INDO Study on the Comparison of the Nonbonded Environments of Q_I (Q_A) and Q_{II} (Q_B) in PSU

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INDO studies have been carried out for the elucidation of the difference in the nonbonded polarizable environment of Q_I (Q_A) and Q_{II} (Q_B) in thylakoid discs in chloroplast (in bacteria). The free energy of reduction of Q_{II} by Q_I^- (-0.13 eV in vivo) has been explained by considering a more polar environment for Q_I. Three environmental features have been accommodated. Each plastoquinone is hydrogen bonded to the imidazole ring of a histidine molecule that serves as the most proximate environment. The RC protein surrounding the plastoquinone has been viewed as a dielectric continuum. As the most logical way of accounting for the difference in the protein environment of Q_I and Q_{II}, two additional peptide fragments have been symmetrically placed on the two sides of the plastoquinone ring plane of Q_I. Our calculation indicates that the protein environment is closer to Q_I than to Q_{II}. The proximity indicates that Q_I must be tightly bound as its escape would be prohibited by steric effect, and Q_{II} would be held much less tightly within a larger cage. If no new bond is formed while Q_{II} acquires an electron, the product Q_{II}^- would be bound to the protein cage more strongly than Q_{II} by an amount of the order of 1 eV.

1. Introduction

In a previous paper we have shown that a major part of the free energy (−0.13 eV) for the process

$$Q_I^-(s) + Q_{II}(s) = Q_I(s) + Q_{II}^-(s) \qquad (1)$$

that takes place in the condensed phase within PSII can be explained if one assumes that Q_I is bonded to the 43 kDa RC protein by a side chain like −NHR, NRR′, NHCOR, NRCOR′, or even an aliphatic hydrocarbon chain.[1] The letter "s" in parentheses indicates the respective species to be in the condensed phase, (that is, solvated in the protein dielectric). The remaining part of ΔG must be attributed to a difference in the environment surrounding, but not directly bonded to, the plastoquinone moiety. Alternatively, the difference in the nonbonded environment for the two plastoquinones may be responsible for the whole change in free energy. The alternative is the subject of this work.

Our objective here is to construct a viable theoretical model for the nonbonded environment of Q_I relative to that of Q_{II}, which can be utilized in the theoretical investigation of the electron transfer processes $Q_I^- + Q_{II} = Q_I + Q_{II}^-$ and $Q_I^- + Q_{II}^- = Q_I + Q_{II}^{2-}$ which take place in chloroplast. In this paper we have made an INDO/2 study on models of plastoquinone (PQ)−protein complexes. These models are based on a probable geometrical arrangement of the protein chain around Q_I and Q_{II}. Although exceedingly simple in nature, we will see that the models are also capable of clarifying why Q_I and Q_{II}^- are tightly bound whereas the affinity of the free plastoquinone molecules for the binding site is very low.[2]

2. Theoretical Models

The INDO/2 method is one of the most powerful techniques for studying the ground state properties of large molecules, especially those containing transition metal ions. The INDO/s technique has been shown to be very successful in explaining the spectroscopic properties of large molecules involved in photosynthesis.[3] The INDO/2 parametrization is ideally suited for the investigation of thermodynamical properties like standard reduction potentials,[4] dissociation constants,[5] and even electron transfer rates.[6] This is why we have relied on the INDO/2 methodology in the present work that involves quantum chemical calculations on molecules containing up to 150 atoms.

Neighborhood of Q_I. The carbonyl oxygens of Q_I in *Rhodobacter sphaeroides* R-26 are within a hydrogen bond distance from the peptide nitrogen of Ala M260 and the side chain of Thr M222.[7] The experimentally determined hydrogen bond distances are 1.55 and 1.78 Å respectively.[8] The carbonyl oxygens of Q_A (menaquinone) are known to form hydrogen bonds to a peptide nitrogen of Ala M258 and the imidazole ring of His M217 in *Rhodopseudomonas viridis*. These ligands (Ala M258 and His M217) are respectively equivalent to the ligands Ala M260 and His M219 in *Rb. sphaeroides*.[7] Not only is Q_I very tightly bound but also the chemistry of Q_I in bacteria is different from that of a free plastoquinone at physiological pH value.[9]

We have resorted to standard tactics of quantum chemistry, which is to simulate an approximate environment by placing counterions, counterdipoles, etc. around the species under investigation. What we have really done is to include in the model (1) the possible attachment of a histidine to the plastoquinone and (2) two peptide fragments symmetrically placed around the plastoquinone such that they form a cagelike orientation facing the quinone ring. Besides, (3) the RC protein surrounding the plastoquinones has been considered to serve as a dielectric continuum. The first two environmental features have been combined together in Figure 1. Energy as a function of the peptide−plastoquinone distance is shown in Figure 2.

Optimization. Optimization of the molecular geometry of plastoquinone PQ (with only one isoprene unit) has been discussed in detail in refs 1 and 4. The molecular geometry of histidine was adopted from its crystal structure.[10] The equilibrium distance between the quinonoid oxygen of plastoquinone and the N(δ) hydrogen of the imidazole ring of histidine was optimized. The optimized distance is 1.514 Å.

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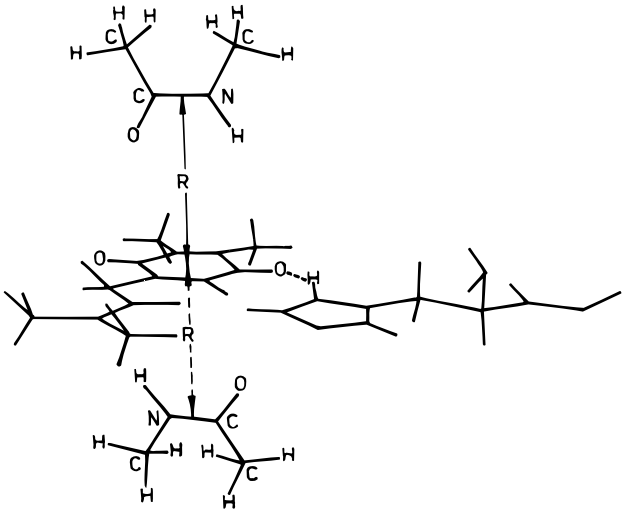

Figure 1. Relative environment of Q_I with two additional peptide fragments facing the quinone ring. Both Q_I and Q_{II} are bound to histidine molecules by hydrogen bonds.

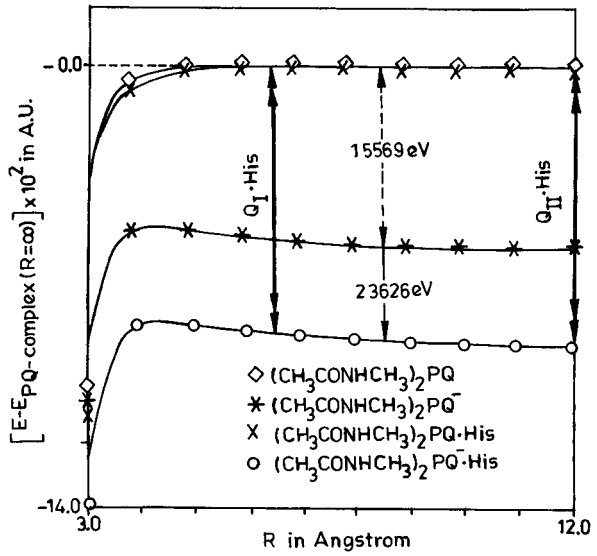

Figure 2. Potential energy diagram for the environment of Q_I: Numerical figures show the free energy of the reduction of Q_I while Q_{II} is not flanked by additional peptide fragments. Another set of possible relative environments of Q_I and Q_{II} is indicated by the positions of the bold lines with two arrowheads at each end.

3. Results and Discussion

The anion Q_I^- is not protonated while the kinetics of protonation of the photosystems is studied.[9] Therefore, we consider the simple reductive process

$$Q_I(s) + e^- = Q_I^-(s) \qquad (2)$$

and a similar process involving $Q_{II}(s)$ and $Q_{II}^-(s)$ to be responsible for the observed redox potential.

In this work we are interested in the reduction potential of Q_I relative to the potential for the corresponding reduction of Q_{II}, that is, in the redox potential for process 1. The relative free energy change is determined by the relative change in internal energy as the $(P\Delta V - T\Delta S)$ term for the oxidation of Q_I^- more or less cancels the same term for the reduction of Q_{II}. Thus the free energy change ΔG is determined by the ΔE of process 2 for $Q_I(s)$ and the ΔE of a similar process involving $Q_{II}(s)$.

The environmental effect on the relative reduction potential will be negligible unless there is a sharp difference between the environment of Q_I and that of Q_{II}. The histidines in our model constitute the immediate environments of Q_I and Q_{II}, so a part of the dielectric relaxation effect is already included in our model. In order to obtain a nonzero relative reduction potential, one must create slightly different environments for Q_I and Q_{II}. In our model we have simulated this difference by placing two peptide fragments at various vertical distances from the Q_I plane (Figure 1).

TABLE 1: Computed INDO Total Energies for Calculating the Standard Reduction Potentials of Q_I/Q_I^- and Q_{II}/Q_{II}^- Systems with a Cagelike Arrangement around Free PQ and the PQ·His Complex

	INDO total energy		
R (Å)	quinone E(Q) (au)	semiquinone $E(Q^-)$ (au)	difference $E(Q^-)-E$(Q) (eV)
	$(CH_3CONHCH_3)_2PQ$		
3.0	−245.6775	−245.6805	−0.0822
4.0	−245.5800	−245.6269	−1.2759
5.0	−245.5759	−245.6272	−1.3956
6.0	−245.5753	−245.6291	−1.4629
7.0	−245.5750	−245.6305	−1.5099
8.0	−245.5749	−245.6316	−1.5440
9.0	−245.5748	−245.6323	−1.5695
∞	−245.5746	−245.6367	−1.6869
	$(CH_3CONHCH_3)_2PQ$·His		
3.0	−362.2306	−362.2598	−0.7946
4.0	−362.1286	−362.2047	−2.0701
5.0	−362.1238	−362.2047	−2.2014
6.0	−362.1228	−362.2062	−2.2694
7.0	−362.1223	−362.2074	−2.3157
8.0	−362.1220	−362.2084	−2.3511
9.0	−362.1218	−362.2091	−2.3756
10.0	−362.1216	−362.2097	−2.3973
11.0	−362.1216	−362.2101	−2.4055
12.0	−362.1215	−362.2105	−2.4191
∞	−362.1213	−362.2129	−2.4926

The rest of the environment that surrounds the plastoquinone and histidine can be considered as a dielectric continuum. It would have more or less the same effect on Q_I and Q_{II} and on Q_I^- and Q_{II}^-. Still we have taken it into account and show that its effect is to cause a change in the relative reduction potential in volts only in the third decimal place.

The neighboring molecule effect is shown in Table 1. The first part of this table indicates that if two peptide fragments are located about 8.51 Å above and below the plastoquinone ring (Figure 1), the reduction potential of the system will be 1.557 V, that is, 0.13 V less than the reduction potential of free plastoquinone, 1.687 V (Figure 2). As both Q_I (Q_A) and Q_{II} (Q_B) are considered to be attached to histidine in *Rb. sphaeroides* R-26,[7] we have investigated the energetics of a similar placement of peptide fragments on both sides of the plastoquinone hydrogen bonded to a histidine molecule (reduction potential 2.493 V). The corresponding results are shown in the second part of Table 1. In this case the optimum distance from the plastoquinone ring is 8.53 Å with reduction potential 2.363 V. Thus compared to Q_{II}, the quinone Q_I would be flanked by two additional peptide fragments at a distance of about 8.5 Å.

Figure 2 also illustrates the situation in a comparative way. For example, if we assume that the Q_{II}·His complex is sandwiched between two peptide fragments at a distance of 12 Å from the quinone ring, the peptide fragments in the Q_I system must be about 6.43 Å away from the ring so as to give rise to a relative reduction potential of −0.13 V. Thus the polar environment around Q_I is considerably closer than a similar environment around Q_{II}.

These values of reduction potentials are liable to change if one considers an additional environment in the form of a

TABLE 2: Energetics of the Reactions Involving Electron Transfers

reaction	ΔE (eV) (without Born stabilization)	ΔE (eV) (with Born energy of ion−medium interaction)[a]
reduction of Q_I		
$(Pep)_2PQ\cdot His + e^- = (Pep)_2PQ^-\cdot His$[b]	−2.362	−3.430
reduction of Q_{II}		
$PQ\cdot His + e^- = PQ^-\cdot His$	−2.492	−3.562
reduction of Q_{II} by Q_I		
$(Pep)_2PQ^-\cdot His + PQ\cdot His = (Pep)_2PQ\cdot His + PQ^-\cdot His$	−0.130	−0.132
$E^\circ_{Q_I/Q_I^-} - E^\circ_{Q_{II}/Q_{II}^-}$ (in V)[c]	−0.130	−0.132

[a] Equation 3 with $\epsilon = 8.5$[5] plus corrections due to hydrogen bonding in the case of neutral species. [b] Two peptide fragments are at a distance of 8.5 Å from the PQ−His plane. [c] $P\Delta V - T\Delta S$ is negligibly small for these models of Q_I and Q_{II}.

dielectric continuum. The solvent dipoles generate a self-consistent reaction field that interacts with the solute molecular dipole, but the relative reduction potential will remain largely intact. The most important effect of the dielectric continuum on the reduction potentials arises from the ion−dielectric interaction that is normally considered (in ab-initio and semiempirical calculations) within the framework of the simple Born model. The latter model gives the ion−medium interaction energy as

$$E_{\text{ion-dipole}} = -\frac{Z^2}{2r}\left(1 - \frac{1}{\epsilon}\right) \qquad (3)$$

where Z is the charge of the ion, r is the ionic radius, and ϵ is the dielectric constant of the medium. The INDO radius r was calculated from the INDO atomic charges for each species. The Born interaction energy of the complex $[Q_{II}\cdot His]^-$ has been calculated by using the INDO radii for the nearest integrally charged isolated ions Q_{II}^- (fractional charge −0.9570; ionic radius 6.253 au) and His^- (fractional charge −0.0430; ionic radius 4.186 au). In the case of $[(Pep)_2Q_I\cdot His]^-$, it is found that practically no charge is accumulated on the two peptide fragments and the charge separation on Q_I and His is almost the same (fractional charge on Q_I, −0.9566; that on His, −0.0434) as that found for the complex $[Q_{II}\cdot His]^-$. Therefore, the Born stabilization energies of these two complexes differ only minutely from each other. The static dielectric constant for the condensed phase has been taken as 8.5. Stabilization of the neutral molecules in the condensed phase has been considered only in terms of hydrogen bonds. We have assigned a stability of −16 kcal mol $^{-1}$ corresponding to effectively two O···H and two N···H hydrogen bonds per histidine or peptide in the condensed phase. As we have used the simplest Born model for the stability of the charged species, hydrogen bonding effects have not been considered for the anions. The relative reduction potential is shown in Table 2. Clearly the change made in the relative reduction potential by the additional environment is negligibly small, only a few millivolts. The change that would be created by the interaction of the molecular electric dipoles with the reaction field would be still more negligible.

In this work we have taken a planar structure of the plastoquinone−histidine complex and a fixed orientation of the peptide fragments around Q_I as shown in Figure 1. A conformational analysis to find the most suitable torsion angles etc. has not been carried out. There are several reasons for avoiding the optimization of conformations. The first reason is to keep the model structures as simple as possible. The second reason is that in the absence of detailed x-ray crystallographic data on most of the PSU's including the green-plant ones, one can never be certain about the orientations of different species in vivo. As the third reason we point out that the flanking peptide fragments merely constitute the quantum chemical model of counterdipoles, and the actual situation may be as different as a polypeptide chain haphazardly wound around the plastoquinone but at a larger distance. There are also pigments, lipids, and other molecules nearby, and these along with the RC proteins have been visualized to form the dielectric continuum.

Although Fe^{2+} is known to be present in the reaction center complex in both PSII and bacteria, replacement of iron by other divalent metals exerts no apparent effect on the electron transfer reactions. The removal of Fe^{2+} only slows down the rate of electron transfer from Q_A^- to Q_B by about 2-fold.[9] EPR studies have established that there is very little interaction between Q_B and Fe^{2+}, and Fe^{2+} in PSII interacts with some exogenous quinone acceptors.[11] The distance between Q_A or Q_B and Fe^{2+} is quite large (7−10 Å). It is fairly clear that Fe^{2+} does not alter the energetics of the individual quinone systems involved in the electron transfer process to a great extent, but it acts as an intermediary in such transfers. Because Fe^{2+} is placed symmetrically between the two plastoquinones, its effect on ΔG and the redox potential will be vanishingly small.

4. Conclusions

It is possible to account for the relative reduction potential −0.13 V solely by assuming that the polarizing environment is more proximate to Q_I than to Q_{II}. Effectively two symmetrically placed peptide units are closer to Q_I by about 5−9 Å.

The proximity indicates that Q_I must be tightly bound to the reaction center protein because steric effect would prohibit its escape and that Q_{II} is rather weakly bound to a much larger cage surrounding it. Table 1 shows a negligibly small difference of the energies of Q_I and Q_{II}. However, Table 2 indicates that if no new bond is formed while Q_{II} captures an electron, Q_{II}^- would be bound to the protein cage more strongly than Q_{II} by an amount of 1.07 eV. This value is liable to change when one takes a more detailed account of the ion−medium interaction energy. It also depends upon the choice of the ionic radii. For instance, the ion−dipole model is known to reduce the ion−medium interaction energy from its value predicted by the Born model by about 30%, whereas the use of van der Waals radius in lieu of the ionic radius calculated from the INDO atomic charges will increase the interaction energy by about 40%. These two effects largely offset each other. Thus the Q_{II}^--cage binding is estimated to be stronger than the Q_{II}−cage binding by an amount of the order of 1 eV.

Earlier we established that the redox potential −0.13 V can be explained in terms of a suitable substituent linking the plastoquinone Q_I to the surrounding protein fold and a very minor difference in the otherwise nonbonded environment.[1] The present study offers an alternative explanation, and it calls for a more accurate experimental determination of the structure in vivo to distinguish between the two possibilities.

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