ISSN 0006-2979, Biochemistry (Moscow), 2021, Vol. 86, No. 1, pp. 1-7. © Pleiades Publishing, Ltd., 2021.
Russian Text © The Author(s), 2021, published in Biokhimiya, 2021, Vol. 86, No. 1, pp. 6-13.

MINI-REVIEW

The Mechanisms of Electrogenic Reactions in Bacterial Photosynthetic Reaction Centers: Studies in Collaboration with Alexander Konstantinov

Olga P. Kaminskaya[1] and Alexey Yu. Semenov[2,3,a]*

[1]*Institute of Basic Biological Problems, Pushchino Scientific Center for Biological Research, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia*
[2]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119992 Moscow, Russia*
[3]*Semenov Federal Research Center for Chemical Physics, Russian Academy of Sciences, 119991 Moscow, Russia*
[a]*e-mail: semenov@belozersky.msu.ru*

Received October 15, 2020
Revised November 11, 2020
Accepted November 21, 2020

Abstract—In this review, we discuss our studies conducted in 1985-1988 in collaboration with A. A. Konstantinov, one of the top scientists in the field of membrane bioenergetics. Studying fast kinetics of membrane potential generation in photosynthetic reaction centers (RCs) of purple bacteria in response to a laser flash has made it possible to examine in detail the mechanisms of electrogenic reactions at the donor and acceptor sides of RCs. Electrogenesis associated with the intraprotein electron transfer from the exogenous secondary donors, redox dyes, and soluble cytochrome (cyt) *c* to the photooxidized dimer of bacteriochlorophyll P_{870} was studied using proteoliposomes containing RCs from the non-sulfur purple bacterium *Rhodospirillum rubrum*. It was found that reduction of the secondary quinone electron acceptor Q_B accompanied by its protonation in chromatophores from *R. rubrum* in response to every second light flash was electrogenic. Spectral characteristics and redox potentials of the four hemes in the tightly bound cyt *c* in the RC of *Blastochloris viridis* and electrogenic reactions associated with the electron transfer within the RC complex were identified. For the first time, relative amplitudes of the membrane potential generated in the course of individual electrogenic reactions were compared with the distances between the redox cofactors determined based on the three-dimensional structure of the *Bl. viridis* RC.

DOI: 10.1134/S0006297921010016

Keywords: bacterial photosynthetic reaction centers, electron transfer, direct electrometric method, membrane potential, cytochrome *c*, quinone

BRIEF REVIEW OF THE PRECEDING STUDIES OF THE ELECTROGENIC REACTIONS IN THE REACTION CENTERS OF PHOTOSYNTHETIC BACTERIA USING DIRECT ELECTROMETRIC METHOD

In 1985-1988, the authors of this review had collaborated with Alexander Alexandrovich Konstantinov to investigate the nature and the mechanisms of electrogenic reactions in chromatophores and photosynthetic reaction centers (RCs) isolated from the non-sulfur purple bacteria *Rhodospirillum rubrum* and *Rhodobacter sphaeroides (*previously classified as *Rhodopseudomonas sphaeroides*) and the sulfur purple bacterium *Blastochloris viridis* (previously classified as *Rhodopseudomonas viridis*) [1-6]. In 1974-1976, L. A. Drachev, A. D. Kaulen, and one of the authors of this review (A. S.) had developed a technique for direct measurement of electric activity of membrane proteins [7, 8]. It was established using this technique that continuous illumination of bacterial chromatophores associated with the artificial planar phospholipid membrane in the presence of redox mediators led to the formation of the transmembrane electric potential difference ($\Delta\psi$) with positive charge inside the chromatophores [9, 10].

It was found that the photoinduced electron transfer in this system was limited to the reduction of the primary

Abbreviations: $\Delta\psi$, transmembrane difference in electric potentials; cyt, cytochrome; DAD, 2,3,5,6-tetramethyl-p-phenylenediamine (diaminodurene); E_m, midpoint redox potential; PMS, phenazine methosulfate; RC, reaction center; P_{870} (P_{960}), bacteriochlorophyll dimer in RC; τ, characteristic reaction time; TMPD, N,N,N′,N′-tetramethyl-p-phenylenediamine; Q_A and Q_B, primary and secondary quinone electron acceptors, respectively.

* To whom correspondence should be addressed.

quinone acceptor Q_A, presumably due to extraction of the secondary quinone Q_B and endogenous ubiquinone pool to the artificial membrane lipid phase [9-11]. Exogenous redox cofactors, electron donors and acceptors [ascorbate, N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD), diaminodurene (DAD), phenazine methosulfate (PMS), 2,6-dichlorophenol indophenol (DCPIP), methylene blue, and artificial quinones] maintained steady state photopotential, providing multiple re-reduction of the photooxidized primary RC donor (dimeric bacteriochlorophyll P_{870}) and re-oxidation of the photoreduced Q_A [10].

The use of collodion film as a support for the planar phospholipid membrane allowed to record the kinetics of the $\Delta\psi$ generation in response to a laser flash with a ~200-ns time resolution [12]. It was demonstrated in the studies conducted by Drachev and coauthors [11-15] that illumination of chromatophores from the purple bacteria with a single laser flash caused $\Delta\psi$ formation that was too fast to resolve it with the used technique. The observed fast reaction step was due to the electron transfer between P_{870} (or P_{890} in the case of chromatophores from the sulfur purple bacteria *Chromatium minutissimum* and *Ectothiorhodospira shaposhnikovii*) and the primary quinone acceptor Q_A. Furthermore, an additional step in the $\Delta\psi$ generation was observed in chromatophores from *C. minutissimum* and *E. shaposhnikovii*, whose RCs contain tightly bound four-heme cytochrome (cyt) *c* subunit. This step was associated with the reduction of the photooxidized P_{890} by cyt *c*. It was suggested that Q_A oxidation occurring either during electron transfer to an exogenous acceptor or during reduction of the secondary quinone acceptor Q_B could contribute to the $\Delta\psi$ generation.

The studies showed that $\Delta\psi$ generated in response to a single flash by chromatophores incorporated into the collodion phospholipid membrane dissipated with the heterogenous kinetics on a scale of tens of milliseconds to seconds [13, 14]. It was suggested that the fast component of the $\Delta\psi$ decay was due to the charge recombination between the photooxidized P^+_{870} and reduced Q^-_A. The slow component of the $\Delta\psi$ decay with the characteristic time $\tau \geq 0.5$ s reflected the process of passive discharge of $\Delta\psi$ through the artificial and the chromatophore membranes. It was found that the $\Delta\psi$ decay slowed down in the presence of reduced PMS.

ANALYSIS OF THE DECAY KINETICS OF THE PHOTOELECTRIC RESPONSE OF CHROMATOPHORES FROM *R. rubrum* AND PROTEOLIPOSOMES WITH *R. rubrum* RCs INCORPORATED INTO THE COLLODION PHOSPHOLIPID MEMBRANE

The studies that were carried out in 1985-1988 in collaboration with A. A. Konstantinov [1-6] were devoted to the detailed investigation of the kinetics of formation and decay of the membrane potential generated by the photosynthetic RCs from purple bacteria in response to a laser flash. S. M. Dracheva and M. D. Mamedov have provided a large contribution to the experimental part of these studies.

It was proven using the double flash technique that the fast component of the laser flash induced $\Delta\psi$ decay with $\tau \sim 70$ ms in *R. rubrum* chromatophores associated with the collodion membrane was due to the back electron transfer from Q^-_A to P^+_{870} [4]. It was demonstrated that the contribution of the slow component ($\tau \geq 0.5$ s) to the kinetics of $\Delta\psi$ decay increased in the presence of efficient electron donors and acceptors capable of reducing or oxidizing components of the ion-radical pair $P^+_{870}Q^-_A$ and providing formation of the long-lived states $P_{870}Q^-_A$ and $P_{870}{}^+Q_A$. It was demonstrated that the relative amplitude of the slow component of the dark $\Delta\psi$ decay could be used to estimate the rates of P^+_{870} reduction and Q^-_A oxidation by exogenous redox cofactors. The redox mediators TMPD, DAD, PMS, and DCPIP were good reductants for P^+_{870}, and 1,4-benzoquinone, soluble analogues of ubiquinone Q-1 and Q-2, as well as ubiquinone 10 (Q-10) added to the phospholipid solutions in *n*-decane used for the impregnation of the collodion membrane, were efficient acceptors of electrons from Q^-_A [4].

The fact that exogenous ubiquinone was capable of fast re-oxidation of Q^-_A, thus preventing recombination of the ion-radical pair, indicated restoration of the Q_B function in the system of incorporated chromatophores. The $Q^-_A \rightarrow Q_B$ reaction was restored in 75% of RCs under saturating concentrations of ubiquinone in the collodion membrane [4]. The deceleration of the $\Delta\psi$ decay caused by addition of Q-10 was completely canceled by o-phenanthroline, an inhibitor of the Q_B site. These experiments corroborated the original suggestion that inhibition of the electron transfer from Q_A to Q_B in the chromatophores incorporated into the artificial phospholipid membrane was due to the extraction of ubiquinone bound at the Q_B site into the hydrophobic bulk of the artificial membrane [9, 10].

The use of membrane-permeable redox mediators as electron donors was required in the experiments with *R. rubrum* chromatophores, because the donor side of the RC protein complex was located in the vicinity of the chromatophore membrane inner surface. We demonstrated that proteoliposomes containing isolated *R. rubrum* RCs could serve as a good model for investigating the redox reaction of P^+_{870} with non-permeable electron donors [3]. Illumination of such proteoliposomes with a laser flash resulted in the generation of the photopotential with the negative charge inside the vesicles indicated that P_{870} was located in the vicinity of the proteoliposome membrane outer surface, which is opposite to its location in *R. rubrum* chromatophores. Addition of non-permeable electron donors (hexammineruthenium and horse

heart cyt *c*) resulted in a significant retardation of the decay of $\Delta\psi$ generated in response to the laser flash. This fact implied fast reduction of the photooxidized P_{870} by exogenous redox cofactors and emergence of the long-lived state $P_{870}Q_A^-$.

ELECTROGENIC REACTIONS AT THE DONOR SIDE OF *R. rubrum* RCs

Investigating electrogenesis associated with the electron transfer between the RC primary donor P_{870} and soluble cyt *c* is challenging in the case of *R. rubrum* chromatophores, because these closed vesicles lose significant part of endogenous cyt c_2 during sonication and, moreover, the donor side of the RC is oriented inside the chromatophore, which makes it inaccessible to the exogenous cyt *c*. Hence, to investigate electrogenic reduction of P_{870}^+ by cytochromes *c* we used proteoliposomes containing isolated *R. rubrum* RCs.

Drachev et al. [3] measured the fast kinetics of the membrane potential generation in the course of electron transfer to P_{870} from the horse heart cyt *c* or from the isolated *R. rubrum* cyt c_2. In the presence of ≥0.1 μM reduced cyt *c*, a kinetic component with the submillisecond to millisecond characteristic time associated with the electron donation from cyt *c* (step C) was observed in the kinetics of $\Delta\psi$ increase in addition to the fast time-unresolvable component A ($\tau < 0.2$ μs) occurring due to the charge separation between P_{870} and Q_A. The relative contribution of this additional component of the $\Delta\psi$ increase to the total electrogenesis was found to be 22-24% in the presence of mitochondrial cyt *c* (at concentration > 5-10 μM) and ~16% in the presence of 7 μM cyt c_2 from *R. rubrum*. Kinetic analysis of the step C indicated saturated second-order reaction between cyt *c* and P_{870}^+ with the maximum value of the reaction rate constant $k_{\psi max} = 6 \cdot 10^3$ s^{-1} and Michaelis constant $K_M = 0.9$ μM at low ionic strength. The rate of the increase of the step C slowed down with the increase in the ionic strength, which is in agreement with the notion on the ionic interactions of the RC protein with cyt *c* during formation of the bimolecular complex.

Understanding the fact that the electrogenic nature of the P_{870}^+ reduction is not specific for the soluble type *c* cytochromes as electron donors but also takes place in the case of reduced forms of redox mediators (TMPD, DAD, and PMS) has become an important step in the investigation of electrogenesis in RCs of photosynthetic bacteria. In the presence of high concentration of PMS and TMPD (>20 μM and >0.5 mM, respectively), an additional component in the $\Delta\psi$ increase was observed on the scale of hundreds of microseconds to milliseconds, which provided 15-18% contribution to the total electrogenesis in *R. rubrum* chromatophores [1]. The characteristic time τ of this additional $\Delta\psi$ component was ~250 μs at the PMS concentration of 50 μM and ~2 ms at the TMPD concentration of 4 mM. The electrogenic nature of P_{870}^+ reduction by low-molecular-weight electron donors indicated electric insulation of the special pair inside the RC protein globule. Based on the fact that the amplitude of the additional phase of $\Delta\psi$ increase provided by the electron transfer from the reduced TMPD or PMS was only slightly less than the amplitude of the step C observed in the presence of cyt *c*, it was concluded that the main contribution to electrogenesis associated with the reduction of P_{870}^+ was due to vectorial electron transfer inside the protein globule of the RC.

GENERATION OF $\Delta\psi$ BY *R. rubrum* CHROMATOPHORES COUPLED TO ELECTRON TRANSFER AT THE ACCEPTOR SIDE OF RC

The possibility of reconstruction of Q_B function in chromatophores associated with the collodion membrane allowed to study electrogenesis in the process of Q_A oxidation by the secondary quinone Q_B [2]. It was demonstrated in our studies that the transfer of the first electron from Q_A to Q_B ($Q_A^-Q_B \rightarrow Q_AQ_B^-$) in *R. rubrum* chromatophores did not result in the $\Delta\psi$ increase. These data were in agreement with the earlier study of electrogenesis in *Rba. sphaeroides* RCs incorporated into the planar bilayer membrane [16] (later, a small pH-dependent electrogenic step was identified that occurred due to the protonation of an amino acid residue in the vicinity of Q_B during Q_B^- formation in response to a single flash [17]). At the same time, we found that reduction of the semiquinone form of Q_B to ubiquinol was associated with the appearance of an additional $\Delta\psi$ component that comprised ~10% of the total response amplitude (step B) [2]. These data were the first evidence of electrogenesis coupled with the protonation of the secondary quinone in bacterial RCs.

The electrogenic step B associated with the Q_BH_2 formation, had τ equal to 130 μs, 250 μs, and ~1 ms at pH 6.5, 7.5, and 9, respectively [2], which was close to the values measured for the electron transfer from Q_A to Q_B in chromatophores from *R. rubrum* and *Rba. sphaeroides* RCs [18, 19] and the rate of antimycin-insensitive binding of H^+ in *Rba. sphaeroides* chromatophores [20] (see also reviews [21, 22]). The fact that protonation of the Q_B during the ubiquinol formation in response to the flash is associated with the generation of $\Delta\psi$ indicates vectorial transfer of H^+ from the external aqueous phase to the electroinsulated quinone Q_B located deep inside the RC protein globule.

The data obtained by Drachev et al. [1-4] on the electrogenic reactions in *R. rubrum* RCs are summarized in Fig. 1 that shows three steps of electrogenesis on the chromatophore membrane coupled with the proton transfer in the RC complex. Step A is the primary charge

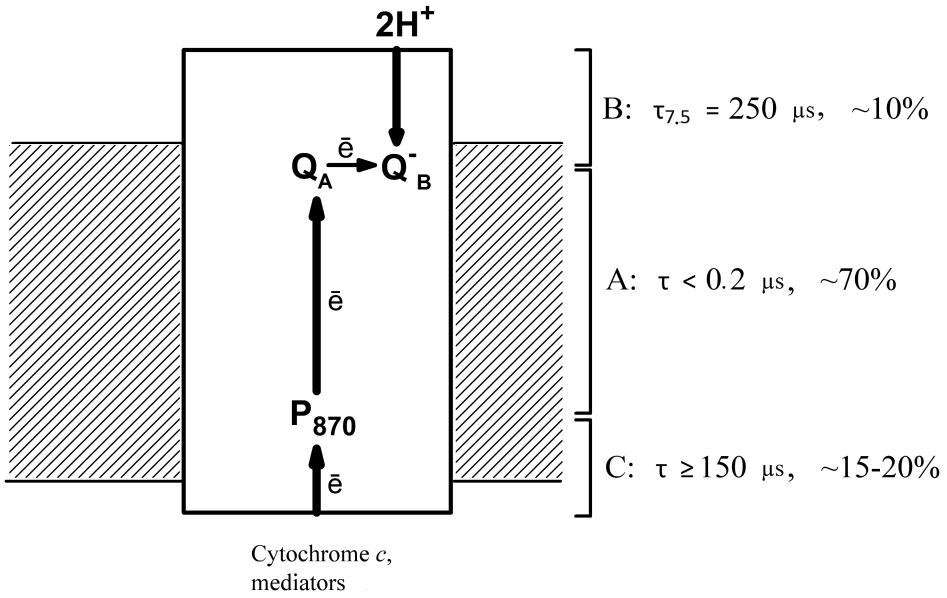

Fig. 1. Electrogenic reactions in *R. rubrum* RCs. Electrogenic reduction of the photooxidized P_{870} by exogenous secondary electron donors is observed in response to each laser flash; electrogenic protonation of the secondary quinone acceptor Q_B occurs in response to every second flash. Characteristic times τ (for the component B – at pH 7.5) and relative contributions of the three steps to Δψ generation are shown.

separation; step B is protonation of the reduced Q_B during ubiquinol formation; and step C is reduction of the bacteriochlorophyll dimer by soluble electron donors.

ELECTRON TRANSFER AND ELECTROGENIC REACTIONS IN *Bl. viridis* RCs

The studies by Dracheva et al. [5, 6] conducted in collaboration with A. A. Konstantinov were devoted to the electron transfer and electrogenic reactions in RCs isolated from the purple sulfur bacterium *Bl. viridis*. The interest in these particular RCs was due to two facts. Firstly, the three-dimensional crystal structure of this pigment-protein complex was elucidated with an atomic resolution [23, 24]. Secondly, in these RCs, the role of the secondary electron donor for the bacteriochlorophyll dimer plays the tightly-bound four-heme cyt *c* rather than to the water-soluble cyt c_2 (as in the RCs from the non-sulfur bacteria *R. rubrum* and *Rba. sphaeroides*).

Prior to these studies, it had been assumed that the four-heme cyt *c* in the *Bl. viridis* RCs contained two equal high-potential and two equal low-potential hemes with the midpoint redox potentials E_m of +340 and 0 mV and absorption maxima at 558 and 552-553 nm, respectively [25, 26]. We demonstrated that neither the high-potential nor the low-potential hemes are identical and that all four hemes exhibit different spectral and redox properties [5, 6], as they were found to have E_m of +380, +310, +20, and –60 mV and absorption maxima at 559, 556, 552, and 554 nm, respectively. It had been shown previously that histidine residues serve as the axial ligands to the heme located second in a row from P_{960} in the *Bl. viridis* RC, which is typical for the low-potential heme [24, 27]. The measurements of the electron transfer kinetics in the *Bl. viridis* RCs using the time-resolved absorption spectrometry demonstrated that under physiological conditions (when hemes c_{559} and c_{556} are reduced), heme c_{559} (τ = 0.3 μs) served as a direct electron donor for the photooxidized P_{960}, while heme c_{556} reduced heme c_{559} (τ = 2.5 μs) [5]. Hence, we suggested that the high-potential and the low-potential hemes were alternating in cyt *c*, and the high-potential heme c_{559} was the closest to P_{960}, while the second high-potential heme c_{556} was the third in row from P_{960}. In this case, the redox centers must be arranged in the following order: $c_{554} - c_{556} - c_{552} - c_{559} - P_{960}$ [6] (Fig. 2).

In order to investigate electrogenic reactions, proteoliposomes containing *Bl. viridis* RCs were incorporated into collodion phospholipid membrane. Analysis of photoelectric signals induced by laser flashes revealed that the kinetics of Δψ generation included three subsequent electrogenic reactions in response to each laser flash: A – time-unresolvable electron transfer from P_{960} to the primary quinone acceptor Q_A (τ < 0.2 μs); C1 – reduction of the photooxidized P_{960}^+ by the most high-potential heme c_{559}^{2+} (τ = 0.3 μs); C2 – re-reduction of the oxidized c_{559}^{3+} by the second high-potential heme c_{556}^{2+} (τ = 2.5 μs); and component B observed in response to every second flash and associated with the protonation of the doubly-reduced secondary quinone acceptor Q_B with the formation of Q_BH_2 (τ = 400 μs) [6]. Relative contributions of

the kinetic steps C2, C1, A, and B to the total electrogenesis were 5, 15, 70, and 10%, respectively (Fig. 2). These results demonstrated that the relative contribution of electrogenic steps increase in the central part of the protein hydrophobic core and decrease at the protein globule periphery.

The first data of the X-ray diffraction analysis of *Bl. viridis* RCs available at the time of these studies [23, 24] allowed to reveal correlation between the amplitudes of the steps of $\Delta\psi$ generation and the distances between the respective redox cofactors. It was found that the relative contribution of the charge transfer reactions to the total electrogenesis was defined not only by the distances between the redox cofactors, but also by the dielectric properties of the respective protein areas [6]. This observation is illustrated in Fig. 2 that shows projections of the distances between the cytochrome hemes c_{556} and c_{559}, c_{559} and P_{960}, P_{960}, and Q_A, as well as between Q_B and the closest protein/water boundary, on the perpendicular to the membrane plane as determined based on the later publication [28]. The indicated distances were 27, 22, 29, and 22% of the distance between the heme c_{556} and the closest protein/water boundary at the acceptor side of the RC protein globule. It can be seen, for example, that the vectorial electron transfer from P_{960} to Q_A inside protein hydrophobic core corresponding to the central part of the membrane, contributes significantly more to electrogenesis than the electron transfer at the same distance between the hemes c_{556} and c_{559} located in the portion of the protein globule protruding from the membrane into the aqueous phase.

It should be emphasized in conclusion that the studies conducted in 1985-1988 in collaboration with A. A. Konstantinov and described in this review have revealed important mechanisms of electrogenic reactions in photosynthetic RCs from the purple bacteria *R. rubrum*, *Rba. sphaeroides*, and *Bl. viridis*. Many features of electrogenesis and electron transfer reactions in bacterial RCs were described for the first time. The kinetics of electrogenic electron transfer between the soluble cyt *c* and the bacterial RC complex was investigated with a high time resolution. It was demonstrated that electrogenesis associated with reduction of photooxidized P_{870} by cyt *c* and artificial redox dyes occurred due to the electron transfer inside the RC protein complex. In addition, the electrogenic nature of the protonation of the double-reduced secondary quinone acceptor Q_B was established. Spectral and redox characteristics of the four hemes in the tightly-bound cyt *c* were determined in the studies of *Bl. viridis* RCs. The rates of P_{960} reduction and electron transfer between the hemes were measured, as well as the steps of electrogenesis associated with the electron transport reactions in this pigment-protein complex were

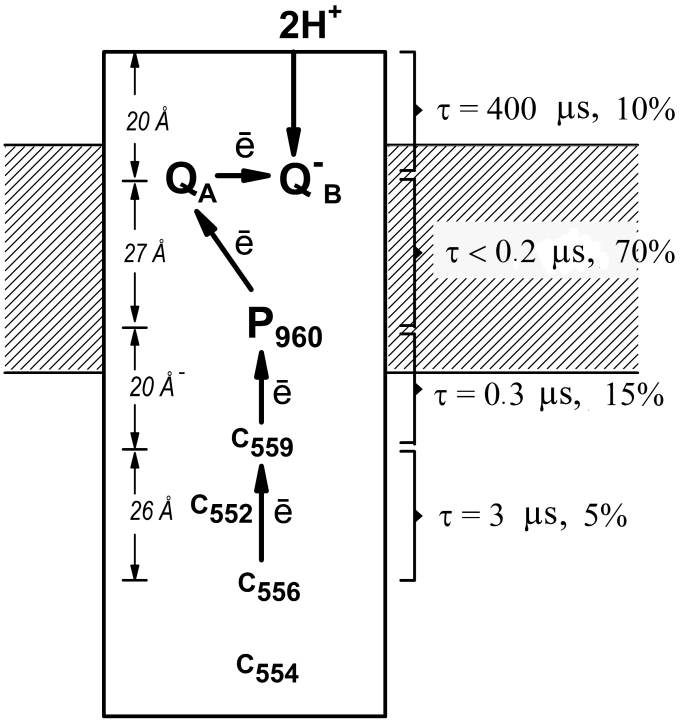

Fig. 2. Electrogenic reactions in *Bl. viridis* RCs. Relative contributions to the total electrogenesis (% $\Delta\psi$) and characteristic times (τ) of individual electron transfer reactions are shown on the right; left panel, projections of the distances between the redox cofactors on the perpendicular to the membrane plane.

recorded. Comparison of the relative contributions of the individual electrogenic steps with the projections of distances between the redox cofactors on the perpendicular to the membrane plane led to the conclusion that the dielectric permittivity inside the RC protein is heterogenous and varies across the protein globule.

Acknowledgments. Alexander Konstantinov had played an invaluable role in the studies described in this review. He was one of the top scientists, a real professional, who valued the beauty of scientific experiments and had brilliant intuition. His broad scientific interests, clear identification of scientific problems, and thorough experiment planning, including selection of optimal experimental conditions, were the hallmarks of his work style. All this has made the results of experiments clear and effective. It was very interesting to work with Alexander Konstantinov. With his help and often with his direct supervision, I. A. Smirnova, S. M. Dracheva, O. P. Kaminskaya, D. L. Zaslavsky, and S. A. Siletsky have received their PhD degrees that involved investigations of electrogenesis mediated by the mitochondrial membrane proteins and reaction centers in photosynthetic bacteria using direct electrometric technique. His colleagues will always cherish the memory of Alexander Konstantinov.

Ethics declarations. The authors declare no conflict of interest in financial or any other sphere. This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

1. Drachev, L. A., Kaminskaya, O. P., Konstantinov, A. A., Semenov, A. Yu., and Skulachev, V. P. (1985) Electrogenic reduction of *Rhodospirillum rubrum* reaction centre bacteriochlorophyll $P870^+$ by redox dyes. Indication of intraprotein electron transfer, *FEBS Lett.*, **189**, 45-49.
2. Kaminskaya, O. P., Drachev, L. A., Konstantinov, A. A., Semenov, A. Yu., and Skulachev, V. P. (1986) Electrogenic reduction of the secondary quinone acceptor in chromatophores of *Rhodospirillum rubrum*. Rapid kinetic measurements, *FEBS Lett.*, **2**, 224-228.
3. Drachev, L. A., Kaminskaya, O. P., Konstantinov, A. A., Kotova, E. A., Mamedov, M. D., et al. (1986) The effect of cytochrome *c*, hexaammineruthenium and ubiquinone-10 on the kinetics of photoelectric responses of *Rhodospirillum rubrum* reaction centers, *Biochim. Biophys. Acta*, **848**, 137-146.
4. Drachev, L. A., Kaminskaya, O. P., Konstantinov, A. A., Mamedov, M. D., Samuilov, V. D., et al. (1986) Effects of electron donors and acceptors on the kinetics of the photoelectric responses in *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides* chromatophores, *Biochim. Biophys. Acta*, **850**, 1-9.
5. Dracheva, S. M., Drachev, L. A., Zaberezhnaya, S. M., Konstantinov, A. A., Semenov, A. Yu., and Skulachev, V. P. (1986) Spectral, redox and kinetic characteristics of high-potential cytochrome c hemes in *Rhodopseudomonas viridis* reaction centers, *FEBS Lett.*, **205**, 41-46.
6. Dracheva, S. M., Drachev, L. A., Konstantinov, A. A., Semenov, A. Yu., Skulachev, V. P., et al. (1988) Electrogenic steps in the redox reactions catalysed by photosynthetic reaction centre complex from *Rhodopseudomonas viridis*, *Eur. J. Biochem.*, **171**, 253-264.
7. Drachev, L. A., Jasaitis, A. A., Kaulen, A. D., Kondrashin, A. A., Liberman, E. A., et al. (1974) Direct measurement of electric current generation by cytochrome oxidase, H^+-ATPase and bacteriorhodopsin, *Nature*, **249**, 321-324.
8. Drachev, L. A., Frolov, V. N., Kaulen, A. D., Liberman, E. A., Ostroumov, S. A., et al. (1976) Reconstitution of biological molecular generators of electric current. Bacteriorhodopsin, *J. Biol. Chem.*, **251**, 7059-7065.
9. Drachev, L. A., Frolov, V. N., Kaulen, A. D., Kondrashin, A. A., Samuilov, V. D., et al. (1976) Generation of electric current by chromatophores of *Rhodospirillum rubrum* and reconstitution of electrogenic function in subchromatophore pigment-protein complexes, *Biochim. Biophys. Acta*, **440**, 637-660.
10. Smirnova, I. A., Konstantinov, A. A., and Skulachev, V. P. (1981) Role of cofactors in the formation of the membrane potential by chromatophores of *Rhodospirillum rubrum*, incorporated into a Teflon filter, *Biochemistry (Moscow)*, **46**, 925-934.
11. Drachev, L. A., Dracheva, S. M., Samuilov, V. D., Semenov, A. Yu., and Skulachev, V. P. (1984) Photoelectric effects in bacterial chromatophores. Comparision of spectral and direct electrometric methods, *Biochim. Biophys. Acta*, **767**, 257-262.
12. Drachev, L. A., Semenov, A. Yu., and Skulachev, V. P. (1979) Generation of electric potential difference by chromatophores induced by laser flash, *Dokl. Akad. Nauk SSSR*, **245**, 991-994.
13. Drachev, L. A., Semenov, A. Yu, Skulachev, V. P., Smirnova, I. A., Chamorovsky, S. K., et al. (1981) Fast stages of photoelectric processes in biological membranes, *Eur. J. Biochem*, **117**, 483-489.
14. Semenov, A. Yu., Chamorovsky, S. K., Smirnova, I. A., Drachev, L. A., Kononenko, A. A., et al. (1981) Kinetics of formation of photo-induced electric potential difference by chromatophores from photosynthesizing bacteria, *Mol. Biol. (Mosk)*, **15**, 622-635.
15. Chamorovsky, S. K., Drachev, A. L., Karagulian, A. K., Kononenko, A. A., Rubin, A. B., et al. (1985) Fast phases of the generation of the transmembrane electric potential in chromatophores of the photosynthetic bacterium *Ectothiorhodospira shaposhnikovii*, *Biochim. Biophys. Acta*, **808**, 201-208.
16. Packham, N. K., Dutton, P. L., and Mueller, P. (1982) Photoelectric currents across planar bilayer membranes containing bacterial reaction centers. Response under conditions of single electron turnover, *Biophys. J.*, **37**, 465-473.
17. Drachev, L. A., Mamedov, M. D., Mulkidjanian, A. Ya., Semenov, A. Yu., Shinkarev, V. P., and Verkhovsky, M. I. (1990) Electrogenesis associated with proton transfer in the reaction center protein of the purple bacterium, *FEBS Lett.*, **259**, 324-326.
18. Wraight, C. A. (1979) Electron acceptors of bacterial photosynthetic reaction centers II. H^+ binding coupled to sec-

ondary electron transfer in the quinone acceptor complex, *Biochim. Biophys. Acta*, **548**, 309-327.
19. Vermeglio, A. (1982) Electron transfer between primary and secondary electron acceptors in chromatophores and reaction centers of photosynthetic bacteria, in: *Function of Quinones in Energy Conserving Systems* (Trumpower, B. L., ed.) Academic Press, New York, pp. l69-180.
20. Petty, K. M., and Dutton, P. L. (1976) Properties of the flash-induced proton binding encountered in membranes of *Rhodopseudomonas sphaeroides:* a functional p*K* on the ubisemiquinone? *Arch. Biochem. Biophys*., **172**, 335-345.
21. Sebban, P., Maroti, P., and Hanson, D. K. (1995) Electron and proton transfer to the quinones in bacterial photosynthetic reaction centers: insight from combined approaches of molecular genetics and biophysics, *Biochimie*, **77**, 677-694.
22. Okamura, M. Y., Paddock, M. L., Graige, M. S., and Feher, G. (2000) Proton and electron transfer in bacterial reaction centers, *Biochim. Biophys. Acta*, **1458**, 148-163.
23. Deisenhofer, J., Epp, O., Miki, K., Huber, R., and Michel, H. (1984) X-ray structure analysis of a membrane protein complex: electron density map at 3 Å resolution and a model of the chromophores of the photosynthetic reaction center from *Rhodopseudomonas viridis*, *J. Mol. Biol*., **180**, 385-398.
24. Deisenhofer, J., Epp, O., Miki, K., Huber, R., and Michel, H. (1985) Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3 Å resolution, *Nature*, **318**, 618-624.
25. Clayton, R. K., and Clayton, B. J. (1978) Molar extinction coefficients and other properties of an improved reaction center preparation from *Rhodopseudomonas viridis*, *Biochim. Biophys. Acta*, **501**, 478-487.
26. Case, G. D., Parson, W. W., and Thornber, J. P. (1970) Photooxidation of cytochromes in reaction center preparations from *Chromatium* and *Rhodopseudomonas viridis*, *Biochim. Biophys. Acta*, **223**, 122-128.
27. Weyer, K. A., Lottspeich, F., Gruenenberg, H., Lang, F., Oesterhelt, D., and Michel, H. (1987) Amino acid sequence of the cytochrome subunit of the photosynthetic reaction centre from the purple bacterium *Rhodopseudomonas viridis*, *EMBO J*., **6**, 2197-2202.
28. Deisenhofer, J., Epp, O., Sinning, I., and Michel, H. (1995) Crystallographic refinement at 2.3 Å resolution and refined model of the photosynthetic reaction centre from *Rhodopseudomonas viridis*, *J. Mol. Biol*., **246**, 429-457.