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Calculation of the Electron Affinities of the Chromophores Involved in Photosynthesis

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Abstract: We examine the electron affinities (EA) of the postulated electron acceptor chromophores of the photosynthetic reaction center in *R. Viridis*. We estimate a difference of EA's between bacteriochlorophyll-*b* and bacteriopheophytin-*b* of 0.19 eV, in excellent agreement with the experimental value reported at 0.20 eV. We estimate this difference in situ at 0.42 eV, compared to an experimental estimate of 0.34 eV. These results support those of Thompson and Zerner (*J. Am. Chem. Soc.* **1991**, *113*, 8210) that in the absence of specific interactions between BChl-L-side and the environment, electron transfer to BChl-L-side might not be directly involved in the overall electron transfer process.

Introduction

The mechanism for the primary electron transfer in bacterial photosynthesis is today a subject of active investigation. Experimentally the electron is observed to move some 17 Å from the special pair (P) to a bacteriopheophytin-*b* (HL)[1−7] (center-to-center) within a time frame of about 3 ps, and always along the L-side of the reaction center.[8] (The protein in which the photosynthetic reaction center (RC) is embedded is composed of several subunits, two of which are designated Large (L) and Medium (M). In this work we designate the special pair (composed of two bacteriochlorophyll-*b* monomers) with a P, the accessory bacteriochlorophylls with a B, and the bacteriopheophytins with an H. The protein subunits that B and H are associated with will be designated with the L and M labels.) Many aspects of this model for the primary charge separation are open to question. There has been, for example, a great deal of discussion as to whether the BL monomer plays a direct role in the initial charge separation step, as it lies between P and HL, Figure 1. To date, though, the photoelectron has not been conclusively detected at the BL monomer. Evidence for this is indirect: either the lifetime of this species is too short to detect, or super-exchange is involved to help explain the speed of the electron transfer.[7,9−11]

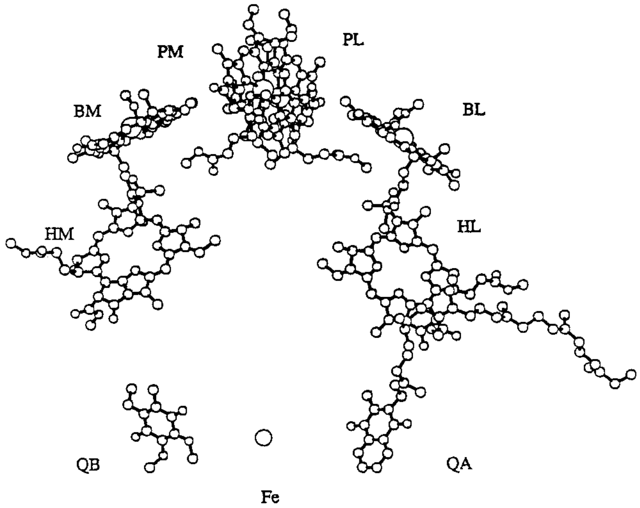

Figure 1. *R. Viridis* reaction center, from ref 15. The original structure was reported in ref 18.

Recent calculations[12−15] have lent support to the idea of a concerted 17-Å jump by the electron from P to HL. These calculations are themselves open to question as their results are dependent upon environmental effects, and these effects can at this time only be approximated.[16,17] Then, as a test of the model used in the above calculations and to lend further support to the results obtained from them, we have chosen to calculate the redox potentials of four chromophores, HL, HM, BL, and BM of the photosynthetic reaction center of *R. Viridis*.[18] (The symbol B_h will indicate the histidine ligating B at the fifth position has been included in the calculation.) Hopefully, if these are well reproduced then the spectroscopic calculations based upon this methodology[13−15] have asymptotic verification, although proviso's that relate this model calculation to the assumed structure still stand. Electron transfer from P to BL and HL must be near competitive processes as small site specific mutations can have remarkable changes on observed spectroscopic properties.[10,19,20]

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Table 1. INDO/S Calculated Transition Energies

	absorption energy (cm^{-1}/10^{3})[a]		
state transitions[b]	vacuum	solvated[c]	estimated relaxed[d]
P* (Q_{y1})	11.6	11.5	11.4
$PH_L \rightarrow P^+H_L^-$	19.7	12.6	7.1
$PB_L \rightarrow P^+B_L^-$	19.8	16.6	14.1
$PB_M \rightarrow P^+B_M^-$	20.2	16.7	14.0
$PH_M \rightarrow P^+H_M^-$	20.6	13.5	8.0

[a] See ref 15 for the details of these calculations. [b] All P and B chromophores have a histidine ligating the fifth position. [c] The excited states are electronically relaxed with respect to the induced polarization of the solvent. No attempt has been made to account for nuclear relaxation in either the solvent or solute. [d] Estimated values with complete dielectric relaxation, see text.

Consider the processes

$$P^*{-}BL{-}HL{-}BM{-}HM \rightarrow P^+{-}BL^-{-}HL{-}BM{-}HM \quad (1)$$

$$\rightarrow P^+{-}BL{-}HL^-{-}BM{-}HM \quad (2)$$

$$\rightarrow P^+{-}BL{-}HL{-}BM^-{-}HM \quad (3)$$

$$\rightarrow P^+{-}BL{-}HL{-}BM{-}HM^- \quad (4)$$

Processes 1 and 2 are of direct interest. The quantum mechanical calculations of ref 15 find that these are roughly of equal energy in the gas phase and some 8000 cm^{-1} above P* (8066 cm^{-1} = 23.06 kcal/mol = 96.44 kJ/mol = 1 eV). (The excited state P* is also called Q_{y1}, and it is the first long axis (y) transition of the four Q_y bands.) When the electronic relaxation of the surroundings is taken into account, as appropriate for *absorption* spectroscopy, process 2 is calculated below (1) by some 4000 cm^{-1}. This situation is summarized in Table 1. Although the model used to calculate the dielectric relaxation of the surrounding protein is crude, the basic physics is likely correct; dielectric relaxation will favor the greatest separation of charge, 17 Å center-to-center for process 2 versus the 8 Å center-to-center separation of process 1, *other things being equal*. Considering the difference between eqs 1 and 2 focuses attention on the electron affinities of B and H. To this end we examine the EA's of the aforementioned chromophores using the same model that was used in ref 15 to examine the RC. The redox potentials of B and H are known in solution.[21] We calculate the EA's using our solvation model to mimic the solvent used in ref 21, this to demonstrate the methods' veracity. We then calculate the chromophore EA's using our model to mimic the environment found in the RC. Finally, we add ligating histidines to BL and BM, as we have found that this does influence the computed redox potentials for these chromophores.

Methods

We use the semiempirical quantum chemical Intermediate Neglect of Differential Overlap method parametrized for spectroscopy (INDO/S).[22−25] In this work the environment was modeled using a Self-Consistent Reaction Field (SCRF) approach.[16,17,26−29] The computed EA's reported herein are of the delta SCF type, well tested for reliability within the INDO/S model.[30] The neutral chromophores are closed-shell, while the ions are doublets, for this reason a small Configuration Interaction Singles excitation (CIS) is performed on the doublets to stabilize the ground state with respect to Brilloiuns' theorem.[31−33] This CIS stabilizes the B-systems by a nearly constant 0.12 eV and the H-systems by 0.14 eV. In addition to reproducing the situation that exists in the results of ref 15, such calculations also tend to restore the balance that makes Koopmans' approximation valid for frontier orbitals; i.e., a cancelation between relaxation and correlation, and, indeed, the orbital energies of the LUMO[30] reflect the results of Tables 2 and 3.

Table 2. Calculated Electron Affinities in Units of eV

system	vacuum	$\epsilon = 9.0$	$\epsilon = 36.7$	expt[a]
HL	−2.47	−3.65	−3.78	−4.00
HM	−2.40	−3.38	−3.51	
BL	−2.56	−3.38	−3.47	−3.80
BM	−2.54	−3.34	−3.43	
BL_h	−2.30	−3.23	−3.32	
BM_h	−2.24	−3.06	−3.13	

[a] From ref 21, measurements taken in a solution of DMF, $\epsilon = 36.7$; 4.50 eV has been added to the experimental values to adjust for the normal hydrogen electrode, see text.

Table 3. Delta EA (H−B) in Units of eV

difference	vacuum	$\epsilon = 9.0$	$\epsilon = 36.7$	expt
HL-BL	0.09	−0.27	−0.31	−0.20[a]
HM-BM	0.14	−0.04	−0.08	
avg	0.12	−0.16	−0.19	
HL-BL_h	−0.17	−0.42	−0.46	−0.34[b]
HM-BM_h	−0.16	−0.32	−0.38	
avg	−0.16	−0.37	−0.42	

[a] From ref 21, measurements taken in a solution of DMF, $\epsilon = 36.7$; 4.50 eV has been added to the experimental values to adjust for the normal hydrogen electrode, see text. [b] See refs 11 and 38.

The structures used in this work were obtained from the crystal coordinates of the *R. Viridis* RC available from the Brookhaven National Laboratory Protein Data Bank (PDB) which is the same data base as that used in ref 15. (The 1PRC structure was used.) The chromophores were extracted from the PDB file, and hydrogens were added, using a CAChe Tektronics work station.[34] We left the phytyl side chains in place as these will help in modeling the environment, and they were not computationally too burdensome to include into the calculations.

Results

We present our calculated results in Table 2. The EA's of the chromophores were computed for three different environments representing a vacuum, a solvent with a dielectric constant (ϵ) of 9.0, and a solvent with $\epsilon = 36.7$. We present the vacuum case for reasons of comparison. The case of $\epsilon = 9.0$ is a best guess[35,36] at the ϵ of the environment surrounding the RC, and the $\epsilon = 36.7$ calculation attempts to reproduce the effect of solvating chromophres in *N*,*N*-dimethylformamide (DMF) for comparison with the experimental work of Fajer *et al.*[21]

The results in Table 2 yield several interesting observations. One is that the comparative difference in the ease with which

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H and B can be reduced in vacuum is reversed by the inclusion of the histidines into the B calculations. This reversal, of B going from more favorably reduced to less favorably reduced than H, is understandable. The inductive effect of the nitrogen lone pair, of the ligating histidine, on the LUMO of B would be expected to raise the LUMO in energy and thus raise the energy cost of reducing B, in this case, to above that of H.

Inspection of the $\epsilon = 9.0$ results, as well as the $\epsilon = 36.7$ results, shows that upon solvation all EA's drop by more than 0.8 eV. This is easy to understand on the basis of the Born model which suggests a stabilization of $-Q^2/2(1 - 1/\epsilon)a^{-1}$, where Q is the charge of the ion, i.e. -1, and a is the radius of a sphere surrounding the chromophore, about 6.0 Å for these systems. (See the Appendix.) Also, with or without the inclusion of histidine into the calculation, H of each (B,H) pair is now energetically preferred for reduction, the difference being greater when histidine is included. (See Table 3.) The reduction of HL is now energetically preferred with respect to the other chromophores by a minimum of 0.27 eV. From Tables 2 and 3 it is seen that L-side reduction is more favorable than M-side reduction, and this preference is increased by the inclusion of histidine into the calculations. This is interesting by itself as it already indicates, just from these structures, a preference for L-side electron transfer over M-side transfer, although based upon thermodynamics alone, we would not be able to rule out modified structures that transfer along the M-side. In addition, we have not modeled the dynamics of this process, although others have done so.[11,37,38] Marchi, Gehlen, Chandler, and Newton have used the gas phase energetics calculated by the INDO model (reported in ref 15) reproducing the experimental charge transfer states,[7] although these calculations have been challenged.[11,38]

We compare our computed results for $\epsilon = 36.7$ with those of Fajer *et al.* by averaging the L-side and M-side results (without histidines), as we do not have a relaxed structure per se, and present this average as our best guess result. In this spirit we find that the average EA of HL and HM is -3.65 eV (-4.0 eV) and that of BL and BM is -3.45 eV (-3.8 eV), where the values in parentheses are those of Fajer *et al.*, and we have added 4.5 eV to correct for the normal hydrogen electrode,[39,40] the reference used in the experiment. The difference in EA's that is of importance in understanding eqs 1−4 is that calculated at -0.19 eV (see Table 3) compared to the experimental difference of -0.20 eV. Note that this difference is independent of the normal hydrogen electrode reference.

Warshel has estimated a difference of -0.34 eV for the EA of the L-side *in situ* chromophores from experimental results.[11,38] This value is best compared with our difference EA(HL) − EA(BL_h) = -0.46 eV, with $\epsilon = 9.0$, or some 2 kcal per mol larger than Warshel's estimate.

Conclusions

We find from a comparison of the calculated EA's of the four possible acceptor chromophores that electron transfer along the L-side of the RC is energetically preferred. We also find that BL would be preferentially reduced over HL in the gas phase, but that this is reversed in condensed matter environments.

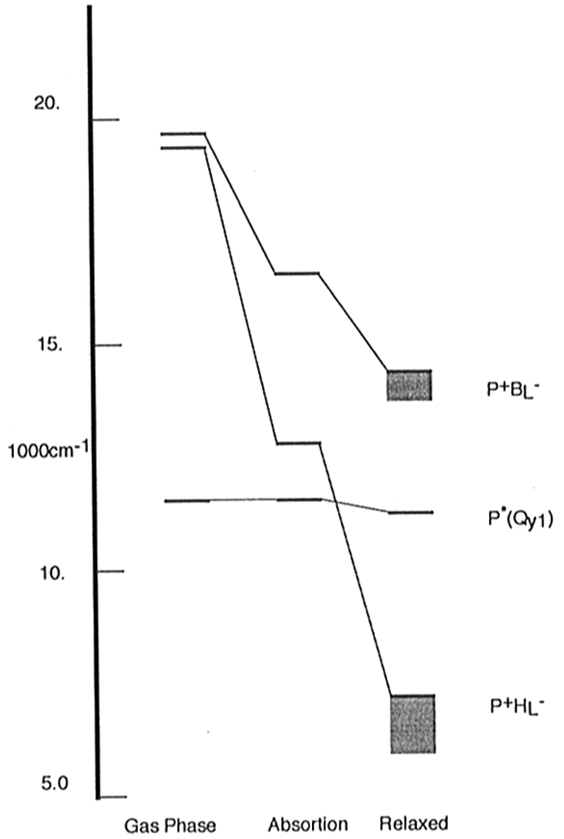

Figure 2. Estimated energy levels for the model reaction center. "Gas phase" refers to the calculation of the absorption spectrum as an isolated supermolecule, "absorption" refers to this same system in a dielectric medium of $\epsilon = 9.0$, $\eta = 1.414$, allowing only for the electronic relaxation of the dielectric medium, and "relaxed" refers to the same calculation in which the dielectric medium is now in equilibrium with the excited state charge distribution.

Our estimated value of -0.19 eV for the differences in EA's of H and B in DMF is in excellent agreement with the experimental value of -0.20 eV. Our estimated value of this difference for the *in situ* HL and BL_h EA's of -0.42 eV is in reasonable agreement with estimates made by Warshel of -0.34 eV.[11,38]

These calculations certainly give credence to similar calculations used to estimate the absorption spectrum for the entire RC in ref 15, partially summarized in Table 1, which suggested that BL is not directly involved in the electron transfer process. (In the calculations of ref 15 only electronic relaxation of the RC environment was included.) Even allowing for a 0.1-eV error (overestimate) of this difference, from the estimate of Warshel, would only lower the excitation energy of the $P^+B_L^-$ state of Table 1 by 800 cm^{-1}, to 15 800 cm^{-1}, still considerably above that of P* and $P^+H_L^-$.

We might estimate the fully relaxed energies of the charge separated states (as opposed to those estimated for absorption) in two ways. The first way is to apply the generalized Born theory, which suggests approximately doubling the gas phase absorption shift, i.e. by the ratio of $(1 - 1/\epsilon)/(1 - 1/\epsilon_\infty)$ or $\simeq 1.8$. From Table 1 this places P* at 11 400 cm^{-1}, $P^+H_L^-$ at 7100 cm^{-1}, and $P^+B_L^-$ at 14 100 cm^{-1}, close to P* but still 2700 cm^{-1} above it, see Figure 2. Fully relaxed $P^+H_L^-$ is believed to be some 2500 cm^{-1} below P*, or to lie at 9000 cm^{-1} from these calculations (to be compared with 7800 cm^{-1} from the experimental value of P* at 10 300 cm^{-1}).[5,6] Assuming

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$P^+H_L^-$ should relax to 9000 cm^{-1} gives a relaxation ratio of 1.51, not 1.8. We can use this to make a second estimate of the relaxed position of $P^+B_L^-$ at ≃15 000 cm^{-1}, still considerably above that of P*. An estimate that the fully relaxed $P^+H_L^-$ state lies below P* by 630 cm^{-1} has been made, but this is for a mutant.[10]

In the absence of a *specific interaction* between BL_h and a fragment of the protein not included in these calculations[9,41,42] that lowers the $P^+B_L^-$ charge transfer state, it is difficult to see how these estimates are in error. Such specific interactions that might change these conclusions might be sought. We concede that we have not examined here the dynamics (rates) of electron transfer. Using the energetics of the gas phase calculations of ref 15, Marchi, Gehlen, Chandler, and Newton[37] have shown that picosecond transfer can occur without $P^+B_L^-$ as an intermediate. In addition, the calculations of Alden, Parson, Chu, and Warshel,[38] although not in concurrence with our suggestion that $P^+B_L^-$ is not an intermediate, indicate a large range of possibilities depending on the details of structure and environmental modeling.

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Appendix

The reaction field model assumes the solute is within a spherical cavity embedded in the solvent.[16,17] The radius *a* of this cavity is somewhat arbitrary. We chose to define it from the *mass density*, i.e.

$$\frac{4}{3}\pi a^3 = \frac{M}{\rho}$$

Where M is the molecular weight and ρ is the solute density. We assumed ρ of the chromophores to be the same as that of porphine, 1.336 g/cm^3.

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