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HYPOTHESIS

Structural Specificity of Photosynthetic Reaction Centers Provides High Efficiency of Excitation Trapping and Conversion

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Abstract—The atomic structures of photosynthetic reaction centers of two species of purple bacteria and two photosystems 2 of cyanobacteria were resolved in the late last century. In this work I put forward the idea that of the huge body of data available thus far, only three structural factors are responsible for the unique function of conversion of physical energy of electronic excitation into electrochemical energy of separated opposite charges in reaction centers at least in purple bacteria and, perhaps, in other photosynthetic organisms.

Key words: reaction centers, structure, mechanism of excitation conversion

The primary photoreaction of chlorophyll in photosynthesis was first discovered by Duysens in two species of purple bacteria [1]. It was later shown by Loach [2] and proved in direct experiments by Parson [3] that this process represented photooxidation of bacteriochlorophyll (BChl). This process takes place in reaction centers (RC). A preparation of RC complexes was first isolated by Reed and Clayton from the purple bacterium *Rhodobacter sphaeroides* R-26 [4]. These preparations were devoid of most of the bulk light-harvesting BChl, and their photochemical activity was found to be extraordinarily high—the quantum yield of photooxidation of the special RC pair (P870) was ≥0.98 [5]. Photochemically active RC preparations were later isolated from other species of photosynthesizing bacteria [6]. The crystallization of RC protein from two species of purple bacteria was largely accomplished in the 1980s, and these crystals were studied by X-ray diffraction with spatial resolution ~ 2.5 Å [7, 8]. It was found that this unique apparatus is arranged in phospholipid membranes as two specific polypeptide subunits with relatively low and medium molecular weight (L- and M-polypeptides, respectively) [6]. It was noted that the mutual arrangements of proteins and cofactors in the RC complexes were remarkably similar. In recent comprehensive studies of German researchers, the three-dimensional atomic structure of photosystem (PS) 2 complexes of the cyanobacterium *Synechococcus elongatus* and their RC was resolved with resolution 3.8 Å [9]. Similar results were obtained by a group of researchers from Japan using PS 2 complexes of the cyanobacterium *Thermosynechococcus vulcanus* [10]. Thus, the molecular structure of these very important microconverters of absorbed light energy has been resolved. The viewpoint that the primary act of charge separation in RC proceeds through the stage of formation of charge-transfer complex (CTC) was substantiated in [11-13]. The idea of the model in a simplified form is illustrated in Fig. 1, which is taken from [11, 12]. Because the distance between the chromophores of two molecules producing the CTC-pair is small and energy of interaction between them is correspondingly high, the electron from the orbital of the first singlet excited state (S_1^*) during photoexcitation of one of the molecules (C) is transferred to the orbital cb* shared between the molecules of the CTC-pair. This corresponds to transfer of a fraction of one electron from molecule C to molecule B. The fact of the existence of CTC states in photosynthetic RC was demonstrated in further studies [14-17]. Within the framework of the contemporary concept of the primary photoreaction in RC of purple bacteria, it is safe to suggest that molecules C and B in Fig. 1 are $P870_A$ and $P870_B$ of special RC pair, respectively, whereas molecule Q is the primary electron acceptor P800. In the case of bacterial photosynthesis, this process can be represented as follows:

Abbreviations: CTC) charge-transfer complex; RC) reaction center; BChl) bacteriochlorophyll; Chl) chlorophyll; P870) special RC pair; EES) electronic excited state.

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$$B875^* + P870 \overset{K_M}{<==>} B875 + P870^* \overset{K_{CT} > 10^{13}\,sec^{-1}}{<==>} [P_A^{+\delta e} \sim P_B^{-\delta e}]^* \overset{K_e}{<==>} P870^+ + A^-,$$

$$K_{d1} \quad K_{d2}$$

$$P_A \sim P_B$$

where B875* is the excited BChl molecule (an asterisk indicates electronic excited state (EES) of corresponding molecule or CTC-pair); $[P_A^{+\delta e} \sim P_B^{-\delta e}]^*$ is the special pair P870 in the CTC state; $P_A^{+\delta e}$ and $P_B^{-\delta e}$ are molecules P870 with charges $+\delta e$ and $-\delta e$, respectively; e is the electron charge, $\delta < 1$; $P870^+$ and A^- is the special pair and the primary electron acceptor in oxidized and reduced states, respectively; K_M, K_{CT}, and K_e are rate constants of EES migration, EES conversion from the singlet state B875* to the CTC-state in RC, and the primary electron transfer, respectively.

The theory of CTC-states in pairs of bound molecules was developed in the 1960s based on a large body of experimental data (see, for example, monographs [18, 19]). An electric dipole is generated in molecules in the CTC-state. No less than 20% of electron charge ($\delta > 0.2$) is transferred in strong CTC-states. The electric asymmetry of these states brings about localization of $-\delta e$ and $+\delta e$ on certain molecules. This property of CTC was employed to explain the well-known fact that of two nearly symmetric branches of electron transport cofactors in RC of purple bacteria only one branch is active [20]. Strong CTC are active charge donors because their excited electron is partially displaced to another molecule, thereby facilitating its further localization or separation. Generalization of the results of a large number of photochemical studies revealed general trends in formation of CTC-complexes. Small-molecule solvents, e.g., CH_3CN, pyrene, etc., with constant electric dipoles and structure close to spherical were used as polar solvents to study CTC. These solvents cause an increase in the yield of ion pairs because their solvation coats around CTC-pairs undergo rapid polarization in the electric field of CTC-dipoles, thereby shielding $D^{+\delta e}$ and $A^{-\delta e}$ and facilitating fixation of an excited electron on molecule $A^{-\delta e}$. The use of these solvents provided an opportunity to obtain CTC pairs whose molecules under conditions of long-term exposure to light (i.e., with a low quantum yield) were able to be converted to ionic forms [18, 21]. For example, it was emphasized in monograph [22] that flat cyclic molecules in CTC state were arranged with their planes parallel to each other and with center-to-center distance 3.3-3.4 Å determined by effective radii of π-electron envelopes. A similar description of this concept was given in [18].

None of photochemical systems studied so far was designed to utilize a considerable fraction of the electronic energy of the CTC-state, which otherwise would be mainly converted into the energy of polarization of solvent molecules in the vicinity of the CTC-pair and dissipated to heat. This problem is solved very effectively in natural photosynthesis.

The goal of this work was to reveal important specific features of microstructure of the special RC pair in photosynthetic organisms that allowed for the development of effective systems of light energy conversion in photosynthesis.

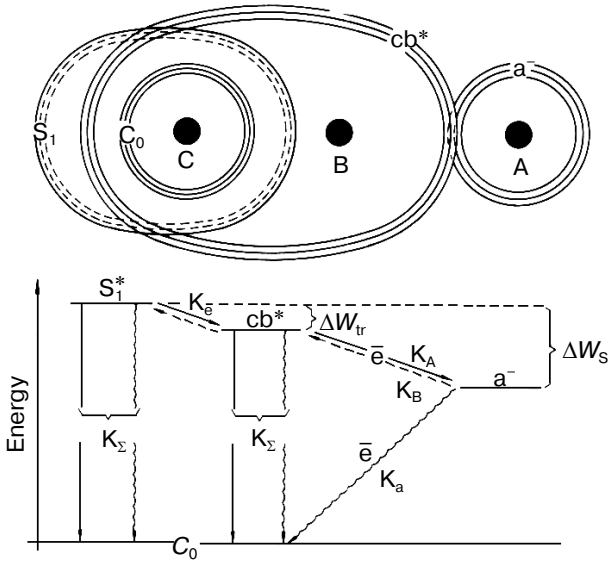

Fig. 1. Model of separation of opposite charges in photosynthetic reaction centers.

SPECIFIC STRUCTURAL FEATURES PROVIDING THE UNIQUE PROPERTIES OF REACTION CENTERS

According to theoretical views and experimental data considered below, these specific structural features are following.

1. Significantly larger distance between centers of the tetrapyrrole rings in special RC pair P875, P960, and P680 compared to numerous CTCs in solution. *Purple bacteria.* Based on the results of the X-ray diffraction studies of the RC structure in the purple bacteria *Rhodopseudomonas viridis* [7] and *Rhodobacter sphaeroides* [8] available from BROOKHAVEN PROTEIN BANK [23], it is possible to estimate the distance between magnesium atoms of BChl molecules in special RC pairs P875 and P960, which can be assumed to coincide with electron density centers $+\delta e$ and $-\delta e$ and to determine the length L_{mg} of the dipole in the charge-transfer complex of the reaction center:

Rhodopseudomonas viridis: $L_{mg} = 7.58 \pm 0.2$ Å,

Rhodobacter sphaeroides: $L_{mg} = 7.62 \pm 0.2$ Å.

Photosystem 2 of cyanobacteria. The X-ray diffraction studies of RC structure revealed that in the cyanobacteria *Thermosynechococcus vulcanus* [9] and *Synechococcus elongates* [10] the distance between the centers of special RC pairs P680 L_{mg} was 8.3 Å.

Thus we come to important conclusion: these distances are 4-5 Å larger than in classical photochemical CTC-pairs dimers in solutions.

2. Remote mutual location of tetrapyrrole rings in chlorophyll molecules of special RC pairs (remote "hand-shaking") providing maximum possible energy of their π-electron exchange interaction. According to the theory put forward in [18, 19, 24], a 1 Å increase in the intermolecular distance brings about an almost one order of magnitude decrease in the energy of electron exchange interaction. According to the theory, a 4 Å increase in the distance in RC pairs P875 and P960 relative to that value in photochemical CTC-pairs should cause a 3.5 order of magnitude decrease in this energy to ~ 1 meV. However, because the pyrrole rings are oriented toward each other, this orientation provides local overlapping of π-electron orbitals of molecules $BChl_A$ and $BChl_B$, which is necessary to produce strong CTC. The value of the energy *in vivo* can be calculated using well-known equation for splitting of 0–0 transition in the absorption band of a pair of interacting homogenous molecules [25]:

$$h\nu_1 \rightarrow S_1^* \rightarrow S_1^* \pm h\Delta\nu = S_1^* \pm W_{int}, \qquad (1)$$

where $h\nu_1$ is the photon energy equal to the electron energy in the singlet state (S_1^*) of a monomer molecule, ν_1 is the optical frequency corresponding to the 0–0 transition of light absorption by monomer molecule $S_0 \rightarrow S_1^*$, $\Delta\nu$ is the splitting value of the band S_1^* of a pair of molecules expressed in frequency units, W_{int} is the electromagnetic energy of interaction in the pair of molecules.

In the P870 pair, this splitting gives rise to two bands: a dominant band at 870 nm and a minor band at 810 nm [8]. According to Eq. (1), this corresponds to a fairly large value W_{int} = 105 meV, which is only a few times less than that value in solutions of dimers of dye molecules with adjacent planes of π-electron systems (!). Approximately the same values are obtained in case of the special RC pair P960 from *Rhodopseudomonas viridis*. Therefore, it is safe to suggest that although the CTC base length provided by the transmembrane polypeptide L- and M-subunits is much longer than the base length in photochemical CTC, special RC pairs indeed produce sufficiently strong CTC.

3. Presence of mobile water molecule(s) in the immediate proximity of the special RC pair. Let us consider the contribution of the structural factors 1-3 listed above to the solution of basic problem of photosynthesis. In other words, let us reveal how the properties of CTC-states listed above enable reaction centers to provide effective trapping of electronic excitation from light-harvesting ensembles of chlorophylls and further transfer of an electron from the CTC-pair to the electron-transport chain with high quantum yield and with simultaneous conservation of a large fraction of the initial energy of the singlet electronic excitation.

Trapping of electronic excitation in reaction centers. It was shown in [26-28] that the conventional model of organization of primary processes of electronic excitation energy conversion in RC of purple bacteria was in conflict with kinetic data on the electronic excitation lifetime in core-BChl. Based on this controversy, it was suggested and substantiated that the conventional model should be upgraded to the water-polarization model of trapping of electronic excitation in the reaction center. According to this model, within fractions of one picosecond after photoexcitation of special RC pair it attains a specific CTC state loosing a portion of electronic excitation energy of 30-50 meV. The resulting energy sublevel decreases the rate of backward migration of electronic excitation in core-BChl by a factor of several times. The energy portion of 30-50 meV is lost because of reorientation of uncompensated charges of mobile hydrogen atoms of water molecule(s) bound to the special RC pair or located in its vicinity [28, 29]. It is obvious that these water molecules will be exposed to the RC CTC field. The volume occupied by the field is proportional to the third power of the length of the CTC dipole $(L_{mg})^3$. This value in the case of photochemical CTC is close to $(3.5\ Å)^3$, i.e., the field is concentrated virtually completely within the special pair molecule itself. This gives rise to a low level of the quantum yield. In the case of photosynthetic RC, in which L_{mg} is equal to 7.5-8.3 Å, these fields are extended to a significant part of the surrounding medium around the CTC-pair and exert a substantial effect on the mobile charges in the CTC interior.

Energy factor. It has been shown in many contemporary monographs devoted to this problem that the energy of the CTC state is generally larger than the energy of the initial singlet state (S_1^*) of a molecule of the pair (Fig. 2). This is due to energy expenditure (W_{qul}) required to overcome electrostatic attraction of charges $D^{+\delta e}$ and $A^{-\delta e}$ during CTC formation. On the other hand, there is a W_{re} decrease in the energy of the CTC-state resulting from rearrangement of the π-electron system of the CTC-pair and dielectric reaction of the medium, i.e., interaction of the constant electric dipole generated in the CTC-state with nearby mobile charged atoms (W_{di}). However, these components alone are usually insufficient to provide complete compensation of W_{qul}.

In contrast to photochemical systems, the following two conditions should be observed to achieve the quantum yield value close to 100% in CTC of photosynthetic reaction centers. First, the time of electronic energy conversion into dielectric polarization of the medium (W_{de}) should be substantially shorter than mean time of electronic excitation energy migration from special RC pair back to antenna BChl.

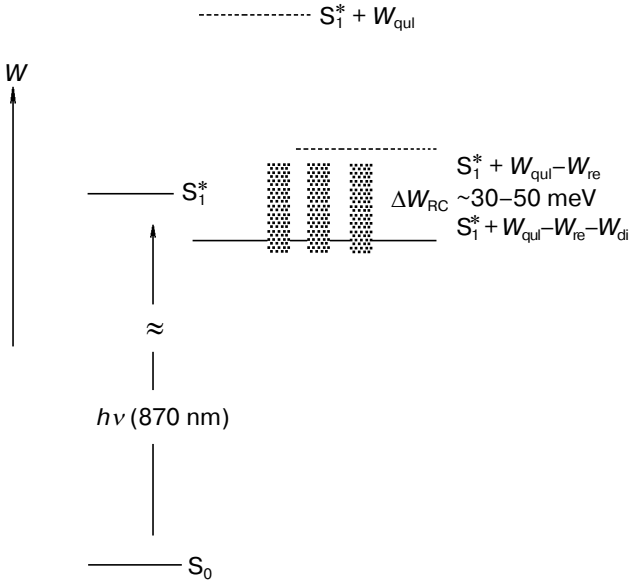

Fig. 2. Scheme of formation of charge-transfer complex during photoexcitation of the reaction center (designations are in the text).

Second, the difference $W_{qul} - W_{re} - W_{di}$ should be negative and small:

$$W_{qul} - W_{re} - W_{di} = -(30\text{-}50)\ \text{meV}, \qquad (2)$$

because larger value of this energy difference inevitably leads to slowing down its converting into vibrations, thereby causing a decrease in the rate of charge separation. As a result, the first condition noted above is not observed within the time range $0.3\cdot10^{-13}$-$0.3\cdot10^{-11}$ sec. The observation of the conditions noted above makes the process of trapping of electronic excitation from core-BChl in special RC pair substantially irreversible [26].

Charge separation in the CTC-state of the special RC pair. To observe the condition for stimulation of the CTC-state: $W_{qul} - W_{re} - W_{di}$ = 30-60 meV, it is necessary to provide a certain value of energy W_{di} spent for local dielectric polarization. However, in contrast to typical photochemical systems, the interior of the RC is very hydrophobic. It contains a few atomic groups with uncompensated charges. Of these groups, only the lightest hydrogen atoms charged to ~0.27*e* in loosely bound water molecules (and, perhaps, in peptide groups O=C–O–H and H–N–H) are able to undergo subpicosecond reorientation [26-29]. Therefore, the structure of the special RC pair should meet specific requirements.

To extend the electric field of the RC-pair CTC to the required number of nearby charged groups (condition 2), the field should be effective within the vicinity of a certain radius. In other words, the length of the RC pair dipole should be of a certain value. Evolution of photosynthesis was perhaps accompanied by a significant increase in the RC dipole length. As a result of significant increase in the length of the CTC base, an electron fraction –δe is displaced from the chromophore $P_A^{+\delta e}$ at a distance 7.5-8.3 Å, which significantly stimulates further electron transfer to the primary acceptor P800, whose π-electron system in the RC species described thus far is remote from $P_B^{-\delta e}$ at a distance comparable with the length L_{mg}. Therefore, it is safe to suggest that the radius of action of the RC dipole (i.e., its L_{mg}) in photosynthetic organisms should be determined by the position of mobile hydrogen atoms in the immediate proximity of the special RC pair. The energy of their reorientation in this case must satisfy the following equation: $W_{qul} - W_{re} - W_{di} = -(30\text{-}50)$ meV. The water-polarization model of trapping and conversion of electronic excitation in energy in reaction centers of purple bacteria was suggested by Fok and Borisov based on CTC-states [29, 30]. According to this theory, the minimum distance between centers of bacteriochlorophyll chromophores of special RC pair, at which the energy of reorientation of hydrogen atoms in the closest water molecule satisfied the condition noted above, was estimated as 5-6 Å.

DISCUSSION

The idea of this work is that the mutual arrangement of L- and M-subunits of reaction centers in the membrane, at least in photosynthetic bacteria, provided specific interaction between special pair bacteriochlorophylls, thereby enabling them to implement unique functions of photoelectric microconverters of electronic excitation *in vivo*. In this context, the most important and decisive factors are:

a) distance between centers of special RC pair chromophores (L_{mg} ~ 7-8 Å) is about 2-2.5 times larger than in CTC-pairs in solutions;

b) pyrrole rings extended toward each other provide the maximal (for such long distances) efficiency of van-der-Waals interaction between the special pair molecules;

c) one or two water molecules with mobile hydrogen atoms are exposed to the zone of action of the electric field of the CTC-state of the special RC pair.

Strictly speaking, the special RC pairs are not dimers because the mutual arrangement of their molecules is determined by the L- and M-polypeptide subunits of reaction centers and differs from that in chlorophyll dimers in solutions. Molecules of such dyes as proflavins, porphyrins, acridines, oxazines, phthalocyanins, and other dyes with planar π-electron systems tend to produce dimers in solutions, the process of dimerization being based on the principle of minimization of potential energy of bonds. This principle corresponds to the maximum efficiency of van-der-Waals contacts in the case of planar alignment of π-electron cycles, which brings the energy

of interaction between dimer molecules to 0.3-0.8 eV [19, 21]. However, the dipole length of CTC-pairs in solutions in such sandwich conformation is ~3.5 Å, and their electric field is mainly localized within the CTC-molecules themselves, whereas in the surrounding medium this field is very weak. As a result, the solvation coat of a CTC-pair rarely undergoes significant rearrangement and charges $-\delta e$ and $+\delta e$ usually "annihilate" with energy loss. This causes low quantum yield of separation of CTC-pair into ions and the requirement of long-term exposure of such systems to light.

In this sense, special RC pairs are not dimers because L- and M-RC polypeptides stretched them into unusual mutual conformation sharply different from conformations of Chl and BChl dimers in solutions. Certainly, the distance from the P870 pair to the monomer P800 and to the next electron carrier, bacteriopheophytin, and mutual orientation of their π-electron systems also contribute significantly to high total quantum yield of transmembrane charge separation in RC. During transition of an electron from P870 to P800 and then to bacteriopheophytin, the dipole length progressively increases, and the volume of the space embraced by its field gradually increases, thereby giving rise to further dielectric stabilization [6].

The idea discussed above provides a new insight into the unusual optical properties of carotenoids, particularly into the extraordinarily short lifetimes of their singlet states (the order of 10^{-12}-10^{-13} sec). Because the π-electron systems of carotenoids are elongated, the corresponding dipole transition moments of their $S_0 \rightarrow S_1^*$ transitions have unusually long length (up to 20 Å!) and, therefore, their fields embrace a significant volume of surrounding medium. Upon excitation of the molecule, fast polarization of mobile charged groups within this volume within the time interval 10^{-12}-10^{-13} sec should cause a significant decrease in the energy level of state S_1^* and corresponding changes in molecular structure. Based on this idea, it is safe to make the following prediction: the lifetime of states S_1^* in carotenoids should significantly increase in solutions with minimal polarizability within the time range 10^{-13}-10^{-11} sec, i.e., in solvents containing a minimum number of light mobile atoms and their groups bearing uncompensated charge (not to be confused with tabular coefficients of dielectric polarization).

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