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Cationic Antiseptics Disrupt the Functioning of the Electron-Transport Chain at the Acceptor Side in the Photosynthetic Reaction Centres of the Purple Bacterium *Cereibacter sphaeroides*

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Abstract—Using electrometric technique, the cationic antiseptic octenidine was revealed to reduce generation of transmembrane electrical potential difference in the chromatophores of photosynthetic bacterium *Cereibacter sphaeroides*. This is also confirmed by measurements of electrochromic shifts of carotenoid absorption bands in chromatophores. In reaction centers (RCs), isolated from chromatophores in the absence of external electron donors and acceptors, the rate of recombination between photooxidized bacteriochlorophyll P_{870} and reduced secondary quinone acceptor Q_B, as measured by absorption changes in the near infrared region, was very weakly dependent on the presence of antiseptics, in contrast to the kinetics in the 400-600 nm spectral range, where absorption changes associated with the oxidation of P_{870} and the formation of semiquinone radicals Q_A^- and Q_B^-, as well as electrochromic shifts of the carotenoid and bacteriopheophytin RC absorption bands, were observed. The addition of cationic antiseptics modified the flash-induced absorbance changes in this region with the formation time of ~100-200 ms and a decay time of ~3 s. In the series: picloxydine – chlorhexidine – octenidine – miramistin, the last one was the most effective. The maximum amplitude of such changes was observed in the absorption region of the semiquinone radical around 460 nm. When electron transfer from Q_A^- to Q_B was blocked by o-phenanthroline, the effect disappeared. Cationic antiseptics are suggested to stimulate protonation of Q_B^- with the formation of a neutral $Q_B^-H^+$ complex.

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INTRODUCTION

Currently, the widespread and uncontrolled use of antiseptics raises a reasonable question about their possible negative impact on the biosphere. Understanding the behavior of these compounds in various environments is important for their application. Previously, we have examined the effect of cationic antiseptics with one or two charged groups (miramistin, octenidine, chlorhexidine, and picloxydine) on energy transfer in the membranes of chromatophores of photosynthetic purple bacteria [1, 2], as well as on isolated nuclear complexes of photosystem II from spinach leaves [3]. Recently, the effect of these antiseptics on fluorescence characteristics and electron transfer was studied in another pigment-protein complex of photosystem I from the freshwater cyanobacterium *Synechocystis sp.* 6803 [4]. For all the abovementioned

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medications, the most noticeable effect was observed in the presence of octenidine. In bacterial chromatophores from *Cereibacter sphaeroides* (previously called *Rhodobacter sphaeroides*), which represent a minimal possible photosynthetic unit which is structurally and functionally intact, the addition of octenidine led to a decrease in the efficiency of electron excitation energy migration from the light-harvesting complex LH2 to the core complex LH1–RC (RC – reaction center) by approximately three times [1]. Miramistin had the weakest effect on this energy migration in *C. sphaeroides* chromatophores. It was suggested that the disruption of effective energy transfer occurs due to the antiseptic binding to the hydrophilic heads of negatively charged cardiolipin molecules located in the rings of light-harvesting pigments on the cytoplasmic surface of the chromatophores.

The photosynthetic electron transport chain in *C. sphaeroides* chromatophores catalyzes a light-driven cyclic electron flow between the RC and the cytochrome bc_1 complex involving a mobile membrane pool of ubiquinones (UQ_{10}) and soluble endogenous cytochrome c_2.

RC is a transmembrane complex comprised of three protein subunits (designated as L, M, and H) with a total molecular weight of about 100 kDa. Redox cofactors in the RC structure are organized into two transmembrane branches, of which only the L branch is functional. A special pair of bacteriochlorophylls (P_{870}) is located near the periplasmic surface of the photosynthetic membrane. The transmembrane protein L subunit contains monomeric bacteriochlorophyll (BChl) a, bacteriopheophytin (BPh) and the primary quinone acceptor Q_A molecules, forming the L branch, while the transmembrane subunit M includes monomeric BChl, BPh, and the secondary quinone acceptor Q_B, constituting the M branch. A molecule of a specific carotenoid is also located in the structure of the M subunit near BChl a. In particular, RCs of *C. sphaeroides* contain spheroidene or spheroidenone as this carotenoid. The latter is synthesized during bacterial growth in the presence of O_2 [5]. Upon light excitation of RCs, electron transfer occurs from the primary electron donor P_{870} to BChl with a characteristic time of ~3 ps, then to BPh in ~0.9 ps, to Q_A (~200 ps) and to Q_B (~100 μs) [6-8]. RCs from *C. sphaeroides* also contain three integral lipid molecules: cardiolipin, phosphatidylcholine, and glucosyl-galactosyl diacylglycerol [9]. This suggests the possible existence of specific interactions between phospholipids and electron transport components in RCs. In particular, it was shown that the thermodynamic and kinetic parameters of electron transfer in the quinone acceptor unit of RCs from purple bacteria depend on the presence of physiologically important lipids in their environment [9, 10]. It has been suggested that electrostatic interactions of lipids with quinone acceptors in RCs affect the stabilization time of separated charges [11]. Other experiments have shown that the packing of RCs in the lipid bilayer of chromatophore membranes appears to increase the pK_a of the singly reduced secondary acceptor of ubiquinone Q_{10}, the semiquinone anion Q_B^-, by almost three units [12] compared to its very low value (about 4.5) in RC samples [13]. It is assumed that the high pK_a value of the secondary quinone promotes the formation of neutral hydroquinone $Q_B^-H^+$ and stimulates the transfer of the second electron to it with the formation of $Q_B^{2-}H^+$. This hypothesis of proton–electron events has been termed “proton-activated electron transfer” [13].

Since cationic antiseptics interact primarily with amphiphilic molecules, it should be expected that not only in membrane preparations but also in isolated RC complexes containing lipid molecules and surrounded by amphiphilic detergent molecules, electron transport processes may depend on the presence of cationic antiseptics. In this regard, the aim of this work was to study the effect of a number of cationic antiseptics on photosynthetic electron- and proton-transport activity in *C. sphaeroides* chromatophore membranes and RC preparations isolated from them by the electrometric method and flash photolysis.

MATERIALS AND METHODS

Isolation and purification of chromatophores and RC preparations from *C. sphaeroides*. Chromatophores from the wild-type purple non-sulfur *C. sphaeroides* bacteria were isolated and purified according to the method described by Woronowicz et al. [14], with minor modifications. In brief, cells were collected and washed in 25 mM Hepes buffer (pH 7.5) and disrupted using a French press at 12,000 psi. After centrifugation of the suspension for 20 min at 4°C and 14,000*g*, the supernatant was loaded onto a 5-35% (*w/w*) sucrose gradient prepared in 25 mM Hepes buffer (pH 7.5) over a 60% sucrose cushion and centrifuged in a VTi 50 vertical rotor for 2 h at 140,000*g*. The bottom (third from the top) band containing chromatophores was collected, dialyzed twice against 25 mM Hepes buffer (pH 7.5) for 2 h and concentrated. The BChl concentration in the chromatophore suspension was determined as described previously [15].

During RC isolation, the chromatophores were incubated for 30 min at 4°C in 10 mM sodium phosphate buffer (pH 7.0) containing 0.5% lauryldimethylamine oxide (LDAO) detergent. The membrane fragments were then centrifuged for 90 min at 4°C and 144,000*g*. The RC fraction contained in the supernatant

was separated by chromatography on a hydroxyapatite column, as described by Zakharova et al. [16]. The applied method made it possible to obtain RC preparations that had retained both quinone acceptors within their structure.

Electrometric method. Ag/AgCl electrodes immersed in a Teflon cuvette were used to measure the transmembrane electric potential difference (Δψ) on chromatophores adsorbed on the surface of a collodion film impregnated with a phospholipid (type IIS phosphatylcholine, Sigma-Aldrich, USA) in decane [17, 18]. The output voltage was connected through an operational amplifier (3554BM, Burr Brown, USA) to a GaGe CS8012 analog-to-digital converter and then to a computer. The method allows direct measurements of undistorted electrical signals with a time resolution of ~200 ns. Each measurement was repeated at least 3 times. The error range of the signals was ~3%. All measurements were performed at 22 ± 1°C. Second harmonic laser flashes of a Nd:YAG laser (532 nm; 12 ns half-width; 20 mJ pulse energy; Quantel, Les Ulis, France) were used for light activation.

To restore the function of the loosely bound secondary quinone acceptor Q_B and the pool of membrane ubiquinones in chromatophores, CoQ_{10} (20 mg/ml) was added to the lipid solution for impregnating the collodion film.

Flash photolysis. Light-induced absorption changes in the suspension of chromatophores and RCs were recorded using the flash photolysis setup described earlier [4]. In the chromatophores, photoelectric responses were recorded by measuring light-induced absorption changes (electrochromic shift of carotenoid absorption bands) at 523 nm and the redox state of P_{870} at 790 nm. An aliquot of concentrated chromatophores was diluted for experiments in 25 mM Hepes buffer (pH 7.5) to a final volume of 1 ml and a final concentration of BChl of ~30 μM. The measurement medium contained 25 mM Hepes buffer (pH 7.5), 2 mM potassium ferrocyanide and 50 μM N,N,N′N′-tetramethyl-p-phenylenediamine (TMPD).

In experiments with RCs in 10 mM Hepes buffer (pH 7.5), the BChl concentration was ~1.5 μM. In this case, exogenous cofactors were not added. Photoinduced absorption changes in the RC suspension were recorded in the visible spectrum range, 400-650 nm, with a step of 10 nm, and in the IR range at wavelengths of 790 and 865 nm. A 1 cm^3 sample in a 10×10 mm glass cuvette was placed in a thermostatted qpod-2e holder (Quantum Northwest, USA) with a set temperature of 21°C. An Nd:YAG laser (532 nm; 7 ns half-width, 10 mJ pulse energy, Lotis-TII, Belarus-Japan) was used for pulsed illumination. The kinetics of absorption changes per flash were analyzed in the time range from 1 μs to 1 s. To improve the signal-to-noise ratio, 50 to 100 single signals were accumulated and averaged. The time interval between flashes was chosen to ensure complete recovery of P_{870}^+ in the darkness; it was 1.5 s for chromatophores and 5 s for RCs. Kinetic data analysis was performed using Origin 9.1 (OriginalLab Corp., USA) or Mathematica 8 (Wolfram Research Inc., USA) software. Absorption spectra were recorded on a modified Hitachi-557 spectrophotometer (Hitachi Ltd., Japan).

Antiseptic preparations. The following ready-made pharmaceutical preparations were used in the studies: chlorhexidine bidigluconate 20%, picloxydine dihydrochloride 0.05% (Vitabact), miramistin 0.01%. The source of octenidine was the commercial preparation “Octenisept” (Schulke & Mayr, Germany), containing 0.1% octenidine dihydrochloride and 2% phenoxyethanol. Preliminary experiments showed that phenoxyethanol, a component of the Octenisept preparation, did not by itself affect the spectral properties and the studied parameters of the analyzed samples in equimolar concentrations.

RESULTS AND DISCUSSION

The cationic antiseptics used in the study had similar effects on the spectral characteristics and the studied functional characteristics of photosynthetic membranes and RCs isolated from them. The magnitude of these effects decreased in the series: miramistin – octenidine – chlorhexidine – picloxydine. At the same time, chlorhexidine and picloxydine had a significantly less pronounced effect compared to miramistin and octenidine. Therefore, the discovered effects are further illustrated using octenidine and miramistin as an example.

Light-induced vector charge transfer (electron and proton) in bacterial membrane vesicles (chromatophores) results in the formation of Δψ on the chromatophore membrane [17-19]. The electrometric method used in this study allows to reveal both the kinetics and dielectrically weighted distances for vector (electrogenic) charge transfer reactions within RC protein complexes [20, 21]. Using this method, electrogenic reactions such as electron transfer from P_{870} to Q_A, re-reduction of P_{870}^+ from cytochrome c_2, and protonation of the doubly reduced quinone acceptor Q_B were previously shown in *C. sphaeroides* chromatophores [17, 18].

A photoelectric signal consists of an unresolved fast phase of Δψ generation (resolution time of the measuring system is ~ 200 ns), which is caused by charge separation between P_{870} and Q_A, followed by Δψ dissipation. Since the primary and the secondary quinone electron acceptors are located parallel to the membrane plane, the stage of electron transfer from Q_A^- to Q_B is not coupled with the formation of Δψ,

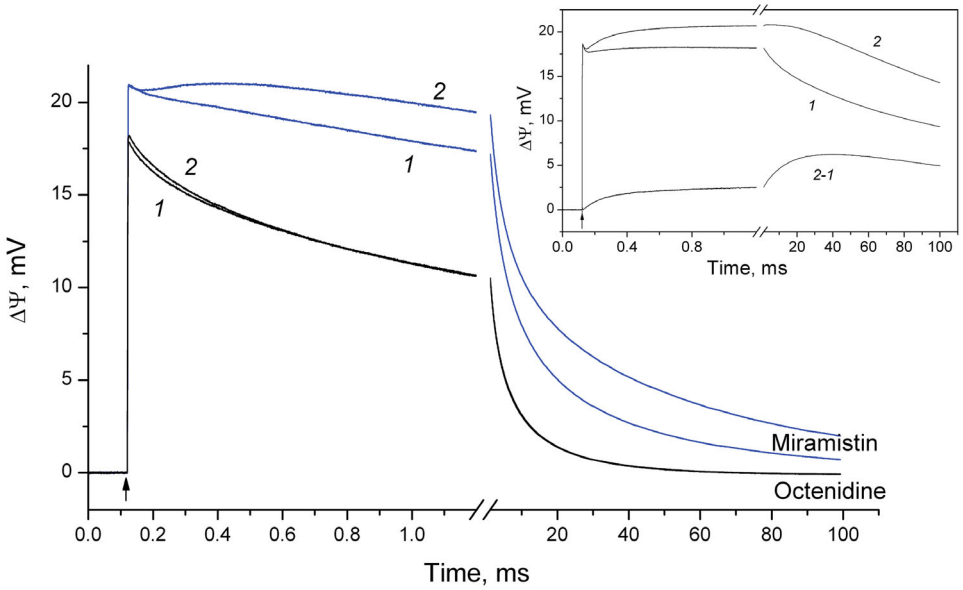

Fig. 1. Photoelectric responses induced by the first (curves *1*) and second (curves *2*) light flashes in the presence of 100 μM octenidine (lower curves) and 100 μM miramistin (upper curves), respectively. Arrows indicate the moment of the laser flash. The incubation medium contains 25 mM Hepes buffer (pH 7.5), 2 mM potassium ferrocyanide, and 50 μM TMPD. 20 mg of ubiquinone Q_{10} was added to 1 ml of the phospholipid solution in decane to impregnate the collodion film. The inset shows the electrical responses of *C. sphaeroides* chromatophores adsorbed on the surface of a collodion film impregnated with a phospholipid solution in the absence of antiseptics induced by the first (curve *1*) and second (curve *2*) laser flashes. The subtraction of curve *2* from curve *1* is marked as *2-1*. The time interval between the first and second flashes is 1 s here and below.

i.e., it is non-electrogenic. In early studies [17, 18], it was shown that the association of chromatophores with a collodion film impregnated with phospholipids in decane leads to the extraction of loosely bound secondary quinone Q_B and a pool of ubiquinones (UQ). Indeed, in the absence of exogenous electron donors and acceptors, the kinetics of Δψ decay caused by the reverse electron transfer reaction between Q_A^- and P_{870}^+ is almost fully completed within 100 ms. In this case, the Δψ signal decay is approximated fairly well by a single exponential with τ ~ 66 ms (not shown). It should be noted that the redox conditions in the medium in the presence of 50 μM TMPD and 2 mM potassium ferrocyanide (the redox potential of the medium is about +320 mV) allow the detection of slow electrogenic stages caused by vector charge transfer involving both RC and the cytochrome bc_1 complex in chromatophores [17, 18]. As can be seen on the inset to Fig. 1, in response to the second light flash, an additional electrogenic phase appears in the kinetics of the photoelectric response with a rise time of ~190 μs (~14% of the amplitude of the fast phase, see the difference between the 2nd and 1st flashes). This submillisecond rise phase is inhibited by o-phenanthroline, which displaces the loosely bound secondary quinone acceptor Q_B from the binding site in the RC protein (not shown). The dissipation time of the photopotential in the darkness under these conditions is ~500-600 ms and reflects passive membrane discharging.

The main part of Fig. 1 shows the kinetic curves of photopotential generation in chromatophores in the presence of 100 μM cationic antiseptics. At a concentration of 50 μM, both antiseptics had a weak effect on the additional submillisecond electrogenic phase in response to the second flash of light (not shown), but octenidine completely removed it already at a concentration of 100 μM, while miramistin was much less effective. It should also be noted that while the photoresponse decay time was greater than 0.5 s in the control sample (insert to Fig. 1), in the presence of antiseptics, especially octenidine, the decay kinetics became faster (<12 ms) (Fig. 1). This is probably due to the disordering effect of the antiseptic on both the RC protein and the chromatophore membrane, resulting in an increase in passive ionic conductivity of the chromatophore membrane.

In parallel experiments, we investigated photoinduced absorption changes in a chromatophore suspension using flash photolysis, choosing a wavelength of 523 nm to monitor the carotenoid shift and a wavelength of 790 nm to monitor P_{870} redox transformations. It is important to note that in bacterial photosynthetic chromatophores of *C. sphaeroides*, measurements of the electrochromic shift at 523 nm in response to single light flashes allow real-time detection of generation and dissipation of the electrical component of both the local and transmembrane fields (Δψ and $\Delta\mu H^+$) [22-24]. Its formation is due to

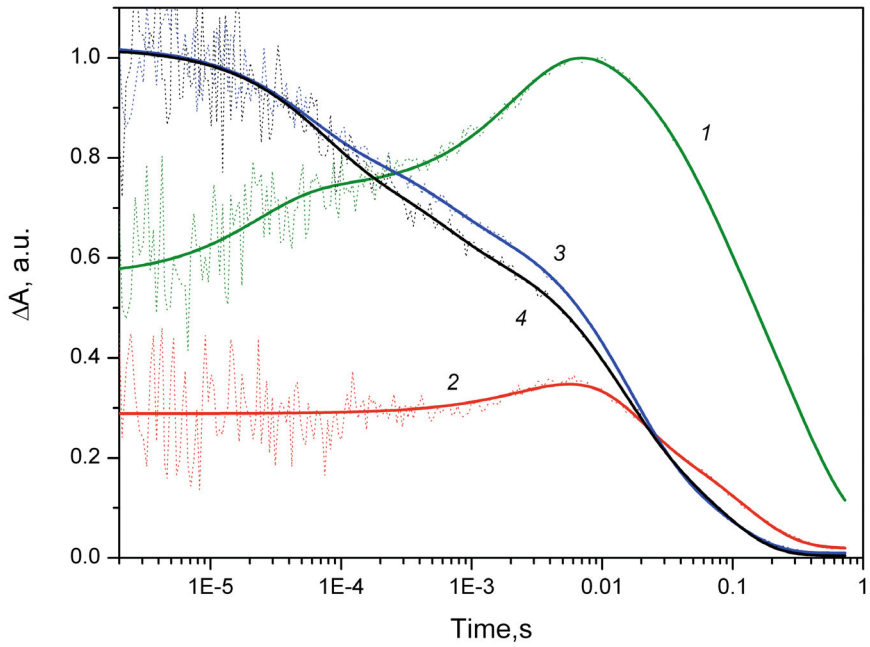

Fig. 2. Kinetic curves of the absorption changes at 523 nm (curves *1* and *2*) and 790 nm (curves *3* and *4*) recorded by flash photolysis in *C. sphaeroides* chromatophore preparations in the absence (control, *1* and *3*) and in the presence of 100 μM octenidine (*2* and *4*). The curves were normalized to the maximum amplitude of absorption changes in the control. The dots represent the experimental values; the solid line is an approximation as the sum of exponentials (details given in the text). The medium was 25 mM Hepes buffer, pH 7.5, 50 μM TMPD, and 2 mM potassium ferrocyanide.

the generation of Δψ as a result of the functioning of both the RC and the cytochrome bc_1 complex, and the decline in the photoelectric response reflects ion fluxes across the chromatophore membrane, including the proton current through the F_O-ATP synthase channel [25] and the passive diffusion of ions across the phospholipid bilayer [24].

It should be noted that just as in the experiments on recording the Δψ generation using the electrometric method, 50 μM TMPD and 2 mM potassium ferrocyanide were also added to the medium for absorption measurements.

The parameters of the approximation of the kinetic curves of the carotenoid shift (Fig. 2, curves *1* and *2*) are given in Table 1. The unresolved fast phase (shorter than 0.1 μs), reflecting the formation of a local electric field during charge separation between P_{870} and Q_A in the control comprises ~60% of the total amplitude. The remaining 40% can be represented by two phases with half-times of ~20 μs and ~2.4 ms, which, in our opinion, are associated with the formation of the transmembrane field. The decline in carotenoid absorption changes occurs over tens to hundreds of milliseconds with characteristic times of about 50 and 300 ms and an amplitude ratio of 3 : 7. When octenidine is added at a concentration of 100 μM, the total signal amplitude drops by about 2.5 times, and significant changes occur in the kinetics of both its formation and decay. The fast phase of formation (<0.1 μs) dominates, accounting for more than 80%, and the microsecond phase (~20 μs) completely disappears. A small-amplitude phase with the length of the order of several milliseconds remains, the nature of which is not entirely clear. And while the dark decay kinetics at 523 nm was much slower than the recovery of P_{870}^+ in the control sample, in the presence of octenidine the dissipation of the carotenoid shift accelerated, and these events occurred in the same time range.

Changes in P_{870} absorption depend weakly on the presence of octenidine in the medium. Both the amplitude of P_{870} photooxidation and the kinetics of dark reduction of P_{870}^+ remain practically unchanged (Fig. 2, curves *3* and *4*). Since the primary photooxidation of P_{870} occurs on a subpicosecond time scale, the instrumental capabilities allowed us to record only the slower processes of dark reduction of P_{870}^+ on a micro-millisecond time scale.

In Table 2 we present the results of fitting the kinetics at 790 nm by a sum of 4 exponentials.

The following assumptions can be made about the nature of individual phases of P_{870}^+ reduction after the activating flash. Since the medium contains reduced TMPD and some preserved amount of cytochrome c_2, it is possible that phases τ_1 and τ_2, lasting about 50-500 μs, reflect electron transfer from cytochrome c_2, and the next two millisecond-range phases, τ_2 and τ_3, are associated with the reduction of P_{870}^+ by the redox mediator TMPD. It is important that the amplitude of changes in P_{870} absorption after the addition of octenidine remains constant and the kinetics

Table 1. The parameters (τ and A, %) of the multiexponential approximation of the kinetics of the photoinduced shift of carotenoid absorption band at 523 nm

Parameters	Formation			Dissipation	
	τ_1 (A_1, %)	τ_2 (A_2, %)	τ_3 (A_3, %)	τ_4 (A_4, %)	τ_5 (A_5, %)
Control	< 0.1 μs (~60)	20 μs (~16)	2.4 ms (~24)	52 ms (~30)	310 ms (~70)
+ 100 μM octenidine	< 0.1 μs (~83)	–	3.1 ms (~17)	18 ms (~50)	117 ms (~50)

Table 2. The parameters (τ and A, %) of the multiexponential approximation of the kinetics of dark reduction of P_{870}^+ at 790 nm

Parameters	τ_1 (A_1, %)	τ_2 (A_2, %)	τ_3 (A_3, %)	τ_4 (A_4, %)
Control	50 μs (~15)	0.5 ms (~15)	14 ms (~46)	79 ms (~24)
+ 100 μM octenidine	50 μs (~20)	0.5 ms (~17)	12 ms (~36)	74 ms (~27)

of dark reduction of P_{870}^+ changes insignificantly, while the carotenoid shift decreases in amplitude, the microsecond phase of its generation disappears from the kinetics, and the dark decline accelerates.

Flash photolysis data obtained on chromatophores showed that octenidine does not affect the primary charge separation between P_{870} and quinone acceptors, but disrupts transmembrane electric field generation. Thus, these observations confirm the conclusion drawn from the experiments with direct measurements of electrogenesis in chromatophores. However, the mechanism of this inhibitory effect in the experiments on chromatophores could not be established, so we studied the action of antiseptics on preparations of RCs isolated from *C. sphaeroides* chromatophores, which are a convenient object for assessing the efficiency of the functioning of quinone acceptors Q_A and Q_B. In the absence of an electron donor in the medium, the kinetics of dark reduction of P_{870}^+ after a flash reflects the degree of electron occupancy of the primary and secondary acceptors. In RC preparations with two quinone acceptors, Q_A and Q_B, after an activating flash of light, a slow recovery due to dark recombination of $P_{870}^+ Q_B^-$ is observed

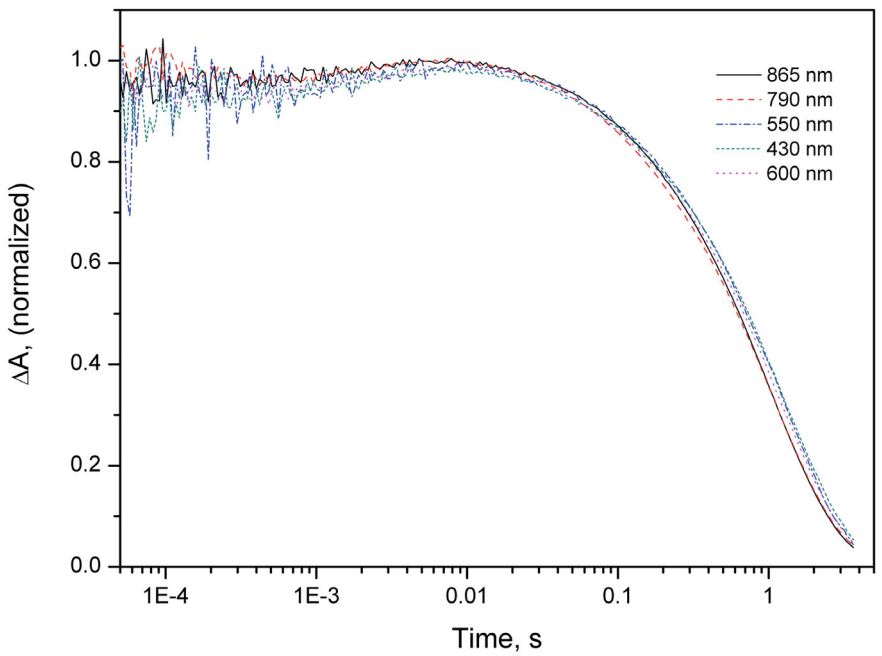

Fig. 3. Kinetic curves of dark decay of absorption changes (normalized by amplitude and expressed as a positive value) in a suspension of *C. sphaeroides* RCs, recorded in the IR (790 and 865 nm) and visible (430, 550, and 600 nm) spectral regions.

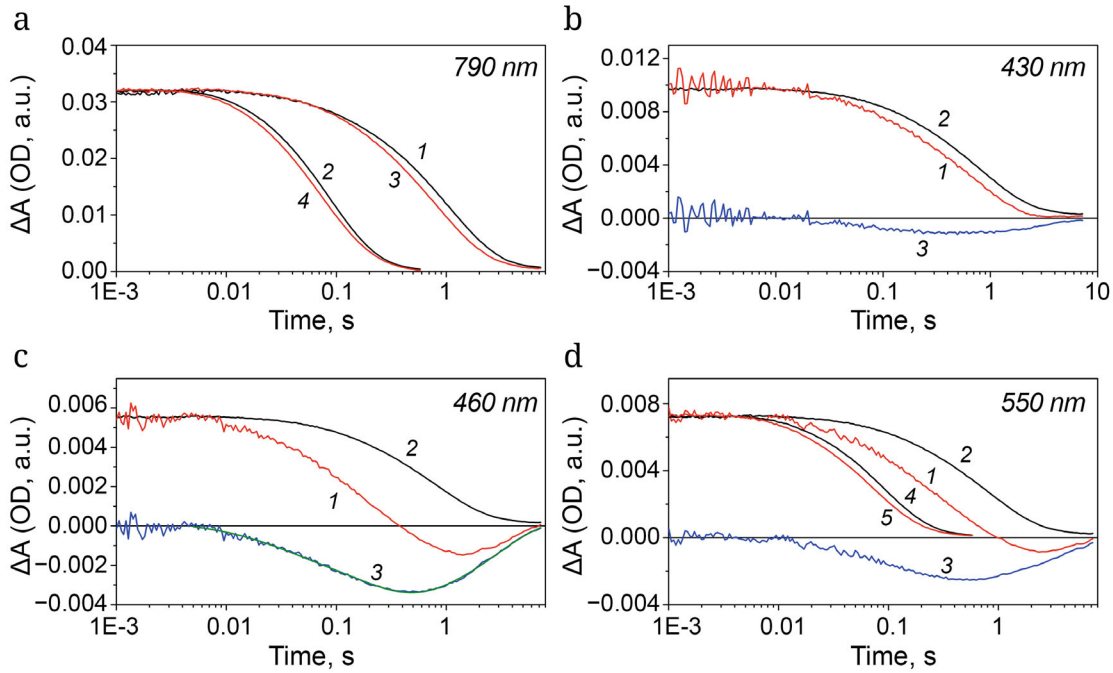

Fig. 4. Kinetic curves of dark decay of absorption changes for a *C. sphaeroides* RC suspension recorded at different wavelengths. a) 790 nm, curves *1* and *2* – control without and in the presence of 5 mM o-phenanthroline, respectively, curves *3* and *4* – after adding 150 μM miramistin to these samples; b-d) 430, 460, and 550 nm, respectively; curves: *1*) in the presence of 150 μM miramistin, *2*) normalized kinetic curves at 790 nm in the presence of 150 μM miramistin, *3*) differential curves "*2* minus *1*". An approximation of the differential curve by a sum of three exponentials is also shown in panel c (description in the text). d) curves *4* and *5*: the kinetics at 550 nm in the presence of 5 mM o-phenanthroline in the control (*4*) and after the addition of 150 μM miramistin (*5*).

with a characteristic half-time of about 1 s (the rate of direct electron transfer from Q_A^- to Q_B is 3 orders of magnitude higher than that of the electron return to P_{870}^+, therefore, all electrons that hit the primary quinone will then go to the secondary). And in preparations where the direct transfer is blocked in some RCs, the recovery of P_{870}^+ will occur from Q_A^- with a characteristic time of about 0.1 s. Since the RC preparations used in this work contained both quinone acceptors and there was no exogenous electron donor, after a flash of light we observed P_{870}^+ recovery from the secondary acceptor Q_B^- with a characteristic time of ~1 s. The kinetics of the dark decay of absorption changes in the visible spectrum (400-650 nm) and near IR range (700-900 nm) were almost identical. The characteristic curves for a number of wavelengths are shown in Fig. 3.

However, when antiseptics are added, the kinetic curves begin to diverge. While in the IR region, only a slight acceleration of the decay kinetics is observed for absorption changes reflecting the transformations of the P_{870} dimer, in the visible region of the spectrum the discrepancies are more significant (Fig. 4).

Fig. 4a demonstrates the kinetics of absorption changes at 790 nm in the control (curve *1*, $\tau \approx 1.1$ s) and in the presence of 5 mM o-phenatroline (curve 2, $\tau \approx 85$ ms). It also shows the kinetic curves for these samples in the presence of 150 μM miramistin (curves *3* and *4*). It should be emphasized that even at very high concentrations of miramistin or octenidine (400 μM), the reduction in recovery time was no more than 10% of this value in the control. In other words, the effect of antiseptics is fundamentally different from the action of o-phenanthroline, which is well known to replace the secondary acceptor at the site of its localization in moderate concentrations, and even displace the primary quinone in high concentrations [26]. It should also be noted that in the presence of o-phenanthroline, the kinetics of photoinduced absorption changes in the visible and IR regions coincided for all the studied concentrations of antiseptics.

In the RC preparations without o-phenanthroline, negative changes appeared in the kinetics of photoinduced absorption changes in the 400-650 nm region a few milliseconds after the activating flash, which distorted the "normal" form of the kinetics, if we consider that the kinetics of transformations at 790 nm adequately reflects the charge separation in RCs between P_{870}^+ and Q_B^- with the formation of a local electric field and the subsequent charge recombination (Fig. 4, b-d). The strongest effect was observed in the presence of miramistin (then, in the descending order – octenidine, chlorhexidine, picloxydine), so we studied the effect of this preparation in most detail.

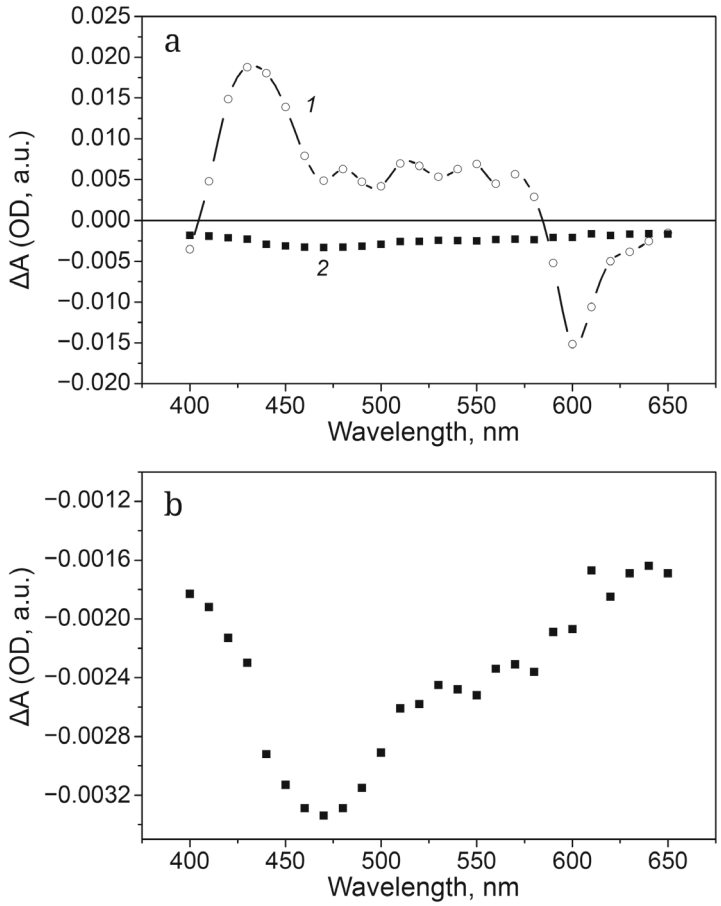

Fig. 5. Single-flash-induced differential absorption "light minus dark" spectra in a suspension of *C. sphaeroides* RCs. a) By the amplitude of absorption changes 1 ms after the flash (*1*) and by the amplitude of negative absorption changes appearing with a delay (*2*), shown, in particular, in Fig. 4, b-d by curves *3*; b) the same (*2*) dependence on an enlarged scale.

Let us recall that the recorded absorption changes in the visible spectrum region reflect a number of processes: oxidation of the BChl dimer, formation of the semiquinone radical, as well as electrochromic shifts of the absorption bands of carotenoids and BPh.

For illustration, Fig. 4, b-d shows photoinduced absorption changes for wavelengths of 430, 460, and 440 nm (curves *1*) and absorption changes at 790 nm normalized by amplitude, which we considered "undistorted" (curves *2*), as well as differential kinetic curves (curves *3*). Fig. 4c shows the fitting of such kinetics by two growth exponents with characteristic times $\tau_1 \approx 25.6$ ms (~30%) and $\tau_2 \approx 191.2$ ms (~70%) and one decay exponent characterized by a time $\tau_3 \approx 3$ s. The kinetic parameters differed little, but the amplitude of the resulting curves depends significantly on the wavelength.

Fig. 5a shows the differential absorption spectrum "light minus dark" in the visible part of the spectrum (*1*), constructed from the signal amplitude 1 ms after the light flash until the moment of negative changes appearing, as well as the dependence of the amplitude of the discussed negative absorption changes on the wavelength (*2*). Fig. 5b presents this dependence on an enlarged scale. We recall that in the 400-600 nm spectral region, multiple absorption changes are observed associated with redox transformations and electrochromic shifts of various RC components: RC BChl (about 430 and 600 nm), BPh (about 540 nm), RC carotenoid (500-600 nm), and semiquinone forms of Q_A and Q_B acceptors (in the 450-460 nm region) [27].

It has been previously shown that the formation of the semiquinone anion Q_B^- in the RC of *Rb. sphaeroides* is accompanied by the appearance of a broad absorption band with a maximum at 450 nm [28]. As follows from Fig. 5b, the amplitude of the absorption changes occurring ~200 ms after the flash reaches a maximum of about 460 nm, which indicates a possible connection with a change in the absorption of the semiquinone anion.

Despite long-term studies, the mechanisms of Q_B reduction, proton binding and its migration in various redox states are not yet fully understood [29]. It was previously suggested that electrostatics plays a decisive role in the interaction of various redox states

of Q_B with the surrounding amino acid residues [30]. Indeed, all models of stabilization of the anionic form of Q_B suggest that this requires a significant rearrangement of charges and dipoles at the Q_B site [31]. At the same time, it is known that in RCs from wild-type *Rb. sphaeroides* bacteria, in which ubiquinone Q_{10} is located at the site of the secondary acceptor Q_B, the formation of its semiquinone anion form is not accompanied by direct protonation, which is explained by the low value of its pK_a ~ 4.5 [13]. Since the environment of Q_B is rich in protons and polar groups, the appearance of a negative charge on Q_B affects the equilibrium of protons in the system of hydrogen bonds involved in stabilizing the electron on the acceptor. In particular, the pK of the amino acid Glu L212 changes [32]. This amino acid, linked through a network of hydrogen bonds with other protonated amino acids and water molecules in the RC structure, plays a key role in donating the first proton in the process of forming hydroquinone Q_BH_2 during two-electron reduction of the secondary acceptor [28]. It is proposed that in response to the electron transfer to Q_B, changes occur in the hydrogen bond network formed by the surrounding water molecules and the polar groups of the amino acids. Calculations show that even small changes in the orientation of these molecular groups can induce shifts in their pK, ensuring the directional proton transfer [33].

The protonation of Q_B^- in RC preparations was successfully recorded in an experiment when ubiquinone Q_{10} was replaced with rhodoquinone, for which the pK_a value was much higher (about 7.3) [13]. At acidic pH values, a decrease in the amplitude of the photoinduced absorption band of the semiquinone anion Q_B^- was observed. As noted in the Introduction, in contrast to purified RC preparations, in chromatophores, this stage of protonation of the secondary acceptor after its one-electron reduction was experimentally observed [12]. This result was attributed to an increase in the pK_a of the secondary quinone Q_{10} by more than 3 units due to RC immersion in the lipid bilayer of the chromatophore membranes.

RC protein relaxation during electron transfer to quinone acceptors is sensitive to protein-bound lipids. It has been shown that electrostatic changes on the protein surface or in the hydrogen bond network surrounding Q_B can lead to changes in pK_a of amino acids near Q_B and the pK of the semiquinone Q_B itself [34]. It can be assumed that the absorption changes we detected reflect the partial disappearance of the absorption band of the semiquinone anion Q_B^- and the appearance of the absorption band of the electroneutral form Q_BH due to the displacement of protons in the immediate environment or upon direct binding with a proton in the medium [12, 13]. The observed effect of amphiphilic cationic antiseptics in RC preparations could be associated with their influence on the pK_a value of the secondary quinone, as it occurs in the membrane lipid matrix of chromatophores. The rate of proton binding in our experiments was quite low; this process takes 100-200 ms. At the same time, the calculations performed for Q_B^- protonation with rhodoquinone give a value of several microseconds [13], since this process must precede the transfer of the second electron from Q_A, which occurs on a microsecond time scale. Apparently, the increase in pK_a in our case is not very large, and therefore, when the measurements were performed with a medium pH of 7.5, the rate constant of the direct protonation reaction is close to the deprotonation constant. Nevertheless, the results obtained indicate that cationic antiseptics (mainly miramistin and octenidine) promote the direct protonation of Q_B^- with the formation of a neutral complex Q_BH. It should be noted that in the structure of the resulting “negative” absorption changes, a certain constant value is present for the entire spectral range of 400-650 nm, which clearly does not belong to the semiquinone anion spectrum (Fig. 5b). Apparently, neutralization of the charge on the secondary quinone partially reduces the local electric field, which reduces the magnitude of the electrochromic shifts of carotenoids and BPh, making a significant contribution to the absorption changes in this wavelength range. Indeed, it has been shown that an increase in the yield of the neutral form of semiquinone Q_BH correlates with a decrease in the amplitude of the electrochromic shift of the BPh absorption band at 760 nm [12].

It should be noted that the analyzed cationic antiseptics, according to various data, exhibit a detergent-like effect in high concentrations [35, 36]. Moreover, in a number of studies, the ability of these compounds to disrupt the integrity, i.e. barrier properties of membranes is considered the basis of their antimicrobial action [37, 38]. Considering the acceleration of the decline in the photoelectric response of chromatophores to single light flashes in the presence of 100 μM octenidine which indicates a marked increase in the passive ionic conductivity of the membrane (Fig. 1), as well as a sharp drop in the amplitude of the carotenoid shift under similar conditions (Fig. 3), the possibility of a significant contribution of the detergent-like effect to the observed effects of cationic antiseptics cannot be excluded.

CONCLUSION

The new experimental data presented in this study complement the general picture of the effects of cationic antiseptics on biomembranes. The presented results indicate that these agents can affect the

cyclic electron transfer in bacterial chromatophores, reducing the generation of the transmembrane proton gradient on the photosynthetic membrane. Experiments on RC preparations showed the effect of cationic antiseptics on local proton equilibria responsible for stabilization of electrons on the secondary quinone acceptor in protein-pigment complexes. The discovered differences in the action of octenidine and miramistin on membrane preparations and preparations of isolated RCs are apparently due to the specific physico-chemical properties of these antiseptics – their amphiphilicity, charge characteristics, and general molecular structure. As a result, octenidine has a greater effect on membrane preparations, influencing their functional activity. Miramistin modifies protein–pigment complexes isolated from membranes more effectively, interacting with the surface of the complex and, possibly, partially penetrating the surface structure of the protein.

The main conclusion from the obtained results is that cationic antiseptics, which are increasingly entering the environment, disrupt the functioning of the quinone acceptor side of the electron transport chain, reducing the energization of the coupling photosynthetic membrane necessary for ATP synthesis. Thus, they can have a negative impact on the global processes of photosynthetic utilization of light energy.

Abbreviations. Δψ, transmembrane electrical potential difference; BPh, bacteriopheophytin; BChl, bacteriochlorophyll; P_{870}, special pair of bacteriochlorophyll, the primary donor of RC; Q_A and Q_B, the primary and the secondary quinone acceptors, respectively; RC, photosynthetic reaction center; TMPD, N,N,N′N′-tetramethyl-p-phenylenediamine.

Contributions. E. P. Lukashev, M. D. Mamedov, P. P. Knox, V. Z. Paschenko – setting the objectives, discussion of the results of the study, writing the text of the paper; E. P. Lukashev, M. D. Mamedov, L. A. Vitukhnovskaya, A. M. Mamedova – conducting experiments, processing results; L. A. Vitukhnovskaya, A. M. Mamedova – preparing the samples for the study.

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