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Involvement of the Protein–Protein Interactions in the Thermodynamics of the Electron-Transfer Process in the Reaction Centers from *Rhodopseudomonas viridis*

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ABSTRACT: Reaction centers from *Rhodopseudomonas viridis* were reconstituted into dimyristoylphosphatidylcholine (DMPC) and dielaidoylphosphatidylcholine (DEPC) liposomes. Freeze–fracture electron micrographs were performed on the samples frozen from temperatures above and below the phase transition temperatures of those lipids (T_c = 23 and 9.5 °C, in DMPC and DEPC, respectively). Above T_c, in the fluid conformation of the lipids, the reaction centers are randomly distributed in the vesicle membranes. Below T_c, aggregation of the proteins occurs. The Arrhenius plots of the rate constants of the charge recombination between P^+ and Q_A^- display a break at about 24 °C in DMPC vesicles and about 10 °C in DEPC vesicles (P represents the primary electron donor, a dimer of bacteriochlorophyll, and Q_A the primary quinone electron acceptor). This is in contrast to what was previously observed for the proteoliposomes of egg yolk phosphatidylcholine and for chromatophores [Baciou, L., Rivas, E., & Sebban, P. (1990) *Biochemistry 29*, 2966–2976], for which Arrhenius plots were linear. In DMPC and DEPC proteoliposomes, the activation parameters were very different on the two sides of T_c ($\Delta H°$ for $T < T_c$ = 2.5 times $\Delta H°$ for $T > T_c$), leading however, to the same $\Delta G°$ values. Taking into account the structural and thermodynamic data, we suggest that, in vivo, protein–protein interactions play a role in the thermodynamic parameters associated with the energy stabilization process within the reaction centers.

The light excitation energy harvested by the antenna of the photosynthetic organisms is converted at the level of the reaction centers into chemical free energy. This occurs via a transmembrane charge separation. In bacteria, the chromatophore membrane is mainly composed by phospholipids, 25% of total lipids being phosphatidylcholine (Niederman & Gibson, 1978; Rivas et al., 1987). The different kinetic steps of the electron transfer within the reaction centers as well as the prosthetic groups involved in these processes have been known for about 15 years. However, a main step for a better understanding of the energy stabilization in the reaction centers

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has been accomplished with the crystallization and X-ray structural analysis of the reaction center protein from the purple bacteria *Rhodopseudomonas viridis* (Deisenhofer & al., 1985; Michel & Deisenhofer, 1988; Michel & al., 1986) and *Rhodobacter sphaeroides* (Allen et al., 1988; Chang et al., 1986; Ducruix & Reiss-Husson, 1987; Komiya et al., 1988; Yeates et al., 1988). The reaction center consists of three polypeptides, L, M, and H, which molecular weights range between 30 and 35 kDa. In *Rps. viridis*, a tightly bound cytochrome (40 kDa) containing four *c*-type hemes is found (Weyer et al., 1987). The L and M polypeptides are almost completely embedded in the chromatophore membrane, whereas H and the cytochrome, which are more hydrophilic, are situated on the cytoplasmic and periplasmic sides of the membrane, respectively. The pigments involved in the electron-transfer process are located on the L and M subunits. The primary electron donor is a dimer of bacteriochlorophylls (P), which, in its electronic excited state P*, transfers an electron to a bacteriopheophytin (I) in less than 5 ps, possibly via a monomeric bacteriochlorophyll (Holzapfel et al., 1990). The P^+I^- state decays in about 200 ps by electron donation to the primary quinone molecule, Q_A. Under normal conditions, the electron present on Q_A is transferred to a secondary quinone molecule Q_B in 20–200 μs, depending on the bacterial species. However, in the presence of electron-transfer inhibitors that compete with Q_B for its site, the $P^+Q_A^-$ state decays by recombination in about 1.5 ms at pH 8 in *Rps. viridis* (Baciou et al., 1990; Sebban & Wraight, 1989; Shopes & Wraight, 1987). The decay kinetics associated with this process are biphasic (Baciou et al., 1990; Sebban & Wraight, 1989). Because they were observed in chromatophores, and also on the $P^+Q_B^-$ charge recombination, which decays in about 500 ms at pH 7, these phases were attributed to two conformational states of the reaction centers, preexisting in the dark (Baciou et al., 1990). In bacterial strains such as *Rps. viridis*, where the energy gap between the initial state P* and $P^+Q_A^-$ is smaller than 0.8 eV (Gunner et al., 1986), $P^+Q_A^-$ recombines via a relaxed state of P^+I^- (Kleinfeld et al., 1985; Gopher et al., 1985; Gunner et al., 1986; Sebban, 1988; Sebban & Wraight, 1989; Shopes & Wraight, 1987; Woodbury et al., 1986). Therefore the Arrhenius plots of the rate constant of $P^+Q_A^-$ charge recombination were found linear in reaction centers isolated in detergent (Sebban & Wraight, 1989; Shopes & Wraight, 1987), reconstituted in phosphatidylcholine (egg yolk) (PC)[1] vesicles and in chromatophores (Baciou et al., 1990). Little is known about the reaction center–lipid interactions and about the influence of lipids on the electron-transfer processes within the reaction centers. In this work we have reconstituted reaction centers from *Rps. viridis* into dimyristoylphosphatidylcholine (DMPC) and in dielaidoylphosphatidylcholine (DEPC) liposomes. We show that the lipid phase transition does affect the thermodynamics parameters associated with the electron-transfer process in the reaction centers. Moreover, in the view of the freeze-fracture electron micrographs we suggest that protein–protein interactions play a role in the energy stabilization function of the reaction centers, in vivo.

Material and Methods

Wild-type *Rps. viridis* cells were grown anaerobically (N_2 and CO_2) in the light in the Hutner medium. Reaction centers were prepared as previously described (Prince & Dutton, 1976). DMPC (14:0/14:0) was obtained from Bachem and DEPC (18:1tΔ^9/18:1tΔ^9) from Avanti. The DMPC proteoliposomes were prepared by using the same method as previously described for PC proteoliposomes, except that the liposome preparation, the reconstitution with the reaction centers, and the elimination of detergent (LDAO) on a Sepharose CL-4B column were all done at about 26 °C for DMPC and about 20 °C for DEPC, i.e., above the phase transition temperatures (T_c). The lipid to protein ratio was 3:1(w/w).

For freeze–fracture electron microscopy, small drops of about 50 μL containing 25–30% glycerol were deposited on conventional Balzers gold planchets and rapidly frozen (about 100000 °/s) in Freon 22 at −160 °C. Fracturing and replication were done with Balzers BAF 301 freeze–etching unit by using platinum–carbon shadowing. The replicas, after digestion of organic material with chromic acid and washing with distilled water, were observed in a Philips 301 electron microscope.

The flash absorption spectroscopy apparatus was the same as previously described (Baciou et al., 1990). The temperature was monitored by using a NiCr–Ni thermometer with a precision of ±0.3 °C. For the activation energy measurements, pH was measured in line and readjusted at different temperatures.

Results

The $P^+Q_A^-$ charge recombination kinetics observed in DMPC and DEPC proteoliposomes were biphasic, as was already mentioned for the reaction centers in detergent (Sebban & Wraight, 1989), in PC liposomes, and in chromatophores (Baciou et al., 1990). In DMPC liposomes, at 298 K, the two lifetimes, measured at pH 9, at 960 nm, are equal to $1/k_{fast} = 0.72 \pm 0.05$ ms (50%) and $1/k_{slow} = 2.48 \pm 0.05$ (50%), in good agreement with our previous data on PC liposomes, for which the lifetimes were 0.68 and 2.2 ms, respectively. The Arrhenius plots of k_{fast} and k_{slow} are represented in Figures 1 and 2 for DMPC and DEPC, respectively. It was previously suggested that, in *Rps. viridis*, at room temperature, charge recombination occurs by a thermally activated process (Shopes & Wraight, 1987). The state via which $P^+Q_A^-$ recombines was suggested to be a relaxed state of P^+I^- (Kleinfeld et al., 1985; Gopher et al., 1985; Gunner et al., 1986; Sebban, 1988; Sebban & Wraight, 1989; Shopes & Wraight, 1987; Woodbury et al., 1986). Because the rate of electron transfer from P^+I^- to $P^+Q_A^-$ is much higher than the deactivation rates from either P^+I^- or $P^+Q_A^-$, it was postulated that thermal equilibrium is established between these states. In other words it was assumed that, for the two components (Sebban & Wraight, 1989)

$$k_{slow} = k_d \exp(-\Delta G°_{slow}/k_B T) + k_{Tslow}$$
$$k_{fast} = k_d \exp(-\Delta G°_{fast}/k_B T) + k_{Tfast} \quad (1)$$

where k_d is the rate constant of charge recombination from P^+I^-. We used $k_d = 2 \times 10^7$ s^{-1} (Holten et al., 1978; Shopes & Wraight, 1987). An inherent hypothesis to eq 1 is that k_d is the same for the fast and the slow component. This is probably correct since it was reported by Woodbury and Parson (1984) that the decay of the absorption changes associated with P^+I^- is fitted by a single exponential (C. E. D. Chidsey, C. Kirmaier, D. Holten, and S. G. Boxer, personal communication; A. C. van Bochove, R. van Grondelle, N. Woodbury, and W. W. Parson, unpublished data). $\Delta G°_{fast}$ and $\Delta G°_{slow}$ are the free energy differences between P^+I^- and $P^+Q_A^-$ for the fast and the slow phase, respectively. At low

[1] Abbreviations: DMPC, dimyristoylphosphatidylcholine; DEPC, dielaidoylphosphatidylcholine; LDAO, lauroyldimethylamine *N*-oxide; PC, phosphatidylcholine.

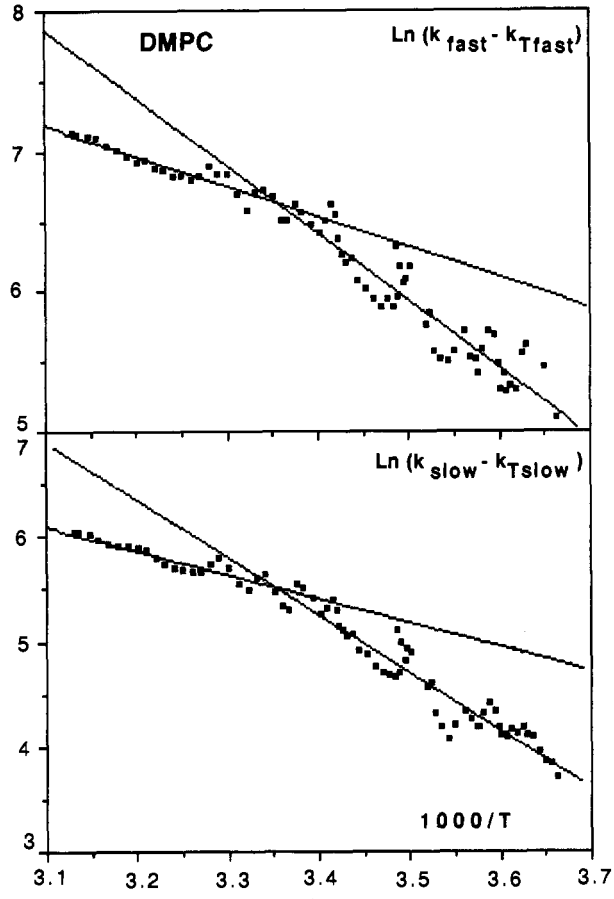

FIGURE 1: Arrhenius plots of the $P^+Q_A^-$ charge recombination kinetics in *Rps. viridis* reaction centers reconstituted in DMPC vesicles. k_{Tfast} and k_{Tslow} were taken equal to 150 and 600 s^{-1}, respectively (see text).

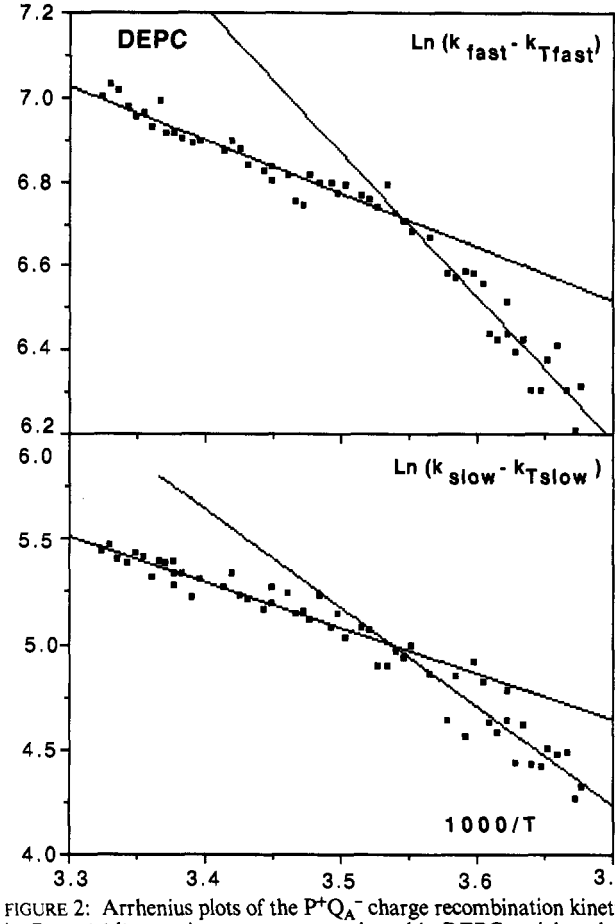

FIGURE 2: Arrhenius plots of the $P^+Q_A^-$ charge recombination kinetics in *Rps. viridis* reaction centers reconstituted in DEPC vesicles. k_{Tfast} and k_{Tslow} were taken equal to 150 and 600 s^{-1}, respectively (see text).

temperature (<200 K), charge recombination from $P^+Q_A^-$ occurs directly to the ground state, via an activationless electron tunneling process (Shopes & Wraight, 1987), with rate constants k_{slow} and k_{fast}. We used here k_{slow} = 150 s^{-1} and k_{Tfast} = 600 s^{-1}, the values measured at 80 K, in *Rps. viridis* chromatophores, in the absence of glycerol (Sebban et al., 1991).

At variance to what was observed for the reaction centers in detergent, in PC liposomes, and in chromatophores, the Arrhenius plots of Figures 1 and 2 display breaks at about 24 ± 2 and 10 ± 2 °C. These values are very close to the phase transition temperatures of DMPC and DEPC, respectively (Small, 1986). This is consistent with unchanged T_c values in proteoliposomes compared to pure lipids. The relatively low protein content used in our experiments is very unlikely to affect the T_c values measured for these lipids in excess water (Small, 1986). To determine the activation parameters above and below T_c, linear regressions were done by using the following data points: for DMPC, below T_c, up to 18 °C, and above T_c, from 27 °C; for DEPC, below Tc, up to 7 °C, and above T_c, from 13 °C. The thermodynamic parameters derived from these plots are shown in Table I. For k_{fast} and k_{slow}, the $\Delta H°$ values obtained above T_c are about 2.5 times as high as below T_c, e.g., in the rigid phase of the lipids. However, by a remarkable compensation effect, the $\Delta G°$ values are the same for k_{fast} and k_{slow}, in the fluid and the rigid phase of the lipids. This must reflect particular shapes of the potential early surfaces of the reactants and products involved in the electron-transfer process, imposed by the protein environment. The same behavior is observed in DEPC vesicles for which the breaks in the Arrhenius plots occur around 10 °C, close to the measured T_c of that lipid (Small, 1986). Interestingly, the

Table I: Activation Parameters, at pH 9, for the $P^+Q_A^-$ Charge Recombination *Rps. viridis*[a]

	component	$\Delta H°$	$-T\Delta S°$	$\Delta G°$
		DMPC Liposomes		
$<T_c$	fast	0.442 (±0.06)	−0.190 (±0.060)	0.252 (±0.06)
	slow	0.490 (±0.05)	−0.210 (±0.050)	0.281 (±0.05)
$>T_c$	fast	0.176 (±0.06)	0.075 (±0.060)	0.251 (±0.06)
	slow	0.190 (±0.05)	0.092 (±0.05)	0.282 (±0.05)
		DEPC Liposomes		
$<T_c$	fast	0.286 (±0.06)	−0.05 (±0.060)	0.236 (±0.06)
	slow	0.392 (±0.05)	−0.118 (±0.050)	0.274 (±0.05)
$>T_c$	fast	0.106 (±0.06)	0.140 (±0.060)	0.246 (±0.06)
	slow	0.181 (±0.05)	0.104 (±0.050)	0.104 (±0.05)
		Chromatophores[b]		
	fast	0.427 (±0.03)	−0.200 (±0.020)	0.227 (±0.03)
	slow	0.470 (±0.025)	−0.206 (±0.025)	0.263 (±0.02)
		PC Liposomes[b]		
	fast	0.176 (±0.03)	0.062 (±0.020)	0.237 (±0.03)
	slow	0.186 (±0.03)	0.085 (±0.020)	0.271 (±0.03)

[a] T = 298 K. For both samples, k_{Tfast} and k_{Tslow}, the low-temperature limiting values for the rate constants, were taken as 150 and 600 s^{-1} as determined by Sebban et al. (1991) at 80 K in the absence of glycerol. [b] Data from Baciou et al. (1990).

$\Delta H°$ and $T\Delta S°$ values (for k_{fast} and k_{slow}) calculated from the DMPC data obtained above T_c are close to the values found in PC liposomes (Table I) (Baciou et al., 1990) and below T_c to the values measured in chromatophores by the same authors. These analogies are partially confirmed by the data obtained in DEPC vesicles (Table I). Whereas the above observations still hold for the slow component, they are not meaningful enough for the fast component to be considered as a general statement. In addition, the quoted error limits, essentially due

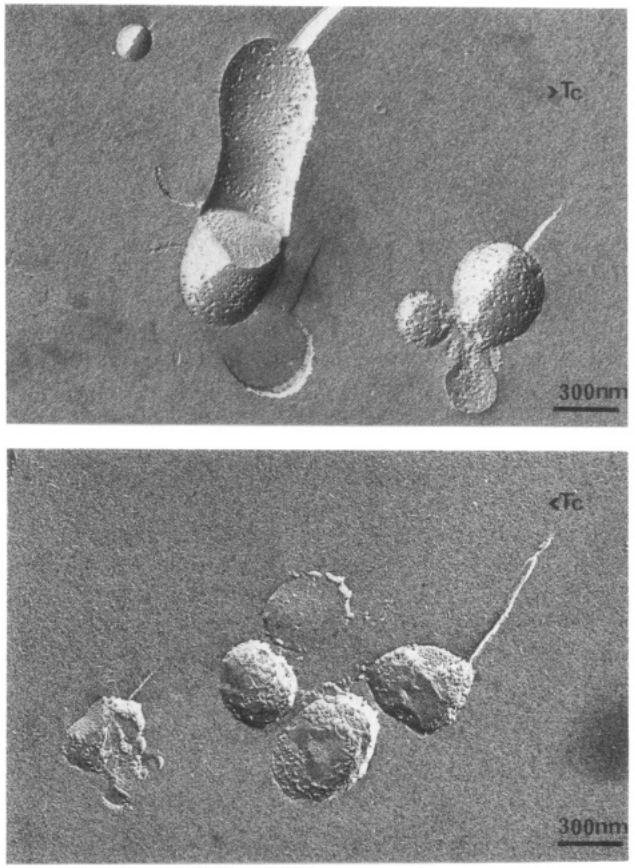

FIGURE 3: Freeze–fracture electron micrographs of *Rps. viridis* reaction centers–DMPC vesicles. Magnification 43000. Lipid/protein ratio, 3:1. $>T_c$, frozen from 30 °C, $<T_c$, frozen from 10 °C.

to data noise (because of the two-exponential decomposition) and calculated from the deviation of different data sets, are too large to allow a definitive equivalence between the parameters obtained below T_c with those derived from chromatophore data. It must also be noted that the activation parameters derived from the plots of Figure 1 and 2 are very sensitive to the limiting values of the rate constants, k_{fast} and k_{slow}, measured at low temperature, especially at lower temperatures of the Arrhenius plots. It could be that k_{Tfast} and k_{Tslow} are slightly different in proteoliposomes and in chromatophores, but we do not expect such a difference that it changes dramatically the relationship between the activation parameters. A complication of the above analysis could come from a possible temperature dependence of k_{Tfast} and k_{Tslow}. To rule out this possibility, we have carefully verified that k_{Tfast} and k_{Tslow} are temperature-independent from 200 to 800 K so that the above analysis makes sense (Sebban et al., 1991).

The substantial differences of the slopes of the Arrhenius plots below and above T_c clearly indicate that the protein–lipid interactions are influenced by the phase transition of the lipids and that this phenomenon affects the thermodynamics of the electron-transfer process within the reaction centers. In order to correlate these observations with possible structural changes, we have performed freeze–fracture electron micrographs of the DMPC proteoliposomes frozen from 10 and 30 °C (Figure 3) and of the DEPC vesicles frozen from 0 and 20 °C (not shown). Above T_c, the proteoliposomes display similar structure as for PC liposomes (Baciou et al., 1990). The reaction centers are randomly arranged in the vesicle membranes, and concave and convex fracture faces are equally observed (Figure 3). Below T_c, a different figure is observed. The reaction center proteins are aggregated and large protein-free domains are observed. A relationship between the activation parameters measured above and the degree of protein–protein contacts clearly appears.

Discussion

From the above measurements, it appears that the activation parameters of the $P^+Q_A^-$ charge recombination kinetics in reaction centers from *Rps. viridis* are substantially influenced by the state of the surrounding lipids, and consequently by the contacts between the proteins. From the freeze–fracture electron micrographs of the vesicles, it is observed that, below T_c, aggregation of the reaction centers occurs. This phenomenon has previously been observed for bacteriorhodopsin (Cherry et al., 1978; Dencher et al., 1983) and for rhodopsin (Davoust et al., 1980) reconstituted in DMPC vesicles. In the former studies, the aggregation state of bacteriorhodopsin was shown to be the same in DMPC vesicles below T_c and in the purple membrane. The photocycle reaction was not affected by the aggregation state of the protein. This observation was not verified for the light–dark adaptation reaction, which yield and rate were reduced by 50% in the monomeric state. It was concluded that for all the reactions for which all-trans bacteriorhodopsins are involved, the monomer is functional but for some processes such as light–dark adaptation, protein–protein interactions regulate the efficiency of the reaction steps. In the case of rhodopsin, it was clearly shown by spin-labeling technique that protein–protein contacts become the major phenomenon below the phase transition temperature (Davoust et al., 1980). That seems also to be the case for the reaction centers from *Rps. viridis*. Our data suggest that in the chromatophore membrane, protein–protein interactions influence the way the energy is stabilized.

Two hypotheses can be drawn to account for such protein–protein contacts in vivo. First, contacts between the antenna polypeptides and the reaction centers could be involved in these processes. Close spatial relationships or direct interaction were demonstrated to exist between the reaction center (H subunit) and the B1020 antenna (Drews, 1985; Jay et al., 1984; Peters et al., 1984; Takemoto et al., 1982). This view is supported by Fourier image analysis techniques showing the reaction centers closely surrounded by six (or 12, i.e., arranged in six dimers) antenna–protein complexes (Engelhardt et al., 1983; Miller, 1982; Stark et al., 1984). In addition to their role of harvesting and focusing the light excitation energy to the reaction centers, the antenna polypeptides could also be indirectly involved in the energy stabilization process.

The second hypothesis arises from possible contacts between reaction center proteins in vivo. This is an attractive hypothesis, which corresponds more to what we observe here in the rigid phase of the lipids. Miller and Jacob (1985) have suggested that, in *Rps. viridis*, the distance between two adjacent membranes is such that it should allow close contact between reaction centers of opposite membranes. Nevertheless, these contacts should essentially be between the hydrophilic parts (H and cytochromes) of the reaction centers. It seems unlikely that the resulting interactions interfere with the electron-transfer process occurring within the hydrophobic part of the protein, in the M subunit. Thus, we come to the conclusion that, if these contacts exist, they are likely to occur between the hydrophobic parts of the reaction centers, closer to the protein area where the electron transfer between I and Q_A takes place. This suggests that reaction centers could be arranged in the membrane not as monomers, but as dimers. It was recently postulated that in *Rb. sphaeroides*, supercomplexes formed by two reaction centers closely associated with a cytochrome c_2 or with a cytochrome c_2 and two cytochrome bc_1 complexes, exist in vivo (Joliot et al., 1989; Lav-

ergne et al., 1989). This was also observed for *R. rubrum* (A. Verméglio, P. Joliot, and A. Joliot, unpublished data). On the basis of Fourier image analysis of the *Rps. viridis* membranes, Stark et al. (1984) have suggested that the photoreceptor unit of this bacterium could be composed of one or two reaction center complexes surrounded by 12 antenna complexes arranged in six dimers. These observations are compatible with the above hypothesis of dimers of reaction centers in the *Rps. viridis* membranes.

Whatever the nature of these protein–protein contacts, either between antenna and reaction centers or between reaction centers, they influence the thermodynamics of the electron-transfer process either by direct electrostatic effects due to interactions between some charged amino acid groups and/or by immobilizing the hydrocarbon chains of the RC's boundary phospholipids.

Structural data at lower resolution on the RC organization within the membrane will probably contribute to a better understanding of these phenomena.

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