



Disproportionate effect of cationic antiseptics on the quantum yield and fluorescence lifetime of bacteriochlorophyll molecules in the LH1-RC complex of *R. rubrum* chromatophores

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Abstract

Photosynthetic membrane complexes of purple bacteria are convenient and informative macromolecular systems for studying the mechanisms of action of various physicochemical factors on the functioning of catalytic proteins both in an isolated state and as part of functional membranes. In this work, we studied the effect of cationic antiseptics (chlorhexidine, picloxydine, miramistin, and octenidine) on the fluorescence intensity and the efficiency of energy transfer from the light-harvesting LH1 complex to the reaction center (RC) of *Rhodospirillum rubrum* chromatophores. The effect of antiseptics on the fluorescence intensity and the energy transfer increased in the following order: chlorhexidine, picloxydine, miramistin, octenidine. The most pronounced changes in the intensity and lifetime of fluorescence were observed with the addition of miramistin and octenidine. At the same concentration of antiseptics, the increase in fluorescence intensity was 2–3 times higher than the increase in its lifetime. It is concluded that the addition of antiseptics decreases the efficiency of the energy migration LH1 → RC and increases the fluorescence rate constant k_{fl} . We associate the latter with a change in the polarization of the microenvironment of bacteriochlorophyll molecules upon the addition of charged antiseptic molecules. A possible mechanism of antiseptic action on *R. rubrum* chromatophores is considered. This work is a continuation of the study of the effect of antiseptics on the energy transfer and fluorescence intensity in chromatophores of purple bacteria published earlier in *Photosynthesis Research* (Strakhovskaya et al. in *Photosyn Res* 147:197–209, 2021).

Keywords Chromatophores · Light-harvesting complex LH1 · Energy transfer · *Rhodospirillum rubrum* · Cationic antiseptics

Introduction

Phototransforming proteins are photoenzymes, the effectiveness of which is inextricably linked with their structural dynamics. As a result, their functioning strongly depends on the state of the protein carriers of photosynthetic pigments (Garbers et al. 1998; McMahan et al. 1998; Paschenko et al. 1998) and the state of the environment, that is the membrane

into which these proteins are embedded (Deshmukh et al. 2011; Ogren et al. 2018). High sensitivity to various factors makes these photosynthetic systems (both isolated proteins and photosynthetic membranes) useful as convenient and informative indicators for studying the mechanisms of action of various physicochemical factors (temperature, humidity, ultraviolet light) (Tokaji et al. 2002; Kaftan et al. 2019; Tandori et al. 1996; Zabelin et al. 2020). They have also been used to detect environmental contamination (for example, in tests for mercury contamination (Asztalos et al. 2012; Sipka et al. 2018)) as well as to study the possible mechanisms of action of certain drugs (Knox et al. 2000, 2001) and the effects of antiseptics on bacterial membranes (Strakhovskaya et al. 2021). It turns out that measuring the influence of such external factors on functional parameters of the photosynthetic apparatus can provide useful information both on the possible mechanisms of action of the agents used and on the structural and dynamic

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organization of the components of the photosynthetic apparatus and the molecular mechanisms of its effective functioning.

In our previous work, we studied the effect of several cationic antiseptics on energy migration from the light-harvesting LH2 complex to the LH1-RC complex in the chromatophores of the bacteria *Rhodobacter sphaeroides* (Strakhovskaya et al. 2021). Antiseptics have been found to reduce the efficient transfer of energy between these complexes. We explained the observed effects by the interaction of antiseptics with anionic phospholipids of *R. sphaeroides* chromatophores, which make up more than 40% of the lipids of these membranes. This leads to structural changes in lipid packing and disrupts the optimal conditions for energy transfer between the pigment rings of light-harvesting complexes in the membrane. It is of interest to continue studying the effects of these antiseptics on energy transfer and fluorescence intensity in photosynthetic membranes with different content of lipids and phototransforming complexes. In this work, we studied the effect of cationic antiseptics on energy transfer and fluorescent intensity in chromatophores of purple bacteria *Rhodospirillum rubrum* containing only LH1-RC complexes. These membranes have a lower molar content of anionic phospholipids than those of *R. sphaeroides*, both in membrane preparations and in LH1-RC complexes isolated from them (Nagatsuma et al. 2019). The light-harvesting complex LH1 of *R. rubrum* chromatophores has a more flexible, dynamic, and labile structure than that of *R. sphaeroides* bacteria (Jamieson et al. 2002; Bahatyrova et al. 2004). It was found that the addition of antiseptics leads

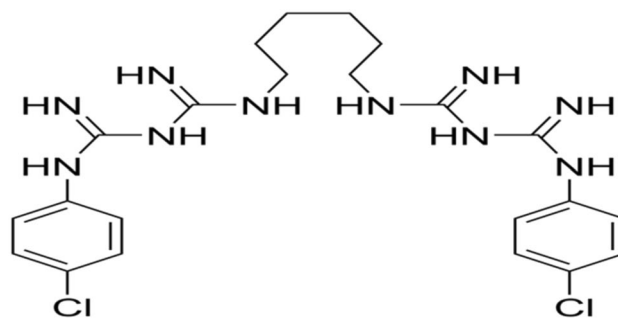
to a disproportionate increase in the fluorescence lifetime and quantum yield of the light-harvesting bacteriochlorophyll (BChl) molecules. The mechanisms leading to the violation of the well-known proportionality relation between the lifetime and the quantum yield of fluorescence are discussed.

Materials and methods

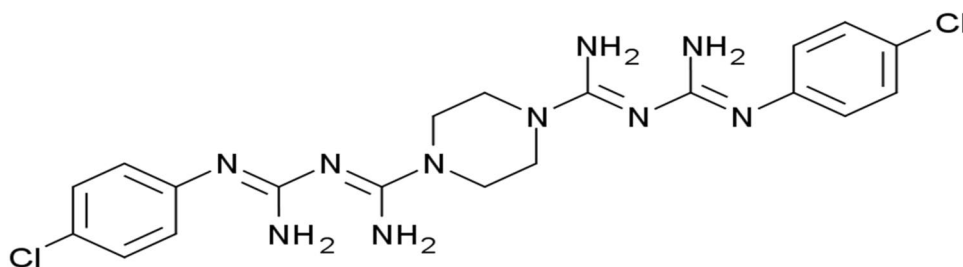
Chromatophores were isolated by suspending cells of *R. rubrum* bacteria in 10 mM Tris–HCl buffer (pH 8.0) and disrupting them using an ultrasonic disintegrator. Intact cells and cell wall debris were removed by differential centrifugation as described in (Makhneva et al. 1997).

The following cationic antiseptics were used:

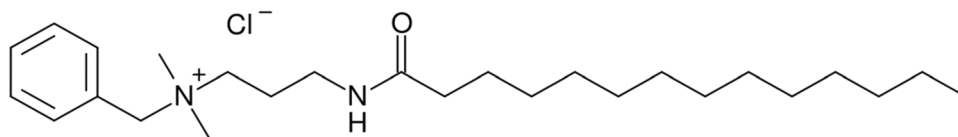
Chlorhexidine



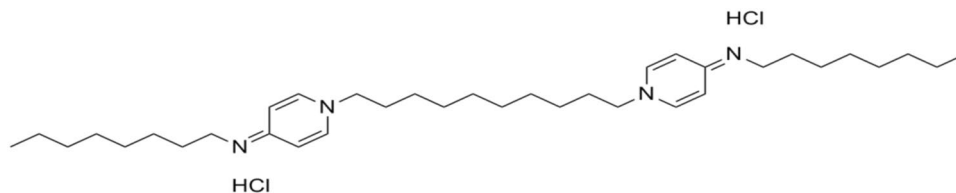
Picloxydine



Miramistin



Octenidine



Ready-made chlorhexidine bigluconate 20%, picloxydine dihydrochloride 0.05% (Vitabact), miramistin 0.01%, and octenidine 0.1% (Octenisept) were diluted with distilled water to obtain antiseptic solutions with concentrations in the range of 5–50 μM . Taking into account that Octenisept contains, in addition to octenidine, 2% phenoxyethanol, phenoxyethanol diluted with distilled water to 1–10 mM was used as an additional control. Chromatophores from stock solution were added to 1 ml of buffer with antiseptic or to the same volume buffer without antiseptic as a control. The measurements were performed after 5 min of chromatophore incubation at room temperature. The final concentration of chromatophores in the samples corresponded to $\sim 10 \mu\text{M}$ of photoactive pigment (P).

Absorption spectra were recorded with a modified Hitachi-557 spectrophotometer; fluorescence spectra, with a Horiba-Jobin-Yvon Fluorolog-3 spectrofluorimeter equipped with high sensitive IR photomultiplier Hamamatsu 5509-72. Spectra were recorded with excitation at 370 nm. The bandwidth was 10 nm for excitation light and 5 nm for emission light.

The fluorescence decay kinetic curves were measured at the maximum of LH1 fluorescence band (890 nm) using Becker & Hickl time correlated single photon counting system (TCSPC) with extremely high dynamic hybrid photodetector HPM-100-07. A Tema-150 femtosecond laser system (Avesta-Project LLC, Russia) was used as a source of exciting light. It generated 300-fs light pulses at 370 nm (repetition frequency, 80 MHz; average radiation power, 2.8 W; single pulse energy, 34 nJ). In the experiments, the energy of the exciting light pulses was reduced using neutral light filters to a level determined by the sensitivity of the recording system. The average radiation power density was $3 \times 10^{-4} \text{ W/cm}^2$. The fluorescence kinetics was approximated using the two-exponential fitting. The decay times τ were calculated using the least-squares fitting algorithm taking into account the instrumental response function (IRF) with FWHM ≈ 16 ps.

The measurements were repeated three times, and the mean values with standard error were used to calculate lifetime (τ) and yield (F) of fluorescence. The dependences of these values on the concentration of the added antiseptic are given in tables and figures. The figures with the fluorescence spectra show the most representative results. All calculation

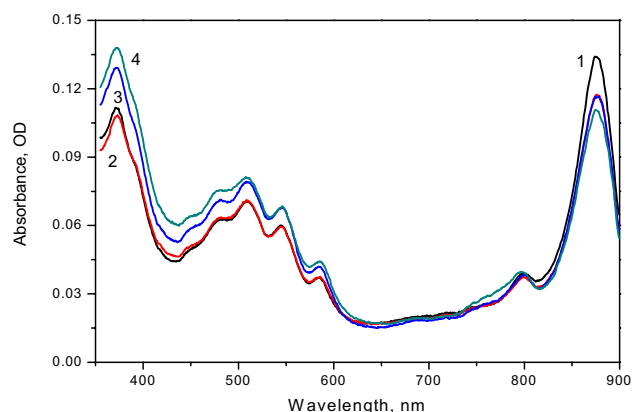


Fig. 1 Absorption spectra of *R. rubrum* chromatophores: control sample (1, black); samples after a 5-min exposure to octenidine at a concentration of 7.5 μM (2, red), 15 μM (3, blue), and 22.5 μM (4, green)

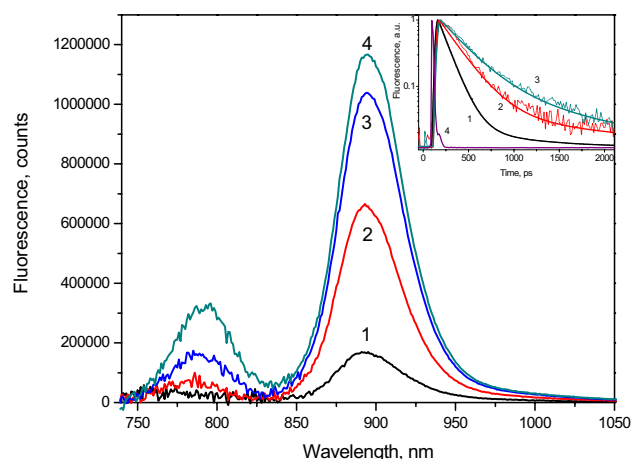


Fig. 2 Fluorescence spectra of *R. rubrum* chromatophores: control sample (1, black); samples with octenidine at a concentration of 7.5 μM (2, red), 15 μM (3, blue) and 22.5 μM (4, green). The optical density of the sample in the absorption band at 880 nm was about 0.15. The time of incubation with octenidine was 5 min. Fluorescence spectra were excited at 370 nm. The inset shows the fluorescence decay kinetics of control sample (1, black) and samples with octenidine at concentration of 7.5 μM (2, red) and 22.5 μM (3, green). Points – data file, solid line – two-exponential fitting. The purple curve (4) on panel shows the instrumental response function with fwhm ≈ 16 ps

and drawing were performed using the Origin 8.1 software (OriginLab, USA).

Results

In a previous work (Strakhovskaya et al. 2021), we studied the effect of four cationic antiseptics at concentrations from 25 to 100 μM on energy transfer in *R. sphaeroides* chromatophores. The effect of antiseptics on energy transfer was found to increase in the following order: miramistin, chlorhexidine, picloxydine, octenidine. At the same time, octenidine at a concentration of 100 μM started to exhibit a pronounced damaging effect on the pigment apparatus of chromatophores. This was manifested in a decrease in the fluorescence intensity of light-harvesting BChl. As a result of the effect of antiseptics, a strong increase in the fluorescence intensity of monomeric BChl and bacteriopheophytin (BPh) was also observed. The sensitivity of *R. rubrum* chromatophores to the applied antiseptics was found to be significantly higher than that of *R. sphaeroides* chromatophores. As in the case of *R. sphaeroides* chromatophores, octenidine has the strongest effect on the efficiency of energy transfer and fluorescence quantum yield.

Figures 1 and 2 show the absorption and fluorescence spectra of *R. rubrum* chromatophores in the control and after the addition of octenidine. The main components of the absorption spectra are as follows: absorption band of porphyrins with a maximum at 370 nm (Soret band), absorption bands of carotenoids in the region of 470–550 nm, Q_X absorption band of BChls with a maximum at ~ 590 nm, Q_Y absorption band of BChl RC at 800 nm, and Q_Y absorption

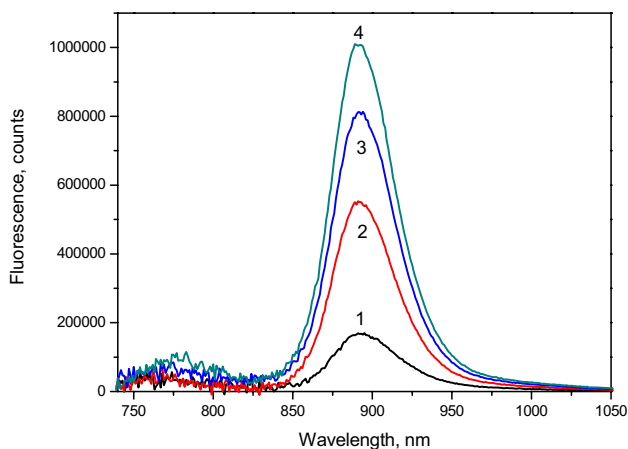


Fig. 3 Fluorescence spectra of *R. rubrum* chromatophores: control sample (1, black); samples with miramistin at a concentration of 10.5 μM (2, red), 20 μM (3, blue) and 30 μM (4, green). The optical density of the sample in the absorption band at 880 nm was about 0.15. The time of incubation with octenidine was 5 min. Fluorescence spectra were excited at 370 nm

band of BChl LH1 and RC at 880 nm. The contribution to the absorption spectrum in the region of ~ 750 nm, which increases with an increase in the antiseptic concentration, is associated with monomeric BPh, and also, according to (Trissl et al. 1999), with monomeric BChl. The reasons leading to the appearance of “free” BPh and BChl molecules under the action of antiseptics are discussed in (Strakhovskaya et al. 2021).

In the fluorescence spectrum, the main maximum near 900 nm is due to the fluorescence of the LH1 complex. It can be seen from Fig. 2 that the intensity of the LH1 complex fluorescence increases with an increase in the antiseptic concentration to 22.5 μM . With further addition of the antiseptic, the fluorescence intensity begins to decrease. The intensity of the band at ~ 770 nm, which appears upon the addition of the antiseptic and is associated with the fluorescence of BPh and monomeric BChl, strongly increases at antiseptic concentrations above 22.5 μM . These changes in the fluorescence intensity are obviously due to a disruption of the intact state of bulk BChl forms with an absorption maximum at ~ 880 nm caused by exposure to the antiseptic at these concentrations.

Unlike in *R. sphaeroides*, miramistin was the second most effective after octenidine in affecting *R. rubrum* chromatophores. The fluorescence spectra of *R. rubrum* chromatophores in the control and in the presence of miramistin are shown in Fig. 3. It should be noted that even in the presence of 30 μM of this antiseptic, there was no distinct band of the degradation products at 760 nm in the absorption spectrum. The intensity of the corresponding fluorescence at 790 nm also changed insignificantly (Fig. 3).

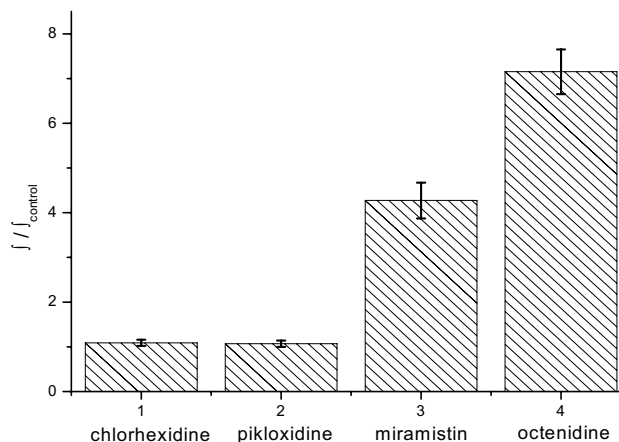


Fig. 4 Integral area of the main fluorescence band of *R. rubrum* chromatophores in the region of 900 nm in samples containing 20 μM chlorhexidine, picloxydine, miramistin, and octenidine, as compared with the fluorescence of the control sample (the area under the fluorescence curve for the control sample was taken as unity). The measurements were repeated three times. The bar shows the standard deviation from mean value

Table 1 Lifetimes (τ_1, τ_2) and amplitude (a_1, a_2) of two components of the fluorescence kinetics of *R. rubrum* chromatophores recorded at 885 nm at various concentrations of octenidine and miramistin ($\lambda_{ex} = 370$ nm)

	Control	Octenidine			Miramistin		
Concentration, μM		7.5	15	22.5	10.5	20	30
τ_1, ps	117 ± 6	214 ± 10	269 ± 13	263 ± 16	179 ± 8	216 ± 13	229 ± 12
$a_1, \%$	98.7 ± 5.3	96.7 ± 4.8	94.2 ± 4.6	88.5 ± 6.2	98.8 ± 5.1	96.5 ± 4.2	96.3 ± 6.9
τ_2, ps	678 ± 21	1254 ± 34	1041 ± 52	908 ± 45	961 ± 36	1189 ± 47	1097 ± 63
$a_2, \%$	1.3 ± 0.2	3.3 ± 0.4	5.8 ± 0.5	11.5 ± 0.7	1.2 ± 0.2	3.5 ± 0.4	3.7 ± 0.3

*The table shows the mean value for the three measurements and the standard error

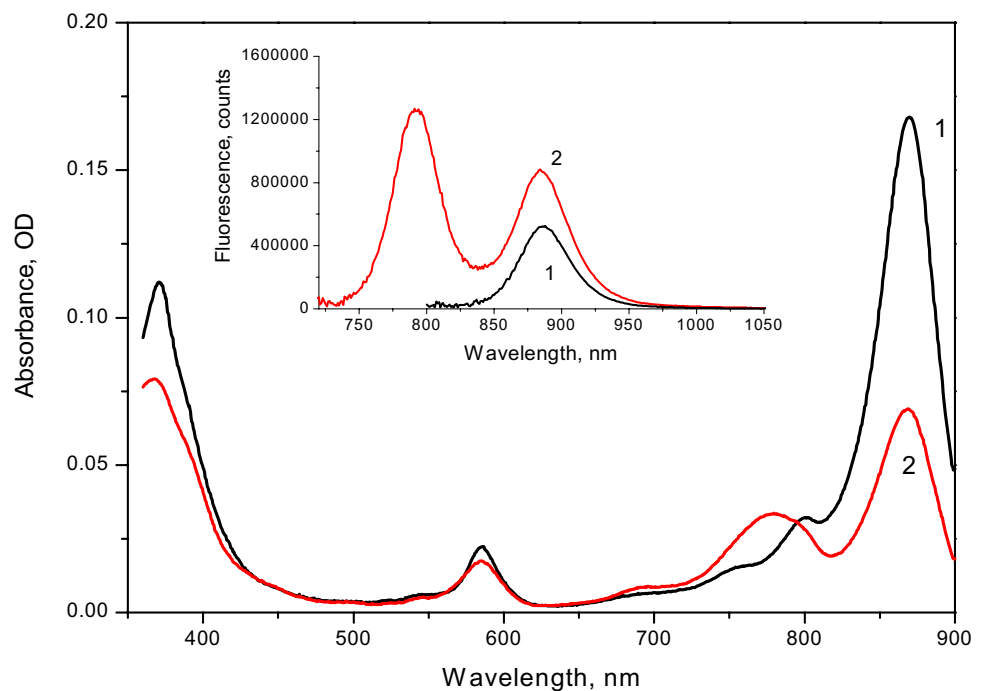
Chlorhexidine and picloxydine had minimal effect on *R. rubrum* chromatophores. As an example, Fig. 4 shows the integral areas of the main fluorescence band of chromatophores in the region of 900 nm in samples containing 20 μM chlorhexidine, picloxydine, miramistin, and octenidine, in comparison with the fluorescence of the control (the area under the fluorescence curve for the control sample was taken as a unit).

As can be seen from the figures, the main effect of adding antiseptics to *R. rubrum* chromatophores is an increase in the intensity of the main fluorescence band of BChl preparations in the region of 900 nm. This indicates, in particular, a decrease in the efficiency of energy transfer from light-harvesting pigments to RC (Strakhovskaya et al. 2021). We measured the fluorescence decay kinetics in chromatophores after the addition of miramistin or octenidine, two antiseptics that cause the greatest increase in fluorescence. Kinetic analysis was performed using a two-exponential fitting. The results of kinetics decomposition into components for

samples containing octenidine or miramistin are given in Table 1. One can see a sequential increase in the duration of the fast component of the fluorescence kinetics (τ_1). Such an increase in the value of τ_1 reflects a decrease in the efficiency of energy migration from light-harvesting pigments to RC upon the addition of the antiseptic (Strakhovskaya et al. 2021). An increase in the duration of the second component τ_2 is also observed.

It should be noted that the presence of carotenoid molecules in chromatophores significantly reduces the effect of antiseptics on the pigment–protein complex. Our studies of *R. rubrum* chromatophores isolated from the G-9 carotenoid-free strain showed that exposure to even moderate concentrations of octenidine or miramistin leads to significant disruption of the structure of BChl molecules. The disruption is accompanied by a significant decrease in the BChl absorption band at 870 nm and an increase in the absorption region of monomeric pigments Bchl and BPh at 760–780 nm. As an example, Fig. 5 shows how the absorption and fluorescence

Fig. 5 Absorption spectra of *R. rubrum* G-9 carotenoidless chromatophores: control sample (1) and after 5-min exposure with miramistin at a concentration of 30 μM (2). Insert—fluorescence spectra of the control sample (1) and samples with 30 μM miramistin (2). Fluorescence spectra were excited at 370 nm



spectra of strain G-9 chromatophores change as a result of exposure to 30 μM miramistin. Under these conditions, BChl fluorescence at 890 nm increases only by $\sim 40\%$; however, an intense band of fluorescence of pigment degradation products appears at 790 nm. This effect is fundamentally different from the effect of miramistin on chromatophores isolated from wild-type bacteria. The exposure of these wild-type chromatophores to the same concentrations of miramistin led to an insignificant change in the absorption spectrum and multiple increases in the BChl fluorescence intensity at 890 nm, while the fluorescence band of degradation products increased only slightly (Fig. 3). The obtained data confirm the stabilizing structural role of carotenoids in photosynthetic proteins (Fraser et al. 2001).

Discussion

As noted earlier, in *R. sphaeroides* chromatophores, the addition of the studied antiseptics led to a decrease in the efficiency of energy transfer from the light-harvesting LH2 complexes to the LH1-RC core complex (Strakhovskaya et al. 2021). This decrease is likely due to antiseptic-induced disturbances in the photosynthetic membrane structure. The effect of the antiseptics on *R. sphaeroides* chromatophores increased in the following order: miramistin, chlorhexidine, picloxydine, octenidine. In this work, we studied the effect of cationic antiseptics on the energy transfer in the core complex in *R. rubrum* chromatophores. Miramistin was the second most effective after octenidine in affecting *R. rubrum* chromatophores.

It is possible that the effect of cationic antiseptics on energy transfer from LH1 to RC in *R. rubrum* chromatophores differs from that in *R. sphaeroides* due to the difference in the lipid composition of the photosynthetic membranes of the two bacterial species. According to Nagatsuma et al. (2019), the chromatophores of *R. sphaeroides* contain 42 mol% of charged lipids [phosphoglycerol (PG) and cardiolipin (CL)] and 58 mol% of uncharged lipids [phosphatidylethanolamine (PE) and phosphatidylcholine (PC)]. In the chromatophores of *R. rubrum*, the fraction of charged lipids is reduced to 33 mol%. This difference is even more pronounced for preparations of the LH1-RC core complex isolated from chromatophores: in *R. sphaeroides*, the fraction of negatively charged phospholipids is 90%; in *R. rubrum*, 60%.

Analysis of the structure of the isolated LH1-RC complexes of *Rhodospseudomonas palustris* showed that on the periplasmic side of this complex there are two PG molecules, 24 PC molecules, and 12 molecules of the detergent used to isolate these complexes. In membrane-bound complexes, these 12 positions are presumably occupied by lipid molecules (Swainsbury et al. 2021). Since the structures of

the core complexes of bacteria *R. palustris*, *R. sphaeroides* and *R. rubrum* are very similar, it can be assumed that in *R. sphaeroides* and *R. rubrum*, dozens of lipid molecules are localized on the periplasmic surface of these complexes.

When the complexes are immersed in the membrane, their lateral surfaces are surrounded by lipids of the membrane bilayer. Bilayer height is ~ 40 Å. According to the structural model of the LH1-RC complex of *R. rubrum* presented in Fotiadis et al. (2004), belts of aromatic amino acids of LH1 proteins interacting with the lipid bilayer surround the outer circumferences of the complex at the border of polar and non-polar regions of membrane lipids on both periplasmic and cytoplasmic sides of the complex. This may be important for the stabilization of the RC within the LH1 complex. Both the light-harvesting BChl molecules in the LH1 ring and the photoactive BChl dimer in the RC are located in the protein at the level of the hydrophobic part of the surrounding membrane bilayer.

When interacting with membranes, the molecules of cationic antiseptics must first be “adsorbed” on negatively charged heads of lipids, both included in the membrane bilayer and covering the surface of the RC. As shown in our previous study of a model membrane containing cardiolipin (Kholina et al., 2020), octenidine promotes the formation of a microdomain in the cardiolipin bilayer, is sorbed on the microdomain as a micellar aggregate, and removes adjacent lipid molecules from the bilayer. The non-polar parts of the molecules of cationic antiseptics can probably penetrate into the hydrophobic regions of photosynthetic membranes and phototransforming complexes and affect their structure and functional state. The fact that octenidine has the strongest disturbing effect on energy transfer in *R. rubrum* LH1-RC is possibly due to the presence of two spatially separated positive charges and two hydrophobic tails at the ends of the molecule. Disruption of the lipid membrane structure upon interaction with an antiseptic molecule was confirmed by molecular dynamics modeling and discussed in our previous work by Strakhovskaya et al. (2021), Kholina et al. (2020). Miramistin molecules carry a single positive charge but have the most extended hydrophobic tail that penetrates to the middle of the bilayer. Thus, miramistin molecules can obviously affect the contacts of LH1-RC complexes with the central hydrophobic region of the chromatophore membrane. The structures of chlorhexidine and picloxydine make them the least lipophilic. Thus, they have a minimal effect on the energy transfer in *R. rubrum* chromatophores.

The observed differences in the effect of the studied antiseptics on the energy transfer from LH1 to RC in *R. sphaeroides* and *R. rubrum* chromatophores may be related to some structural and dynamic differences between these complexes. Thus, the LH1 ring in *R. rubrum* complexes is sufficiently flexible to be able to take both circular and elliptical conformations (on both periplasmic and cytoplasmic

sides) (Jamieson et al. 2002). It is assumed that this is necessary for the release of the reduced quinone from the fully closed ring into the membrane. There is no such need for *R. sphaeroides* ring structures due to the presence of PufX, a special protein involved in the exchange of quinones with the membrane. The flexibility is evidently provided by some features of the structure and packing of the core complexes of *R. rubrum* and *R. sphaeroides*. In particular, in *R. rubrum*, BChl molecules have geranylgeraniol tails instead of phytol tails. The geranylgeraniol tail accounts for ~30% of the molecular weight of the pigment and is believed to play an important structural role in the assembly and stability of the core complex (Bullough et al. 2009). As noted in (Bahatyrva et al. 2004), there are “relatively weak associations that govern the aggregation of the protomers ($\alpha 1\beta 1\text{Bchl}2$) comprising the LH1 complex. The demonstration that the linkage between adjacent protomer units is flexible and can even be uncoupled at room temperature in a detergent-free membrane bilayer provides a rationale for the dynamic separation of individual protomers”. Such flexibility of the *R. rubrum* LH1 structure (“breathing motions of the ring”),

possibly, determines the greater sensitivity of the LH1-RC complex of *R. rubrum* to the action of antiseptics.

The transfer of excitation energy from LH1 to RC BChl (P) occurs in a horizontal plane parallel to the plane of the photosynthetic membrane (Hunter et al. 1989). Possibly, geometric distortions of the closed circuit of the light-harvesting BChl chain, violation of the planarity of this system with respect to the energy acceptor (P) upon binding to photosynthetic membranes, and the introduction of hydrophobic parts of antiseptic molecules into the membranes lead to the observed changes in energy transfer.

As noted above, the experimental fluorescence decay kinetics was approximated using a two-exponential fitting (Table 1). According to numerous studies, Borisov et al. (1985), Somsen et al. (1994), Bernhardt and Trissl (2000), we attributed the fast component τ_1 to the fluorescence of active LH1, and the slow component τ_2 to the fluorescence of those LH1 complexes that lost their RC complexes during the isolation process (Scheuring and Sturgis 2009). Figure 6 shows the dependences of τ_1/τ_0 and F_1/F_0 on the concentrations of octenidine and miramistin. Here, τ_0 and F_0 denote,

Fig. 6 Curves of τ_1/τ_0 and F_1/F_0 ratios for *R. rubrum* chromatophores vs. octenidine and miramistin concentrations. τ_0 and F_0 are, respectively, the fluorescence lifetime and yield for the LH1-RC complex in the absence of the antiseptics; τ_1 and F_1 are respectively the fluorescence lifetime and yield for the complex exposed to various concentrations of the antiseptics. The measurements were repeated three times. The bar shows the standard deviation from mean value

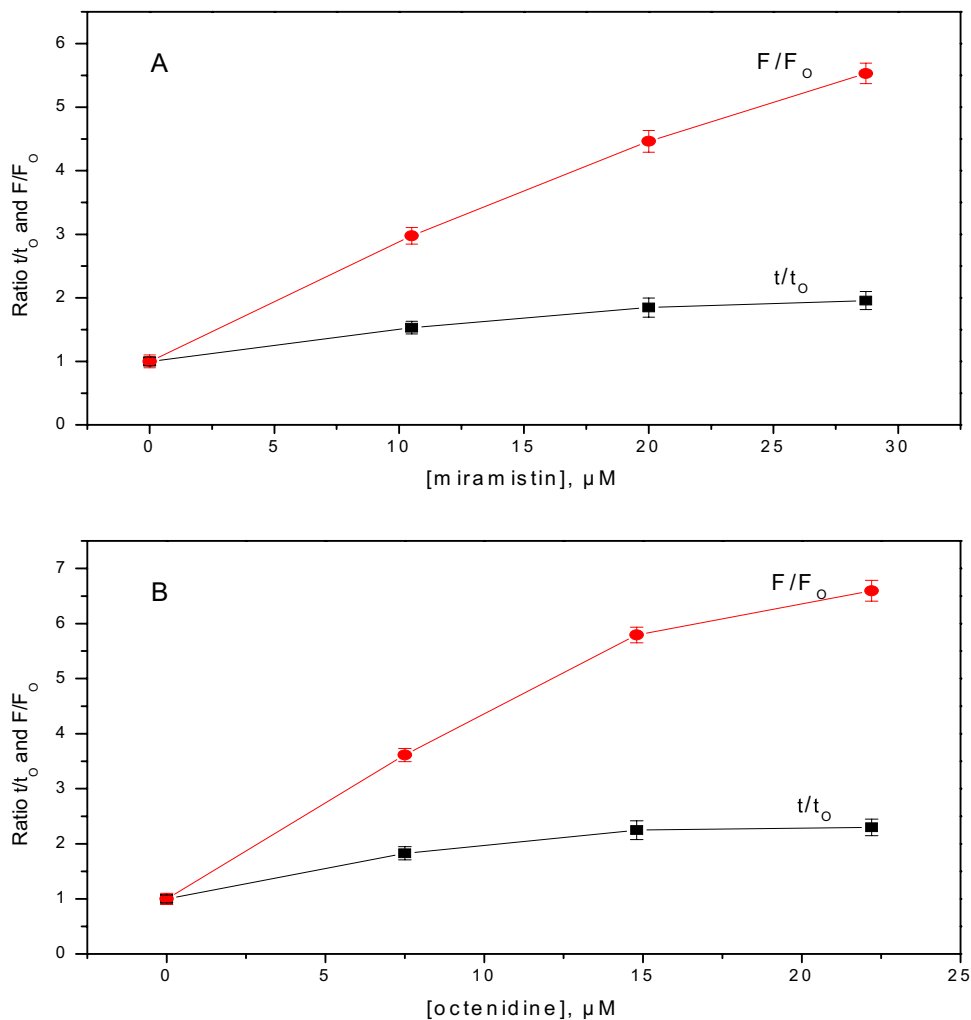


Table 2 The fluorescence lifetime of fast component (τ_1) and τ_1/τ_0 and F_1/F_0 ratios for *R. rubrum* chromatophores at different concentrations of octenidine and miramistin

Octenidine				Miramistin			
μM	τ_1 , ps	τ_1/τ_0	F_1/F_0	μM	τ_1 , ps	τ_1/τ_0	F_1/F_0
0	117 ± 6	1 ± 0.07	1 ± 0.1	0	117 ± 6	1 ± 0.07	1 ± 0.1
7.5	214 ± 10	1.8 ± 0.12	3.6 ± 0.12	10.5	179 ± 8	1.5 ± 0.1	3 ± 0.13
15	269 ± 13	2.3 ± 0.15	5.8 ± 0.14	20	216 ± 13	1.9 ± 0.15	4.50 ± 0.17
22.5	263 ± 16	2.2 ± 0.17	6.6 ± 0.19	30	229 ± 12	2 ± 0.14	5.5 ± 0.16

τ_0 and F_0 are respectively the fluorescence lifetime and fluorescence yield for the LH1-RC complexes in the absence of antiseptics; τ_1 and F_1 are the fluorescence lifetime and fluorescence yield of the complexes exposed to various concentrations of antiseptics; $\lambda_{\text{ex}} = 370$ nm, $\lambda_{\text{reg}} = 885$ nm

*The table shows the mean value for the three measurements and the standard error

respectively, the fluorescence lifetime and yield for the LH1-RC complex in the absence of antiseptics; τ_1 and F_1 , the fluorescence lifetime and yield for the same complexes exposed to various concentrations of miramistin or octenidine. The values of τ_1/τ_0 and F_1/F_0 for various concentrations of antiseptics are given in Table 2.

Figure 6 shows that the addition of miramistin leads to an approximately twofold increase in τ_1/τ_0 and an approximately 5.5-fold increase in F_1/F_0 . For octenidine, these values increase ~2.2 and ~6.6 times, respectively. The obvious conclusion is that the addition of antiseptics leads to a disproportionate change in the lifetime and quantum yield of fluorescence of the LH1-RC complex.

It is generally accepted that the drastic decrease in the fluorescence lifetime τ of light-harvesting complexes of photosynthetic organisms to tens of picoseconds, as compared with the fluorescence lifetime of pigments (Chl, BChl) in a solution (several nanoseconds), occurs due to efficient energy migration (characterized by the energy migration rate constant k_m) from the light harvester molecules to the RC. In the case under consideration, $k_m > 1/\tau$, where τ is the fluorescence lifetime of an energy donor in the absence of an acceptor, which ensures a high (up to 100%) efficiency of photosynthetic conversion of solar energy. It is generally assumed that the reduction in τ due to the energy migration to the RC is accompanied by a proportional decrease in the fluorescence yield φ , described by the equation $\varphi = k_{\text{fl}} \cdot \tau$. Here, k_{fl} is the fluorescence rate constant (assumed to remain unchanged). Changes in τ and φ are taken to occur as a result of a change in the energy migration rate constant k_m . It can be seen from Fig. 4 and Table 2 that with the addition of antiseptics the fluorescence intensity increase rate F_1/F_0 significantly (2–3 times) outstrips the fluorescence lifetime increase rate τ_1/τ_0 . Consequently, in the described experiments, a violation of the $\varphi = k_{\text{fl}} \cdot \tau$ relationship was found, which is valid for the mechanism of dynamic fluorescence quenching.

Apparently, the effect of antiseptics on the lifetime (τ) and yield (φ) of LH1-RC fluorescence can be considered as two complementary processes. On the one hand, the addition of

the antiseptics leads to a decrease in the efficiency of the interaction between the LH1 and RC complexes and, as a consequence, to a decrease in the rate constant k_m of energy migration from LH1 to RC. In this case, the decrease in k_m should be accompanied by an increase in the fluorescence yield.

The value of k_m for chromatophores can be easily determined from the well-known equation $\tau = 1/(\sum k_i + k_m)$, where $\sum k_i$ is the sum of constants of intramolecular deactivation processes. According to Table 2, for control samples, $\tau^{\text{contr}} = 117$ ps, $\sum k_i \approx 680$ ps; for samples treated with 22.5 μM octenidine, $\tau^{\text{oct}} = 260$ ps, $\sum k_i \approx 900$ ps. Thus, in the presence of the antiseptic, a significant decrease in the migration rate constant is observed: $k_m^{\text{contr}} = 7 \cdot 10^9 \text{ s}^{-1}$, $k_m^{\text{oct}} = 2.7 \cdot 10^9 \text{ s}^{-1}$. Treatment of chromatophores with miramistin at a concentration of 30 μM results in $k_m^{\text{mir}} = 3.3 \cdot 10^9 \text{ s}^{-1}$. These calculations were performed under the strict assumption that the change in the fluorescence yield φ of the samples is proportional to the fluorescence lifetime.

As can be seen from Fig. 4 and Table 2, in complement to an increase in the fluorescence yield due to a decrease of k_m , an additional increase in the LH1 fluorescence intensity is observed at each concentration of the antiseptic. This additional increase is not associated with a change in the efficiency of the interaction between LH1 and RC. Thus, with the addition of miramistin until the final concentration of 30 μM is reached, an approximately twofold increase in τ_1/τ_0 and an approximately 5.5-fold increase in F_1/F_0 are observed. If a twofold increase in φ can be easily explained by dynamic quenching of fluorescence ($\varphi = k_{\text{fl}} \cdot \tau$), then an increase in F_1/F_0 to 5.5 requires separate consideration.

We assume here, as before in the work of Strakhovskaya et al. (2021), that an additional increase in the fluorescence intensity that occurs upon treating chromatophores with antiseptics is associated with an increase in the intramolecular fluorescence rate constant k_{fl} . It should be noted that, despite an increase in k_{fl} , the fluorescence lifetime does not change. In other words, exposure of the BChl molecule to the antiseptic agents leads to a redistribution of the probabilities

of intramolecular deactivation of the excited state in favor of the fluorescence rate constant k_{fl} . In isolated Chl (BChl) molecules, transitions to the triplet state (~66%) and fluorescence (~32%) are the main channels for deactivation of the excited state (Gurinovich et al. 1976; Connolly et al. 1982; Becker et al. 1991). Thus, for monomeric chlorophyll molecules in the absence of quenchers, the approximate equation $k_{ST} + k_{fl} \approx 1$ holds well. Therefore, the redistribution of the probabilities of intramolecular transitions in BChl molecules of the LH1 complex implies a decrease in the probability of the singlet–triplet conversion k_{ST} and a corresponding increase in the probability of a transition accompanied by fluorescence k_{fl} . At the same time, we believe that the sum of $k_{ST} + k_{fl}$ is the same for samples treated with antiseptics and for the control samples.

What could be the reason of the increase in k_{fl} ? It is well known that the properties of the S^* and T_1 states of chlorophyll-like molecules are largely determined by the transition that occurs upon excitation of the molecule. In the Chl (BChl) molecule, the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions can occur. The energy levels S^* and T_1 states are closer to each other in the $n-\pi^*$ state, while the probability of radiative transitions is low. In non-polar solvents, the $n-\pi^*$ level of Chl is lower than the $\pi-\pi^*$ level and $n \rightarrow \pi^*$ transitions prevail. In polar solvents, the energy level of the $\pi-\pi^*$ state becomes lower than that of the $n-\pi^*$ state. As a result, $\pi \rightarrow \pi^*$ transitions prevail and the fluorescence yield becomes large, while the fluorescence lifetime remains virtually unchanged Heath (1969). It is possible that the addition of charged antiseptic molecules, especially octenidine and miramistin, enhances the polarization of the medium in the microenvironment of the BChl molecules of antenna complex causing thereby an increase in the fluorescence yield. It should be noted that the octenidine molecule has two spaced positive charges, while the miramistin molecule has only one. Therefore, the polarizing effect of octenidine should be higher. This difference in the enhancement of polarization by these agents is manifested in a greater increase in the fluorescence intensity under the action of octenidine as compared to miramistin (Fig. 6).

Concluding remarks

In this work, we continued to study the effect of various cationic antiseptics on the fluorescence intensity and the efficiency of energy migration in bacterial chromatophores. The study was carried out on *R. rubrum* chromatophores. The difference between this object of study and the previously studied *R. sphaeroides* chromatophores Strakhovskaya et al. (2021) is that the *R. rubrum* chromatophore has only one light-harvesting complex LH1, which efficiently transfers the

electron excitation energy to RC. It was found that among the studied antiseptics, octenidine and miramistin exert the strongest influence on the efficiency of the interaction between LH1 and RC. Exposure to these compounds led to a 5.5- to 6.6-fold increase in the fluorescence intensity of the LH1 complex at 880 nm, while the rate constant of energy migration $LH1 \rightarrow RC$ decreased only by a factor of 2.0–2.2. Therefore, the increase in the fluorescence intensity is 2.5–3 times faster than the increase in the lifetime of fluorescence of light-harvesting BChl. The effect of the antiseptics on the lifetime and yield of the fluorescence of the LH1-RC complex is considered in this work as resulting from two complementary processes. On the one hand, exposure to antiseptics leads to a decrease in the efficiency of the interaction between the LH1 and RC complexes and, as a consequence, to a decrease in the energy migration rate constant k_m and an increase in the fluorescence lifetime τ . Simultaneously, the fluorescence intensity increases by a factor of 2.5–3. In accordance with the known ratio $\varphi = k_{fl} \cdot \tau$, the actual increase in φ is by a factor of 5.5–6.6. In our opinion, the additional increase in the fluorescence intensity φ is due to an increase in the intramolecular fluorescence rate constant k_{fl} of light-harvesting BChl. An increase in k_{fl} can occur as a result of a change in the polarity of the microenvironment of BChl molecules included in the LH1 complex. A similar violation of the proportionality of the τ -to- φ ratio for monomeric molecules Chl *a*, Pheo *a*, and BChl *a* in solvents with different polarities was observed earlier in Connolly et al. (1982), Becker et al. (1991).

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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