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Photoelectrical properties of green bacteria cells and cell fragments located in electrochemical cell

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Abstract

Photopotential action spectra as well kinetics of their generation and decay for green bacteria *Prosthecochloris aestuarii* cells and cell fragments were measured. The sample was located between two transparent electrodes, both semiconducting or one semiconducting and second one metallic. The electric properties of the cells in darkness and under illumination were also established. In the photopotential action spectrum the maximum about 750 nm, responsible for the bacteriochlorophyll *c* oligomers absorption, is absent. It shows that bacteriochlorophyll *c* molecules located in chlorosome oligomers are not taking part in the photopotential generation. From the action spectra it follows that predominantly desaggregated bacteriochlorophyll *c* and bacteriopheophytin, and may be also some chlorophyll *a*—like pigments are responsible for a photopotential generation. In the 670 nm region the desaggregated bacteriochlorophyll *c* as well the bacteriopheophytin absorption are located, whereas the Soret band is characteristic rather for the bacteriopheophytin, but some contribution from the desaggregated bacteriochlorophyll *c* cannot be excluded. With the sample pheophytinization the photopotential amplitudes increase. The complex kinetic of the photopotential generation suggests the superposition of at least two processes: the fast one, occurring just after light absorption, which probably is due to charge separation in the reaction centers and the second one—slower—may be related to some diffusion of the charge generated in the antenna pigments through the cell membrane. Due to slower photopotential decay and higher concentrations of the antenna pigments than that of the reaction centers, the contributions from the desaggregated antenna pigments, at used in the experiments frequencies of light modulation, predominant in the photopotential action spectra. For each sample the sign of the photopotential signal changes with the change of the side of illumination of the electrochemical cell. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Bacteriochlorophyll *c*; Bacteriopheophytin *c*; Chlorosome; Current–voltage characteristic; Electrochemical cell; Light gradient effect; Oligomer; Photopotential action spectrum

1. Introduction

The illumination of the pigmented membranes of photosynthetic organisms generates a difference in the electrical potential between the membrane surfaces [1–4]. As a result, some ions can be transferred through the membranes more easily. The effect is initiated by the light absorbed by the pigments embedded in the membranes. Similar effects have been observed in model systems containing parts of photosynthetic organisms [5–9]. The green sulphur bacterium *Prosthecochloris aestuarii* contains antenna bodies—chlorosomes, predominantly containing bacteriochlorophyll (BChl) *c* with a small amount of BChl *a* [10–13]. The chlorosomes differ from other giant antenna bodies because their internal structure is predominantly generated by the aggregation of the BChls [14] and there is no significant involvement of protein [15]. BChls *c* in chlorosomes form large aggregated structures (oligomers) which are responsible for the spectral properties of the chlorosome [16]. We wanted to check if such oligomeric forms of the pigments were able to participate in the photopotential generation. The main role of these oligomeric structures is to harvest the light energy and to transfer it to a reaction center (RC) where it is used for the charge separation. It was shown by Skulachev [7], that in the case of purple bacteria, the antenna pigments contribute to photogenerated potential but with much lower efficiency than RC pigments. The ratio of observed contributions from the antenna pigments to these given by RCs depends on the method of photopotential measurements, because of different kinetics of these two processes.

2. Materials and methods

P. aestuarii (*Chloropseudomonas ethylica*) 2K strain was grown anaerobically in a culture medium described by Holt

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et al. [17] at illumination 1400 lux in 28°C temperature. The bacteria were centrifuged directly from the culture medium. The bacteria fragments were obtained by sample sonication in 45 min at 4°C. The dense suspension of the bacteria cells or their fragments in 10 mM Tris–HCl buffer (pH 7.4) was located between electrodes and the photoelectrochemical cell was sealed. The photoelectrochemical cell used in our experiments consists of two transparent electrodes: both semiconducting (In_2O_3) or one semiconducting and the second metallic (gold). The teflon spacer had a thickness of 60 μm. The details of the construction of the electrochemical cell as well as methods of photopotential measurements were described previously [8]. Fig. 1 shows schemes of the arrangements used for the photopotential investigations. Two methods of photopotential measurements were applied: in the first one (Fig. 1A), the continuous illumination was switched on and off by a shutter [8,9];, in the second one (Fig. 1B), the acting light was modulated by a chopper [18]. The photopotential values were established for given wavelength by gathering counts during 3 s. Wavelength interval between adjacent points of measurements was 5 nm. The intensities of the light illuminating the sample for various wavelengths were measured in Watt/cm^2 by a photometer made by Ealing Electro-Optics. The photopotential values were recalculated on the number of light quanta reaching illuminated part of sample in the unit of time.

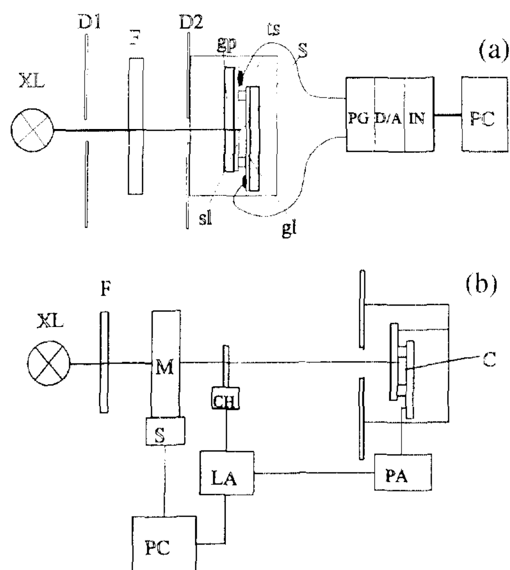

Fig. 1. (a) Scheme of apparatus for photocurrent measurements under continuous light illumination. (XL, xenon lamp; F, absorption filter; D1,D2, diafragrams; S, sample; gp, glass plate; sl, semiconductor layer; gl, gold layer; ts, teflon spacer; PG, programmable potentiostat-galvanostat; D/A, digital/analog converter; IN, interface; PC, computer). (b) Scheme of apparatus for photopotential action spectra measurements with modulated light. (XL, xenon lamp; F, absorption filter; M, monochromator; S, power supply; CH, chopper; LA, Lock-in amplifier; PA, preamplifier; PC, computer; C, electrochemical cell).

3. Results and discussion

Fig. 2 shows the absorption and photopotential action spectra of whole bacteria and bacteria fragments measured in various times after sample preparation. The absorption spectrum changes in time, especially for bacteria fragments (Fig. 2A). This change is similar to that observed by Steengaard et al. [19] during acid treatment of chlorosomes isolated from other green bacteria (*Chlorobium tepidum*). Whole bacteria cells are more stable in time, but when subjected to longer storage time or prolonged illumination, they undergo similar degradation. The maxima located at about 675 nm and in the Soret band at about 428 nm increase during sample denaturation. The decrease of the absorption of oligomeric form of BChl *c* is followed by the shift of red maximum from 747 nm to 757 nm. It agrees with the results obtained by Steensgaard et al. [19] that the different pigment aggregates present in chlorosomes are desaggregated with various kinetics. It

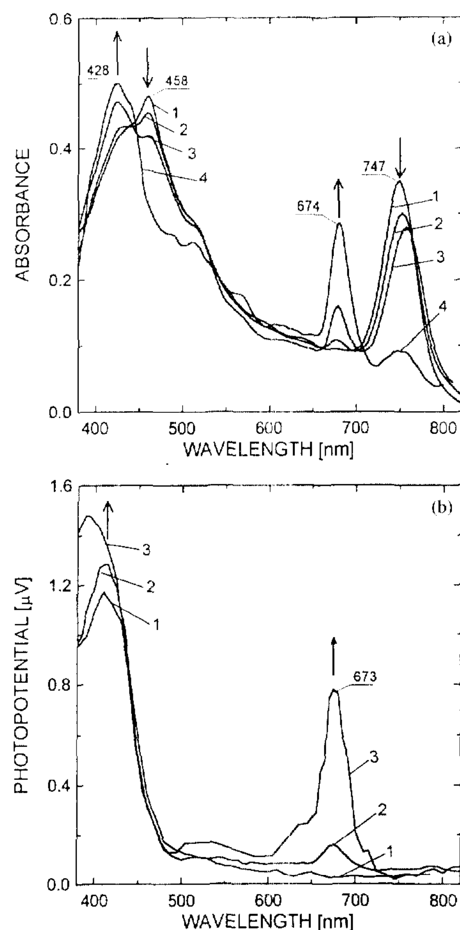

Fig. 2. (a) The absorption spectra of the samples: curve 1, native whole cells of bacteria; curve 2, bacteria fragments measured directly after sample preparation; curve 3, bacteria fragments measured 3–4 h after sample preparation; curve 4, bacteria fragments 24 h after sample preparation. (b) The photopotential action spectra of the sample: curve 1, native whole cells of bacteria; curve 2, bacteria fragments measured 3–4 h after sample preparation; curve 3, bacteria fragments 24 h after sample preparation. All the samples located in the electrochemical cell with two semiconducting (In_2O_3) electrodes.

cannot be excluded that some contributions to the absorption are due to desaggregated but still unpheophytinized BChl *c*. Monomeric BChl *c* located in chlorosome exhibits absorption at 675 nm and 450 nm. It is possible that aggregates have to be previously desaggregated and than the pigment is efficiently, already irreversibly pheophytinized. As what follows from our previous results [20], the desaggregated or denaturated pigments are still located in chlorosomes because they exhibit the orientation. It is in agreement with the reversible desaggregation and aggregation of BChl *c* in chlorosomes due to hexanol treatment observed by Matsuura and Olson [21].

The photopotential action spectra shown in Fig. 2B are recalculated on the same number of quanta (10^{13} quanta/cm^2 s) reaching the surface of the sample. The action spectra of the native and partially pheophytinized bacteria cells (Fig. 2B) located between two semiconducting electrodes are different than the absorption spectra of the same samples (Fig. 2A). In the native sample, the red absorption band located at about 750 nm in the photopotential action spectrum is not observed. The red band at about 675 nm is formed as a result of the pigment desaggregation and pheophytinization. It is known [19,22] that bacteriopheophytin *c* (BPhe *c*) located in chlorosome has a higher Soret:red band absorption ratio than the same pigment in a solution. Moreover, in the Soret band, the absorptions of all other (bacterio)chlorophyll-like pigments are superimposed. The Soret band maximum of the photopotential action spectrum is located at 410–415 nm, which strongly suggests BPhe participation.

Table 1 shows the values of the amplitudes of photopotentials established at modulated light and recalculated on the same number of quanta of the acting light, but measured for two different frequencies of the light modulation using the arrangement shown in Fig. 1A. The change in a frequency of the light modulation changes the ratio of the Soret to the red band photopotential amplitude in the action spectrum (Fig. 2B, Table 1). At 10 Hz it is equal to 15 whereas at 30 Hz, it is 10. It suggests the superposition of the processes occurring with various kinetics. It is very characteristic that in the photopotential action spectrum in the Soret band, the component in the region of 458 nm (which belongs to BChl *c* oligomers) is totally absent. The ratio Soret:red band photopotential amplitude also change with sample denaturation (Fig. 2B), which suggests that other pigments beside BPhe (exhibiting different kinetics) take part in the photopotential generation.

Table 1
The amplitudes of photopotential (A) in the region of the main absorption maxima: at 415 nm—Soret band (S) and at 674 nm—red band (R), recalculated on the same number of quanta (10^{15} cm^{-2} s^{-1})

		S (μV)	R (μV)	S/R
A	ν = 10 Hz	0.45	0.03	15
	ν = 30 Hz	0.20	0.02	10

ν, Frequency of the light modulation.

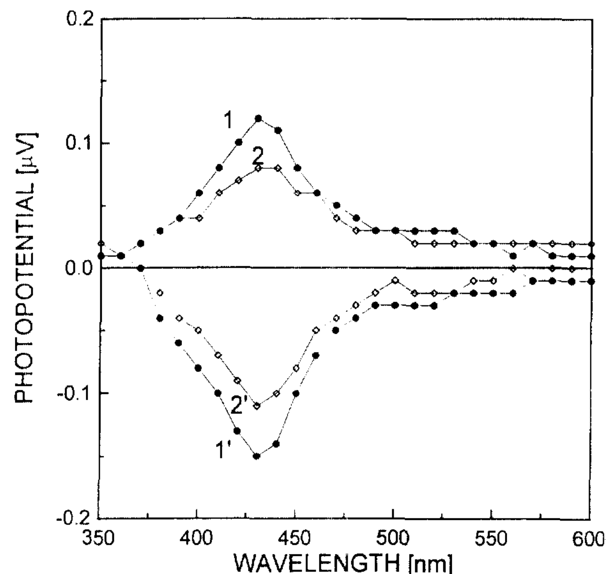

Fig. 3. The 'light gradient effect' of two independent samples (marked 1 and 2) with partially pheophytinized bacteria cells. 1,2: Front illumination; 1',2': back illumination. Frequency of the light modulation ν = 10 Hz. The samples in the electrochemical cell with two semiconducting (In_2O_3) electrodes.

The same conclusion follows from the shift of the Soret band as a result of this process. The maximum at 675 nm increases as a result of the illumination of the sample during the photopotential measurements. This maximum value could contain not only BPhe but also BChl *c* monomers and chlorophyll (Chl) *a* isomers [23]. It is certain that the photopotential generation is not due to the absorption of light by BChl *c* oligomers.

In the suspension of chloroplasts and thylakoids, the light gradient effect is observed because the front and back borders of the thylakoids are illuminated differently [1,24,25]. The similar effect for whole photoelectrochemical cell, for two independent samples, is shown in Fig. 3. The strongly-illuminated semiconducting electrode gives higher signal (electric potential). Hence, the change in the side of illumination, without changes of electric circuit, causes the change in the sign of the photopotential. The dissimilar amplitudes obtained at the illumination from two sides (Fig. 3) is due to the slightly different properties of the two semiconducting electrodes [26].

Fig. 4A shows the kinetics of photocurrent generation and decay taking for the cell with two different electrodes: one semiconducting and second one gold. Such a cell gives higher amplitude than the cell with two semiconducting electrodes. After the light is switched on in the native samples, a very short jump of the photocurrent occurs. Later, the slower decay of this signal is observed. The sign of the signal is positive. In partially pheophytinized sample, a quick jump of photocurrent is also exhibited but it is superimposed on the slower increasing signal. This slower signal later slowly increases with the saturation value. It is not possible to establish the kinetic of the short signal on the basis of the results obtained

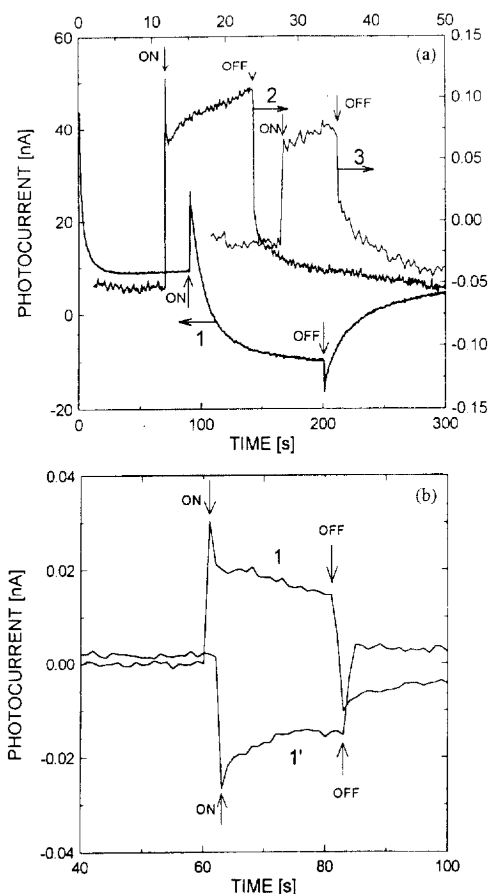

Fig. 4. (a) The kinetics of photocurrent generation and decay in the electrochemical cell: curve 1, native whole cells of bacteria; curve 2, bacteria fragments measured 3–4 h after sample preparation; curve 3, bacteria fragments 24 h after sample preparation. Power of acting light was 150 mW/cm^2. The samples located in the electrochemical cell with one semiconducting (In_2O_3) electrode and the second one-metallic (gold). (b) The 'light gradient effect' of photocurrent kinetics of the sample with bacteria fragments in the electrochemical cell with two semiconducting (In_2O_3) electrodes. 1: Front illumination; 1′: back illumination.

on the used apparatus. After the light is switched off, the quick non-uniexponential decay is observed. In strongly pheophytinized sample, the quick jump of the signal is almost visually undetectable. The complex kinetic of the photocurrent generation suggests the superposition of at least two processes: (i) the fast one, occurring just after light absorption, which probably is due to charge separation in the reaction centers, and (ii) the slower one, which may be related to some diffusion of the charge generated in the antenna pigments through the cell membrane.

Fig. 4B shows the influence of the 'light gradient effect' (i.e., the influence of the side of the sample illumination) on photocurrent kinetics of partially pheophytinized sample in the symmetrical cell with two semiconducting electrodes. It supports the results presented in Fig. 3.

Figs. 5 and 6 show the current–voltage characteristics for partially degradated sample (with absorption presented in Fig. 2A, curve 3). This sample exhibits both 750 nm and 674 nm maxima. The current–voltage dependence were measured

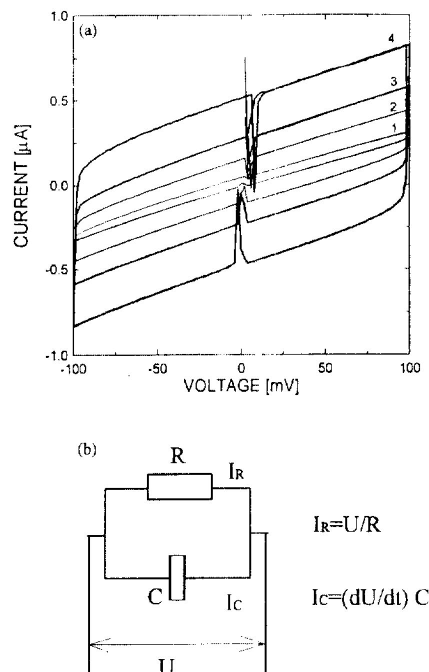

Fig. 5. (a) The current–voltage characteristics of the sample with bacteria fragments in the electrochemical cell with two semiconducting (In_2O_3) electrodes. The absorption of this sample is given in Fig. 2A, curve 3. Speeds of voltage change: curve 1, 1 mV/s; curve 2, 5 mV/s; curve 3, 10 mV/s; curve 4, 20 mV/s. (b) Scheme of simple circuit imitating electrical properties of the electrochemical cell used in the measurement. (R, resistance; C, capacity; I, current; V, voltage; t, time).

at the constant speed of the voltage changes (dV/dt = const, where V is an external voltage applied to the cell and t is time). The loop observed in such a voltammogram depends on the speed of changes of applied voltage (at low speed (1 mV/s) the loop is narrow). It becomes wider with the increase in the dV/dt value (Fig. 5A). From voltammograms, the resistance and capacity of the cell under simplified suppositions (explained in Fig. 5B) can be obtained. The resistance is almost unchanged with the speed of voltage change, the capacity is constant and equal to 25 nF. The change in the loop width with the applied speed shows that the slow processes, such as ions diffusion, occur. Such processes are not able to follow the quick changes of external potential. The capacitance obtained from the voltammograms (C = 25 nF) is much higher than that obtained from the cell geometry and dielectric constant of the sample (C = 1.2 nF). For other photoelectrochemical cells with biological samples, even larger differences between these capacities were observed [9]. This difference is due to the charge distribution in the cell-capacitor. The electric properties of the cell with bacteria fragments differ strongly from these of the cell filled with the solution of chlorophyll in nematic liquid crystal [9,18]. In the last case, the resistance of the cell depends on the speed of voltage change and the capacity decreases with the increase of the voltage speed. The differences between the cells with a liquid crystal solution [9,18] and the cells with bacteria fragments (Fig. 5A) are due to a different charge distribution in such cells. In the first case, the regularly charged layers in

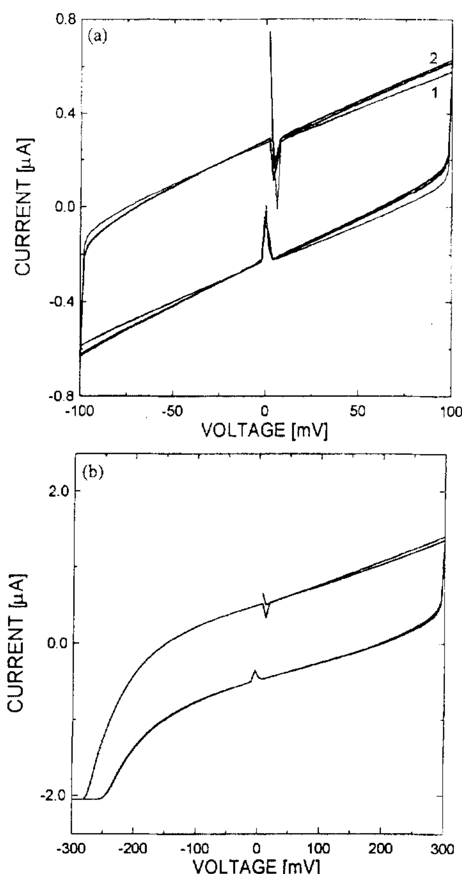

Fig. 6. (a) The current–voltage characteristics of the sample with bacteria fragments in the electrochemical cell with two semiconducting (In_2O_3) electrodes. curve 1, sample in the darkness; curve 2, illuminated sample. Speed of voltage change: 10 mV/s. (b) Voltammogram in larger region of applied voltage.

the whole volume of electrochemical cell are formed. In the second one the charge is shifted in the bacteria cells and than the electrons or ions have to be transferred trough the biological membranes.

The cell illumination has only a small influence on the electrical properties of the sample (Fig. 6A). The value of current increases faster in the illuminated sample compared to the dark one.

Voltammograms deliver information about redox reaction occurring at the semiconductor/sample interface [27]. Most efficient redox compounds exhibit strong asymmetry of $I(V)$ curve. The asymmetry is observed at larger regions of applied voltage (Fig. 6B).

4. Conclusions

On the basis of these results, we can conclude that oligomers of BChl *c* cannot be ionized as a result of illumination by visible light. In the electrochemical cell, only pigments located in the additional local electric field due to the presence in vicinity of either the semiconducting electrode or some charged molecules can be ionized. It is because in ionization, it is necessary to supply about 5 eV of energy, whereas in visible light the quanta have only about 3 eV of energy. The observation that BChl *c* located in oligomers does not take part in the photopotential generation shows that the illumination of the oligomers is not followed by the generation of an adequate local electric field in the vicinity of BChl *c* molecules. BChl *c* molecules located in the oligomers are much more photostable than the monomers of BChl *c* and isomers of Chl *a* because the molecules in the oligomers are efficiently taking part in the chain of the excitation energy transfer to RC [11] whereas the monomeric pigment molecules are more isolated from this chain. Observed signal, measured in modulated light, is due predominantly to the desaggregated antenna pigments. Skulachev [7] showed for purple bacteria that photopotential signal for antenna pigments is one order lower than that due to charge separation in reaction centers. In our experiments, the quick signal is eliminated as a result of sample degradation. Therefore, the quick signal observed in the photocurrent kinetics of the fresh sample (Fig. 4) is probably related to RC.

5. Abbreviations

BChl	bacteriochlorophyll
BPhe	bacteriopheophytin
RC	reaction center

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