*, 3606–3615. (b) Hoffmann, M.; Wanko, M.; Strodel, P.; König, P. H.; Frauenheim, T.; Schulten, K.; Thiel, W.; Tajkhorshid, E.; Elstner, M. *J. Am. Chem. Soc.* **2006**, *128*, 10808–10818.
(23) Koutalos, Y.; Ebrey, T. G.; Tsuda, M.; Odashima, K.; Lien, T.; Park, M. H.; Shimizu, N.; Derguini, F.; Nakanishi, K.; Gilson, H. R.; Honig, B. *Biochemistry* **1989**, *28*, 2732–2739.
(24) Sheves, M.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 4033–4039.
(25) Kito, Y.; Azuma, M.; Maeda, Y. *Biochim. Biophys. Acta* **1968**, *154*, 352–359.
(26) Shichida, S.; Tokunaga, F.; Yoshizawa, T. *Biochim. Biophys. Acta Bioenerg.* **1978**, *504*, 413–430.

JA105050P