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Review Letter

Energy transduction by the photosynthetic reaction center complex from *Rhodopseudomonas viridis*

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Comparison of data of (i) the high-resolution X-ray analysis of the *Rhodopseudomonas viridis* reaction center complex and (ii) direct (with a voltmeter) measurement of charge displacement in the same complex is presented. It is revealed that 95% of the energy stored in the form of the membrane electric potential is due to electron and 5% to proton transfers. Five stages of the overall process were found to contribute to the light-induced membrane charging, namely electron transfers from excited bacteriochlorophyll dimer to bacteriopheophytin, from bacteriopheophytin to MQ, from heme *c*-559 to bacteriochlorophyll dimer, from heme *c*-556 to heme *c*-559 and H^+ transfer from cytoplasmic water to the bound CoQ. The contribution of each stage to the energy storage depends upon the dielectric constant value in the respective part of the complex rather than upon the distance covered by the electron or proton. It appears to be higher, the deeper the redox groups involved are immersed into the membrane.

Reaction center complex; Membrane potential; Fast kinetics; *(Rps. viridis)*

1. STRUCTURE OF THE REACTION CENTER COMPLEX

In 1984 Michel and his colleagues [1] succeeded in crystallizing the *Rhodopseudomonas viridis* reaction center complex and published its X-ray structure at 0.3 nm resolution (see also [2,3]). Later, the same group obtained complete amino acid sequences of all subunits of the complex [3–5]. Thus, for the first time, atomic resolution of the structure of a membranous energy transducer was presented.

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The complex proved to be composed of four polypeptides, namely of the 40.5 kDa tetraheme *c*-type cytochrome (336 amino acid residues), the 35.9 kDa M-subunit (323 residues), the 30.6 kDa L-subunit (273 residues) and the 28.3 kDa H-subunit (258 residues). The 13 nm long complex is arranged in a transmembrane fashion, being composed of two hydrophilic domains (tetraheme cytochrome of *c* type and H-subunit) and the hydrophobic central part (mainly M- and L-subunits). It is the M- and L-subunits that bear the bacteriochlorophyll dimer $(BChl)_2$, two bacteriochlorophyll monomers (BChl), two bacteriopheophytins (BPheo), menaquinone (MQ), CoQ and a non-heme iron. Comparing the X-ray data with previous observations, one may assume that cytochrome *c* faces the periplasm, the H-subunit – cytoplasm, while the M- and L-subunits are

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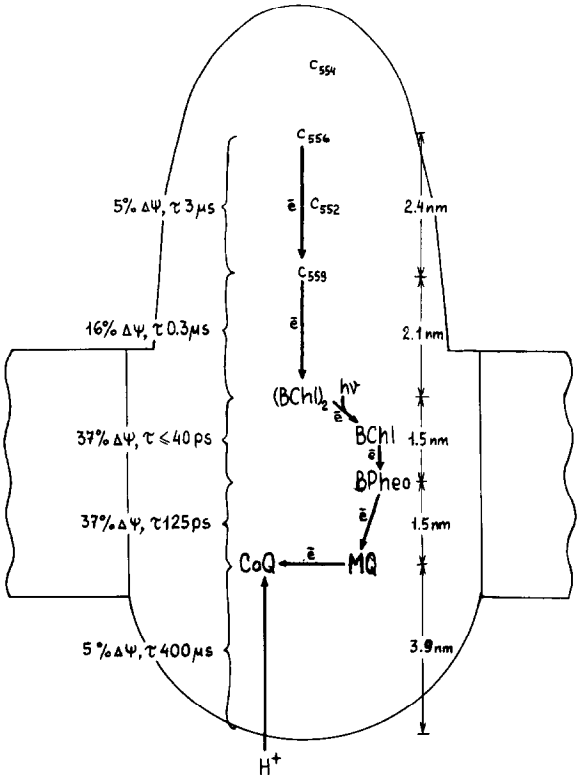


Fig.1. Structure-function relationships in *Rps. viridis* reaction center complex. Contributions of five electrogenic steps (%) to the overall $\Delta\psi$ formation and corresponding rates (τ) are from Dracheva et al. [13] and Deprez et al. [15]. The distances along the axis normal to the membrane plane have been calculated from the X-ray data of Deisenhofer et al. [1].

plugged through the hydrophobic core of the cytoplasmic membrane.

Eleven 4 nm long hydrophobic α-helices were revealed, five belonging to the M-, five to the L- and one to the H-subunit. All the α-helices are oriented roughly parallel to the long axis of the complex, being localized in its membrane part. The α-helices of the M- and L-subunits form two groups of five, which are related to the local symmetry axis.

The single hydrophobic α-helix of the H-subunit serves as an anchor binding this hydrophilic polypeptide to the membrane. Another peripheral subunit, cytochrome *c*, is attached to the membrane by contacts with other subunits and by two fatty acyls covalently bound to a glycerol residue forming a thioester bond with the SH group of the N-terminal cysteine [6].

Four cytochrome *c* hemes are located above the bacteriochlorophyll dimer. The Fe atom of the lowest heme is situated 2.1 nm above two Mg atoms of $(BChl)_2$. Tyrosine residue 162 of the L-subunit (Tyr L162) is localized between this heme and $(BChl)_2$. The porphyrin groups of $(BChl)_2$ are oriented parallel to the long axis of the complex.

According to optical studies, $(BChl)_2$ is arranged perpendicularly to the plane of the *Rps. viridis* membrane [7,8]. This means that the long axis of the complex is oriented across the membrane. Two BChl monomers are arranged slightly below $(BChl)_2$ (the Mg–Mg distance is 1.3 nm and the angles between porphyrins ~70°). Below BChl monomers, there are BPheos. The corresponding distances and angles are 1.1 nm and 64°, respectively. 1.8 nm below BPheo bound to the L-subunit, MQ is localized which lies above Trp M252. The symmetric position below another BPheo seems to be occupied by CoQ lying above Phe L216. Lower, Glu H177 is localized which is presumably involved in H^+ transfer from the cytoplasm to CoQ (see below).

Halfway from MQ to CoQ, there is a non-heme iron. Four iron ligands are formed by imidazoles of the histidines of the L- and M-subunits [1–3].

In fact, the data of the X-ray analysis confirmed the chromophore arrangement scheme inferred by Shuvalov and Asadov [9] in 1979 from measurements of the linear and circular dichroism of *Rps. viridis* reaction centers and from some related spectral observations. They are also in agreement with many more recent results obtained via indirect methods.

2. ELECTRON TRANSFER EVENTS

Light excitation of $(BChl)_2$ results in its oxidation by BPheo bound to the L-subunit (L-BPheo), the process being in some way mediated by L-BChl. It is completed within 20 ps. M-BChl and M-BPheo do not participate in the light-induced electron transfer. Their role still awaits an explanation.

MQ reduction by $BPheo^{\cdot -}$ represents the next step of the process. This takes about 230 ps. Another product of the primary charge separation

process, i.e. $(BChl)_2^{+}$ is reduced by tetraheme cytochrome c (τ = 320 ns). The latter is, in turn, reduced by the water-soluble cytochrome c_2 [10,11].

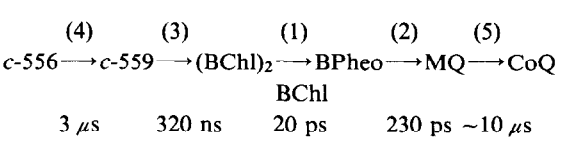
As shown in our group by Dracheva et al. [12,13], the heme of the tetraheme cytochrome c, serving as the $(BChl)_2^{+}$ reductant, is characterized by an α-band at 559 nm and a midpoint redox potential (E_m) of 380 mV. One may assume that this heme (c-559) is localized just above $(BChl)_2$. Heme c-559, in turn, is reduced by another heme c-556 of E_m = 310 mV (τ = 3 μs). It is c-556 that seems to be reduced by cytochrome c_2. The role of the two other hemes c, c-552 and c-554 (E_m 20 and −60 mV, respectively) remains obscure. They cannot be maintained in the reduced form when cytochrome c_2, the electron donor for tetraheme cytochrome c in the cyclic electron transfer system, serves as the reductant, since the redox potential of cytochrome c_2 is much higher than those of c-552 and c-554. On the other hand, some other natural reductants such as sulfur may be considered as possible electron donors for c-552 and c-554.

A study of the possible functions of c-552, performed in our group [13], showed that reduction of c-552 significantly accelerates the c-559 $\longrightarrow$ $(BChl)_2$ electron transfer.

According to Weyer et al. [15], two histidine imidazoles serve as the fifth and sixth ligands of the heme which is the second one assuming that the first is the heme closest to $(BChl)_2$. In the other three hemes, methionine is the sixth ligand. In other known cytochromes, two histidine ligands are always inherent in low-potential hemes. This is why the second heme seems to be c-552 or c-554. Bearing in mind the above-mentioned effect of c-552 reduction upon electron transfer via c-559, we suggested that the second heme is identical to c-552.

The slowest step in the electron transfer reaction sequence is MQ $\longrightarrow$ CoQ oxidoreduction. Its duration varies, depending on the pH, from several microseconds to tens of microseconds [14].

Thus, the above information can be summarized as follows:



3. ENERGY TRANSDUCTION STEPS

As already mentioned, tetraheme cytochrome c is localized on the periplasmic side of the *Rps. viridis* membrane while bound CoQ is rather close to its cytoplasmic side. Therefore, an electron uniport from cytochrome c to CoQ, directed across the membrane, should be electrogenic.

Unfortunately, generation of membrane electric potential ($\Delta\psi$) by the *Rps. viridis* reaction center complexes has not been studied until the present time. In fact, this bacterium does not exhibit any carotenoid electrochromic band-shift which has been most widely used to monitor fast electrogenic events in membranes of photosynthetic bacteria. It is only recently that fast monitoring of the light-induced charge displacements in the *Rps. viridis* cells was performed by Trissl and co-workers [15] using electric measurement of the light-gradient type [16]. Electrogenic effects measured in this way are lowered in magnitude by a factor of at least 100 but the time resolution appears to be as good as 40 ps. Two electrogenic phases ($\tau_1 \leqslant$ 40 ps, τ_2 = 125 ps) of almost equal contributions were observed. Such values correspond to the $(BChl)_2 \longrightarrow$ BPheo and BPheo $\longrightarrow$ MQ electron transfers, respectively. The data are in good agreement with the position of BPheo halfway from $(BChl)_2$ to MQ [1].

Slower electrogenic phases were discovered when we studied, in cooperation with Dr V.A. Shuvalov's laboratory, *Rps. viridis* reaction center proteoliposomes adsorbed onto a phospholipid-impregnated collodion film [13]. $\Delta\psi$ was measured with two electrodes separated by the film as described previously [17,18]. In this system, a single very short laser flash (τ = 15 ns) was found to generate a $\Delta\psi$ of about 100 mV. The τ value of the main electrogenic phase proved to be smaller than the time resolution of our electrometer (100 ns). In the system studied, the electron transfer from $(BChl)_2$ to MQ is the only one which is faster than 100 ns.

An additional electrogenic effect of much slower rate (τ = 400 μs) was revealed when the second flash was added 0.5 s after the first one. Its contribution to the overall electrogenesis was about 10%. It required the film-impregnating decane solution of phospholipids to be supplemented with CoQ_{10}. (As previously shown by our group

[19,20], decane extracts bound CoQ from the proteoliposomes attached to the film.) This phase of the photoelectric response proved to be sensitive to *o*-phenanthroline inhibiting electron transfer at the quinone level.

We concluded that the 400 μs phase can be accounted for by H^+ transfer from the cytoplasmic membrane surface to the site inside the reaction center complex at which CoQ is localised. As a matter of fact, protonation of CoQ requires its double reduction to CoQ^{2-}, since the pK of the single reduction product, $CoQ^{\cdot -}$, is at an acidic pH so that $CoQ^{\cdot -}$ cannot bind H^+ at pH 7. This is why only the second flash results in H^+ transfer and protonation of reduced CoQ. In agreement with such reasoning, CoQ^{2-} protonation in *Rps. viridis* takes 500 μs [14]. On the other hand, the MQ $\longrightarrow$ CoQ electron transfer proceeds 10–100-times faster. No electrogenic event of such a rate was found so that this process seems to be electrically silent [13]. This conclusion is consistent with X-ray data predicting MQ $\longrightarrow$ CoQ oxidoreduction to occur along, rather than across, the membrane [1].

To study the contribution of tetraheme cytochrome *c* to $\Delta\psi$ formation, we varied the redox potential of the medium and, hence, the degree of reduction of hemes *c*. To avoid the electrogenic effect of CoQ^{2-} protonation, we did not add CoQ_{10} to the collodion film-impregnating solution.

At 380 mV redox potential, *c*-559 heme was found to be half-reduced whereas three other hemes were completely oxidized. Under these conditions, an additional electrogenic phase was revealed (15% of the total photoelectric response if calculated for 100% *c*-559 reduction). Its value (300 ns) was shown to coincide with that of the *c*-559 $\longrightarrow$ $(BChl)_2$ electron transfer. The 300 ns phase disappeared at 440 mV when all four hemes were oxidized.

One more electrogenic phase (τ = 2.5 μs) was observed at 220 mV redox potential (*c*-559 and *c*-556 are reduced, *c*-552 and *c*-554 are oxidized). Its contribution was about 5%. The above-mentioned τ value was very similar to that of the *c*-556 $\longrightarrow$ *c*-559 electron transfer [13].

Thus, summarizing our data [13] and those obtained by Trissl and associates [15], we may conclude that there are five electrogenic steps in the overall process catalyzed by the *Rps. viridis* reaction center complex. Four are associated with the following electron transfer steps: (i) $(BChl)_2$ $\longrightarrow$ BPheo, (ii) BPheo $\longrightarrow$ MQ, (iii) *c*-559 $\longrightarrow$ $(BChl)_2$ and (iv) *c*-556 $\longrightarrow$ *c*-559. In response to the second flash, one more (v) electrogenic phase is revealed which is related to H^+ transfer from water to the bound $CoQ^{\cdot -}$. The photoelectric response to the second flash appears to be composed of phases (i)–(v) of the following contributions: 35, 35, 15, 5 and 10%. Since there is no phase (v) in response to the first flash, the average contribution of this phase per single turnover of the system should be reduced from 10 to 5%. The contributions of the other phases should be correspondingly increased. Thus, the improved contributions of the above phases are 37, 37, 16, 5 and 5%. The contribution of each phase may be a function of (i) the distance covered by an electron or proton and/or (ii) the dielectric constant value in the respective membrane region. It is obvious that the contribution must be higher the greater the distance and the lower the dielectric constant. In principle, a system may be organized in such a fashion that the dielectric properties vary only slightly and it is the distance that mainly determines the electrogenic efficiency.

According to the X-ray data [1], the distances along the axis normal to the membrane plane are: $(BChl)_2$–BPheo, 1.5 nm; BPheo–MQ, 1.5 nm; *c*-559–$(BChl)_2$, 2.1 nm; *c*-556–*c*-559, 2.4 nm; CoQ–inner surface of the complex, 3.9 nm. The same value expressed in percentages will be 13, 13, 18, 22 and 34. This differs strongly from the above values of the relative contributions of the respective phases to the generation of transmembrane $\Delta\psi$. Thus, one may conclude that the efficiency of electrogenic phases depends first of all upon a factor other than the distance between the redox groups involved. It seems reasonable to assume that the factor in question is the value of the dielectric constant. In fact, this value must increase as the protein complex protrudes from the membrane into water.

Moreover, according to the X-ray analysis [1–3], the middle (intramembrane) part of the reaction center complex is composed of hydrophobic amino acid residues. Charged amino acids were absent from this region, being localized in peripheral domains. This must also increase the dielectric constant when moving from the core of

the complex to its periphery. Therefore, it is not surprising that the main electrogenic phase responsible for almost 3/4 of the $\Delta\psi$ generation is due to electron transfer from $(BChl)_2$ to MQ although the distance covered by an electron seems to be as small as 1/4 of the overall path of electron and proton translocation inside the complex.

Comparison of the *c*-556 $\longrightarrow$ *c*-559 and *c*-559 $\longrightarrow$ $(BChl)_2$ steps provides another example of the same kind. The distances are 2.4 and 2.1 nm whereas the contributions of $\Delta\psi$ proved to be 5 and 16%, respectively, in agreement with the fact that the latter process occurs closer to the membrane core than the former.

In the 1960s, Mitchell [21] postulated in his chemiosmotic hypothesis that an electron moving from cytochrome *c* to CoQ via bacteriochlorophyll crosses the hydrophobic membrane barrier (the electron transfer half-loop), and this results in a transmembrane charge separation. This assumption, quite speculative at that time, has now been directly proved. The only amendment which appeared to be necessary to Mitchell's original scheme involves, besides the electrogenic steps associated with electron transfer from cytochrome *c* to CoQ, a small but measurable electrogenic step resulting from the proton movement in the opposite direction, namely from cytoplasmic water to the bound CoQ.

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