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EXPERIMENTAL ARTICLES

Photosynthetic Activity and Components of the Electron Transport Chain in the Aerobic Bacteriochlorophyll *a*-Containing Bacterium *Roseinatronobacter thiooxidans*

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Abstract—Bioenergetics of the aerobic bacteriochlorophyll *a*-containing (BCl *a*) bacterium (ABC bacterium) *Roseinatronobacter thiooxidans* is a combination of photosynthesis, oxygen respiration, and oxidation of sulfur compounds under alkaliphilic conditions. The photosynthetic activity of *Rna. thiooxidans* cells was established by the photoinhibition of cell respiration and reversible photobleaching discoloration of the BCl *a* of reaction centers (RC), connected by the chain of electron transfer with cytochrome c_{551} oxidation. The species under study, like many purple bacteria and some of the known ABC bacteria, possesses a light-harvesting pigment–protein (LHI) complex with the average number of 30 molecules of antenna BCl *a* per one photosynthetic RC. Under microaerobic growth conditions, the cells contained bc_1 complex and two terminal oxidases: cbb_3-cytochrome oxidase and the alternative cytochrome oxidase of the a_3 type. Besides, *Rna. thiooxidans* was shown to have several different soluble low- and high-potential cytochromes *c*, probably associated with the ability of utilizing sulfur compounds as additional electron donors.

Key words: aerobic bacteriochlorophyll *a*-containing bacteria, *Roseinatronobacter thiooxidans*, photosynthesis, cytochromes.

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Aerobic bacteriochlorophyll *a*-containing bacteria (ABC) are a group of chemoorganotrophs capable, along with respiration, of anoxygenic photosynthesis. About 30 genera of ABC bacteria are known, and the number of newly described species is continuously growing [1]. These microorganisms, belonging mainly to the α-proteobacteria, are genetically related to facultatively phototrophic non-sulfur purple bacteria are similar to them in physiological characteristics [2, 3]. BCl *a* synthesis in ABC bacteria is possible only under aerobic or microaerobic conditions and is most intensive in the dark. Due to their inability to assimilate CO_2 via ribulose bisphosphate carboxylase (RuBisCOBPC), light energy is used mainly for ATP synthesis [4]. Photosynthetic activity has been confirmed experimentally only for a few species, and ABC bacteria are as of yet the least studied in respect to energy exchange among all the known prokaryotic phototrophs [5].

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ABC bacteria include freshwater and marine species. Extremophiles existing at high temperatures, high salt concentrations, or extreme pH values are of particular interest [1]. *Roseinatronobacter thiooxidans* is the first of the discovered obligately alkaliphilic species, which grows at pH 8.5–10.4 and is capable, like some other species of ABC bacteria, of lithoheterotrophy with thiosulfate as an electron donor [6].

According to the data on phylogenetic analysis, the ABC bacteria *Rna. thiooxidans* and *Rna. monicus* [7], together with two species of alkaliphilic purple nonsulfur bacteria (PNB) of the genus *Rhodobaca* form a common cluster [8]. The presence of BCl *a* in combination with oxygen respiration, nitrate reduction, sulfur-oxidizing activity and alkaliphily imply that *Rna. thiooxidans* possesses a unique combination of energy exchange properties [6, 7].

The goal of the present work was to study the photosynthetic activity and components of the electron transport chains (ETC) in *Rna. thiooxidans*.

MATERIALS AND METHODS

Cultivation of *Rna. thiooxidans* was performed as described [6, 7], under stationary conditions at 28°C in a medium containing the following (g/l): NH_4Cl, 0.4; KH_2PO_4, 0.5; $NaNO_3$, 0.4; $MgCl_2$, 0.2; Na_2SO_4, 0.5; yeast extract, 1; Na acetate, 1; Na pyruvate, 1; NaCl, 40; $Na_2S_2O_3 \cdot 5H_2O$, 1; KCl, 0.5; $NaHCO_3$, 10; Na_2CO_3, 5; vitamin B_{12}, 10 μg; solution of trace elements, 1 ml [9]. $NaHCO_3$ (10%), Na_2CO_3 (10%), yeast extract (5%), Na acetate (10%), Na pyruvate (10%), and Na thiosulfate (10%) solutions were prepared and sterilized separately and then added to the basic medium immediately before inoculation. The final pH value was adjusted at 9.5. In order to achieve maximal accumulation of the pigments, the culture was grown in the dark and used in the late logarithmic growth phase.

Photosynthetic activity of *Rna. thiooxidans* cells was registered by two methods. Differential "photooxidized minus reduced" absorption spectra, which characterize the primary charge separation in the photosynthetic RC and electron transfer from the RC-bound cytochromes, were recorded in the cell suspension in a single-beam spectrophotometer as described [10]. The optical density of the samples in a 2-mm cuvette was 0.2 at 870 nm. Each point in the differential spectra was obtained by exposing the cuvette to light (intensity, 16 mWt/cm^2; wavelength, ≥620nm) for 20 s. In the second method, reversible photoinhibition of respiration (PIR, induced by activation of ETC of photosynthesis, the competitive respiratory chain) was measured by exposing the cell suspension in a polarographic cell to pulsed light of a different spectral composition as described previously [11]. Decrease in the rate of O_2 exchange in the samples under flashes of monochromatic light (0.2 μM quantum/m^2 s; spectral half-width of a slot, 3 nm; duration, 1 s; dark intervals, 20–60 s) was recorded in the spectral range from 400 to 930 nm. The optical absorption cross-section of the light-harvesting antenna for determination of its size, i.e., the number of BCl *a* molecules per one reaction center, was obtained by measurement of the light saturation curves for the photoreaction of respiration inhibition in pulsed monochromatic light as described [11].

Absorption spectra were recorded with a Hitachi 557 spectrophotometer. Differential absorption spectra for cytochrome detection were obtained by subtracting the spectrum of a sample oxidized by ferricyanide (1 mM) from the spectrum of a sample reduced by ascorbate (5 mM) or dithionite (100 mM) in the absorption region of α-β cytochrome bands (500–610 nm) and γ band in the region around 400 nm. The content of hemes in the samples was assayed by absorption spectra of pyridine hemochromes with extinction coefficients of 23.9, 24.0, and 25.0 mM^{-1} cm^{-1} for hemes C, B, and A in the α-bands of 550, 556, and 588 nm, respectively [12].

Fluorescence spectra were measured at 77K in a luminescent installation allowing the registration of radiation in the near infrared region [13]. The optical density of the samples was 0.1 in the infrared maximum of BCl *a* absorption.

Cytochrome oxidase activity was determined by a decrease in absorption at 615 nm of *N*,*N*,*N*′*N*′-tetramethyl-*p*-phenylenediamine, an artificial electron donor added to the samples [14]. For detection of cytochrome oxidases containing cytochrome a_3, the cell suspension was exposed to soft bubbling with carbon monoxide (6 min). Then the sample in a polarographic cell was exposed to pulsed light of a different spectral composition with a photoreversible release of CO and parallel registration of increasing oxygen consumption [15] on a device for PIR registration [11].

Protein electrophoresis in PAG. SDS electrophoresis (15% of acrylamide) was performed in the Laemmli system [16] or with 6 M urea in the Schagger–von Jagow system [17]. Protein zones were stained with a colloid solution of Coomassie G-250. Cytochromes *c* in PAG were detected by staining with 3,3',5,5'-tetramethylbenzidine (TMBZ) of heme C covalently bound with the protein [18].

Separation of cell membranes and water-soluble cell fraction. The cells were precipitated by centrifugation at 1000 *g*, washed twice with a buffer solution (50 mM Tris-HCl, pH 8.0; buffer A). Precipitated cells were treated with 0.1% lysozyme solution for 30 min at room temperature (buffer A with 2 mM EDTA) followed by an addition of DNase up to 0.01% and $MgSO_4$ up to 5 mM. The mixture was incubated for 10 min on ice and centrifuged for 30 min at 140000 *g*. The precipitate was resuspended in buffer A and the cells were sonicated twice for 15 s. The coarse membrane fragments and remainders of undestroyed cells were removed by centrifugation at 15000 *g* for 20 min. The membranes from the obtained supernatant were precipitated for 90 min at 140000 *g*. Both supernatants (140000 *g*) were combined and used for the study of water-soluble cytochromes.

Analysis of water-soluble cytochromes. The combined supernatants were successively supplemented with ammonium sulfate (AS) up to 20 and 35% (wt/vol) and stirred for 15 min; the precipitates were then collected by centrifugation (15000 *g*, 10 min). The precipitates were dissolved in buffer A; the cytochrome content in the solution was determined by spectrophotometry and by staining the protein zones separated by electrophoresis in PAG. The 35% fraction was additionally separated by hydrophobic chromatography in a butyl-TSK fractogel column (Merck, Germany). Proteins were eluted from the column by reverse AS concentration gradient (1.5–0.0 M) in buffer A.

Analysis of membrane cytochromes. Cell membranes in buffer A (10 mg/ml) were solubilized by an addition of dodecylmaltoside from 10% solution to the 1 : 1 weight ratio with the protein. The mixture was incubated on ice for 1 h with periodic stirring, laminated on a sucrose concentration gradient (10–30% in

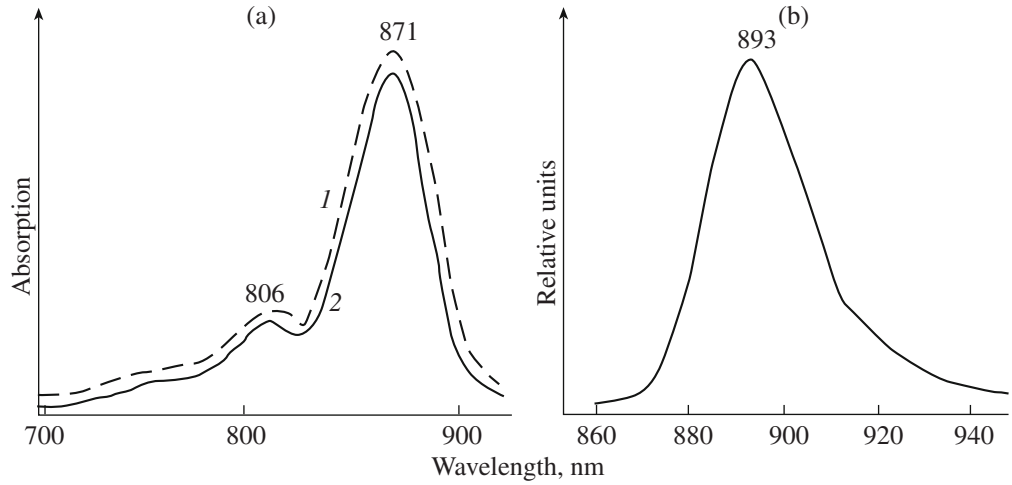

Fig. 1. Absorption and fluorescence spectra of *Rna. thiooxidans*: a, infrared absorption spectra of cell membranes (*1*) and RC-LH1 of the pigment–protein light-harvesting complex (*2*); b, low-temperature (77K) cell fluorescence spectrum. Fluorescence excitation at 600 nm; correction was made for the spectral sensitivity of the fluorescence device.

buffer A with 0.1% dodecylmaltoside), and centrifuged in a bucket rotor Ti-60 at 40000 rpm for 12 h. The obtained sucrose fractions and the initial mixture were analyzed, like water-soluble cytochromes, by spectrophotometry and by electrophoresis in PAG.

RESULTS AND DISCUSSION

Photosynthetic pigment apparatus. The content of BCl *a* in *Rna. thiooxidans* grown in the dark as described above was 3 nmol/mg of protein [6], which corresponds to the level of BCl *a* in other ABC bacteria: 1–4 nmol/mg [4]. The typical features of ABC bacteria are the quantity of BCl *a*, lower by an order of magnitude than in anaerobic purple bacteria, and suppression of pigment biosynthesis by low-intensity effective light [19].

The absorption spectrum of intact membranes of *Rna. thiooxidans* in the near infrared region had a high-intensity band at 871 and a low-intensity band at 806 nm (Fig. 1a) [6], which is typical for BCl *a*. The maximum of 871 nm corresponds to the presence of a LHI or B870 light-harvesting BCl *a*-containing pigment–protein photosynthetic complex in the cells. The absence of intensive maxima at 805 and 835–850 in the absorption spectrum implies that the membranes of *Rna. thiooxidans* have no LHII light-harvesting photosynthetic complex, which complements the LHI complex in a number of purple bacteria [20]. These data are confirmed by the fact that only one pigment–protein complex was obtained from the solubilized membranes of *Rna. thiooxidans* in the sucrose density gradient, with the absorption spectrum in the near infrared region (Fig. 1a) practically coinciding with the spectrum of intact membranes. The low-temperature spectrum of cell fluorescence has a single clearly marked maximum at 893 nm, which corresponds to the 871-nm absorption band and is typical for the LHI complex (Fig. 1b). Thus, similar to most of the studied ABC bacteria [4], the membranes of *Rna. thiooxidans* carry only the LHI light-harvesting complex.

Photosynthetic activity. The first evidence for photosynthesis in ABC bacteria was registration of reversible photobleaching of reaction centers and associated cytochrome oxidation in *Roseobacter denitrificans* [21] and then in other studied strains of these microorganisms [19, 22].

Photoinduced changes of absorption of *Rna. thiooxidans* cells typical of the photosynthetic RC were recorded in the wavelength range from 400 to 910 nm (Fig. 2). The maxima at 860 and 810 nm and the band at 605 nm observed in the "light minus darkness" differential spectrum demonstrate that the studied species contains a RC that does not differ in spectral characteristics from the RC of purple bacteria and other known ABC bacteria. In addition to the above, the differential spectrum has a cytochrome Soret band at 420 nm and an α-band at 551 nm typical for the *c*-type cytochromes (Fig. 2), which is in agreement with the previous data [23] on cytochrome c_{551} in ABC bacteria as an analogue of cytochrome c_2 of purple bacteria acting as an electron donor for the RC of photosynthesis.

The second evidence for RC photosynthetic activity is registration of the PIR spectrum of *Rna. thiooxidans.* At the exposure of intact cells to the light absorbed by BCl *a*, the rate of cell respiration was shown to decrease. This effect results from the presence of the common competitive regions of respiration and photosynthesis ETC [11]. Coincidence between the PIR spectrum and the spectrum of BCl *a* absorption in vivo (Fig. 3) is indicative of a photosynthetic process performed by the cells of *Rna. thiooxidans.* Photoinhibition of respiration in white light had been registered previously in the bacteria *Rsb. denitrificans, Erythro-*

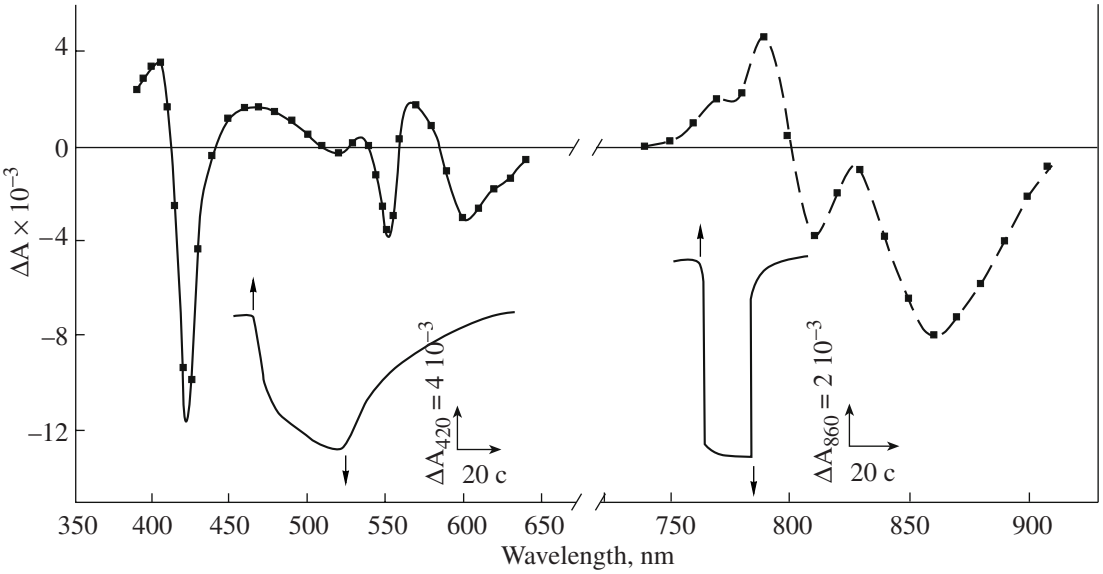

Fig. 2. Differential "light minus darkness" spectra of *Rna. thooxidans* cells. Reversible photobleaching of the photosynthetic RC in the infrared range (750–910 nm) and associated cytochrome oxidation in the visible range (400–650 nm) are presented. The kinetic curves of reversible change of absorption at 806 nm (the infrared band of RC absorption) and 420 nm (the Soret band of cytochromes *c*) are presented additionally. Arrows indicate the moments of turning the light on (↑) and off (↓).

bacter longus [21], and *Acidiphilium rubrum* [24], which was preliminary evidence for photosynthesis in ABC bacteria in the absence of action spectra.

The optical cross-section of the PIR reaction [11] determined for *Rna. thiooxidans* corresponds to the harvesting of the excitation energy from an average of 30 BCl *a* antenna molecules per each RC. These data absolutely correlate with the size of photosynthetic antenna in the RC-LHI complexes of purple bacteria [25], which implies similar if not identical organization for the pigment apparatus of photosynthesis in anaerobic and aerobic phototrophic bacteria.

The presence of carotenoids in *Rna. thiooxidans* is demonstrated by three typical overlapping bands at 480, 520 and 545 nm in the absorption spectrum (Fig. 3). The membranes of ABC bacteria are known to be rich in these pigments [4, 5, 19]. The hypothetical descent of ABC bacteria from purple bacteria is associated with the cells neutralizing the inhibiting effect of O_2 on the photosynthetic apparatus, including the increased biosynthesis of carotenoid pigments; the antioxidant activity of the latter correlates with the obligate aerobicity of ABC bacteria [2, 26]. In the PIR action spectrum of *Rna. thiooxidans* (Fig. 3), carotenoid bands are much less intensive than in the absorption spectrum; this finding is in line with the suggestion that carotenoids perform mainly non-photosynthetic functions in the cells of ABC bacteria [2].

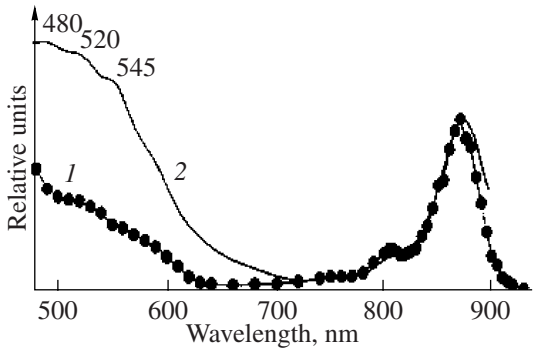

Fig. 3. The PIR spectrum (*1*) characterizing the photosynthetic activity and absorption spectrum (*2*) of intact cells of *Rna. thiooxidans*. The absorption bands of carotenoids are marked.

Membrane cytochromes. Solubilized by fractionation in the sucrose density gradient cytochromes from the membranes of *Rna. thiooxidans* were separated from the RC-LHI complex (Fig. 4a). Absorption spectra of the pyridine hemochromes of sucrose fractions had α-bands at 556 and 550 nm indicating the presence of hemes B and C, respectively, in the cytochrome proteins. The curves of distribution for these hemes had different maxima (Fig. 4a), indicating the presence of at least two cytochrome complexes in the membranes of *Rna. thiooxidans.* The measured cytochrome activity was maximal in fractions 5–7 of the gradient. In the collected fractions of the gradient, similar to the total preparation of solubilized membranes, four cytochromes *c* were distinguished by their contents: 22, 24, 30, and 32 kDa (Fig. 4b).

The polypeptide with the molecular mass of 22 kDa corresponds to the well known subunit of cytochrome c_1 within the bc_1 complex, while distribution of the

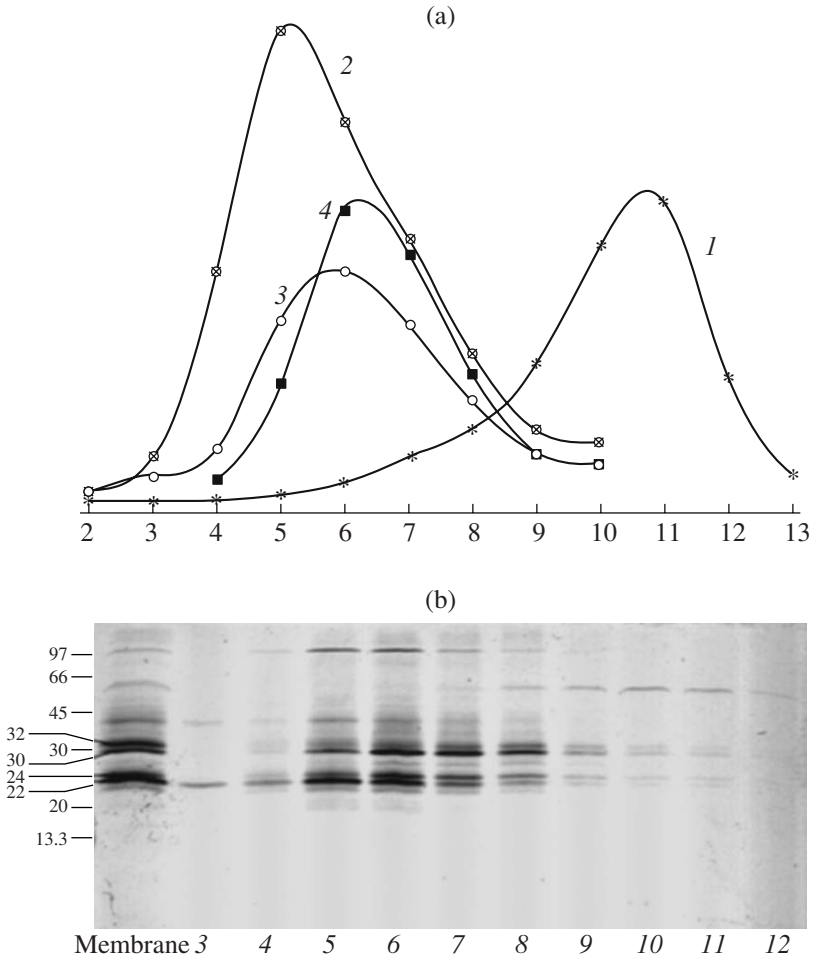

Fig. 4. Composition of membrane cytochromes of *Rna. thooxidans*: a, distribution of the RC-LHI pigment–protein complex (*1*), cytochrome *b* (*2*), cytochrome *c* (*3*), and cytochrome oxidase activity (*4*) in the fractions of solubilized membranes in sucrose density gradient. The RC-LHI complex was registered by absorption at 870 nm. The content of cytochromes *b* and *c* was calculated on the basis of absorption spectra of pyridine hemochromes. Cytochrome oxidase activity was determined by intensity of staining of the fractions with TMPD. Fraction numbers are given on the abscissa axis; b, electrophoresis of resultant fractions in the Laemmli system; polypeptide staining in PAG with TMBZ. Vertically at the left: molecular masses of the markers and revealed cytochromes *c*. Numbers of electrophoretic lanes (*3–12*) correspond to the numbers of chromatographic fractions; the first lane demonstrates the total composition of membrane cytochromes.

22-kDa cytochrome zone among the gradient fractions, including the first fractions lacking cytochrome oxidase activity (Fig. 4b), correlates with the distribution of heme B (Fig. 4a). Together, these data demonstrate the presence of the bc_1 complex in *Rna. thiooxidans.* This complex, being a common component of the respiratory and photosynthetic electron transfer chains, is a necessary ETC component in all phototrophs with the exception of filamentous green bacteria [27]. The presence of bc_1 complex has been demonstrated previously by different methods in the purple bacteria and some ABC bacteria [22].

Distribution of the cytochrome oxidase activity correlated with the distribution of two cytochrome bands: 24 and 30 kDa (Fig. 4a and 4b). In the genome of the sequenced species of ABC bacteria, *Rsb. denitrificans* [28], the genes of cbb_3 oxidase encode polypeptides with the same molecular masses (24.1 kDa with one covalently bound heme *C* and 28.8 kDa with two hemes *C*). It is known that cbb_3 cytochrome oxidase is typical for proteobacteria (including the species under study, *Rna. thiooxidans*) and is expressed under microaerobic conditions used in our experiments. Based on these data, one can assign the polypeptides of 24 and 30 kDa to the cbb_3 cytochrome oxidase and suggest the presence of this terminal electron acceptor in *Rna. thiooxidans.*

The absorption spectrum of pyridine hemochromes of the sucrose fraction 6 had a minor peak at 590 nm indicating the presence of heme A (the curve of distribution for this heme is not presented in Fig. 4a because of its low quantity). The finding of heme A was indicative of a type *a* cytochrome oxidase. The presence of a minor cytochrome *a*-oxidase in *Rna. thiooxidans* was

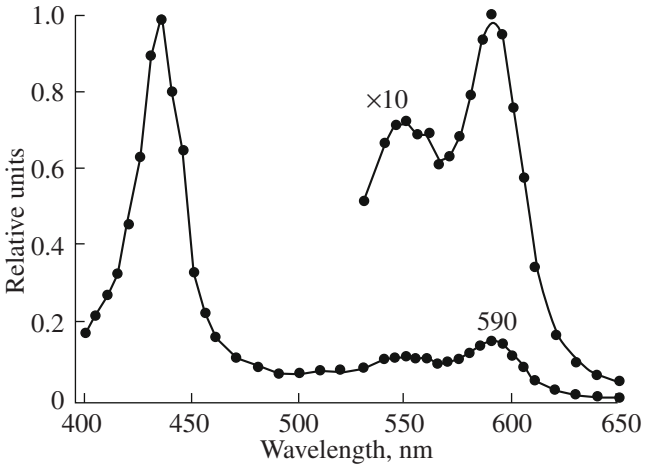

Fig. 5. The action spectrum of O_2 consumption in the suspension of *Rna. thooxidans* cells inhibited by carbon monoxide. In the pulsed monochromatic light, the molecules of inhibited cytochrome oxidase were released from CO, recovering the respiration activity of the ETC. The typical maximum of the spectrum at 590 nm indicates the presence of a terminal cytochrome oxidase of type a_3.

confirmed by reversible inhibition of the dark respiration of the cells by carbon monoxide, which was eliminated by exposure to light. The action spectrum of photodissociation of the cytochrome aa_3–CO complex had a typical peak at 590 nm (Fig. 5).

Cytochrome oxidase of type aa_3 was earlier isolated from the ABC bacterium *E. longus* [29]. The existence of two cytochrome oxidases in facultatively aerobic purple bacteria, which have characteristics similar to those of ABC bacteria, has been long known [30]. It is still difficult to correlate the 32-kDa cytochrome, as well as some other minor cytochromes *c* discernible by electrophoresis (Fig. 6b), with other known membrane complexes. The affiliation of any of them with nitrate reductase is not improbable: *Rna. thiooxidans* can reduce nitrates to nitrites, though nitrate reduction does not support growth [6].

Water-soluble cytochromes. Soluble cytochromes of *Rna. thiooxidans* were preliminarily separated by divided salting-out with 20 and 35% AS. The 20% AS fraction was shown to contain a cytochrome *c* with the molecular mass of 6 kDa (Fig. 6a) and absorption α-band at 551–552 nm (Fig. 7a). The cytochrome is a low-potential one, since it is insignificantly reduced by ascorbate (Fig. 7a) [27].

After hydrophobic column chromatography of the proteins salted out with 35% AS, the only unadsorbed cytochrome had the mass of 15 kDa (Fig. 6a) and the absorption α-band at 550–551 nm. This cytochrome is a high-potential one, since it is reduced by ascorbate almost to the same extent as by dithionine (Fig. 7b). According to its characteristics (position of the absorption maximum, molecular mass, and level of the redox potential), it is cytochrome c_{551} revealed in the photoinduced differential spectrum (Fig. 2), which is the known mobile periplasmic mediator of electron transfer from bc_1 complex to the photosynthetic RC and cytochrome oxidase.

The main part of soluble cytochromes was present in the material adsorbed on the column. Eluted chromatographic fractions contained four cytochromes *c* with the molecular masses of about 20, 40, 50, and 80 kDa. The 50-kDa cytochrome was concentrated in fraction 6, while three other cytochromes were distributed in the same proportion, with the maximum content in fractions 3–5 (Fig. 6b). In the differential absorption spectrum of these fractions, the absorption α-band was at least double: the dominant high-potential component had a maximum of 556 nm and an arm of 551 nm, while the low-potential component had a maximum of 553 and an arm of 558 nm (Fig. 7c). The differences between the absorption α-bands in all the above cytochromes were leveled at their conversion into reduced pyridine hemochromes, yielding a standard maximum of 550 nm, which confirmed the affiliation of these cytochromes with the *c* type (the data not shown). One may suppose that three polypeptide subunits of 20, 40, and 80 kDa belong to a single cytochrome complex that does not include the 50-kDa cytochrome.

The composition and functions of water-soluble cytochromes in ABC bacteria, apart from cytochrome c_{551}, are practically unexplored [22]. In the case of *Rna. thiooxidans*, the possible nature of cytochromes is indicated by the ability for oxidation of reduced sulfur compounds: sulfide, thiosulfate, elemental sulfur, and sulfite [6]. The low redox potential of the 6-kDa cytochrome and a number of other cytochromes described is in agreement with the data on low potentials of soluble cytochromes in alkaliphilic bacteria [31]. In accordance with molecular masses, the revealed periplasmic cytochromes, with the exception of cytochrome c_{551}, may be referred to as the sulfur oxidation system. Two such systems are known. A bacterial cell may have one or both respective electron transport chains [32, 33]. One of them includes two cytochromes encoded by the *Sor*A and *Sor*B genes with the molecular masses of 8.5 and 41 kDa (the data for the facultatively autotrophic *Starkeya novella*) [32]. By their detachment from other cytochromes and molecular weights, the 6- and 50-kDa cytochromes show a good correlation with derivatives of the *Sor*AB genes. The second enzymatic system, which has been most thoroughly studied in the facultatively lithoheterotrophic α-proteobacterium *Paracoccus pantotrophus* [33], includes a complex of four mono- and diheme cytochromes *c* with the masses of 15 to 40 kDa encoded by the *Sox* genes [33]. The cytochromes of *Rna. thiooxidans* (20, 40, and 80 kDa), forming a single complex, are probable derivatives of the *Sox* system. From this point of view, the cytochrome of 80 kDa identified electrophoretically may be a dimer or a more complex protein aggregate. Due to the complexity of the water-soluble cytochrome

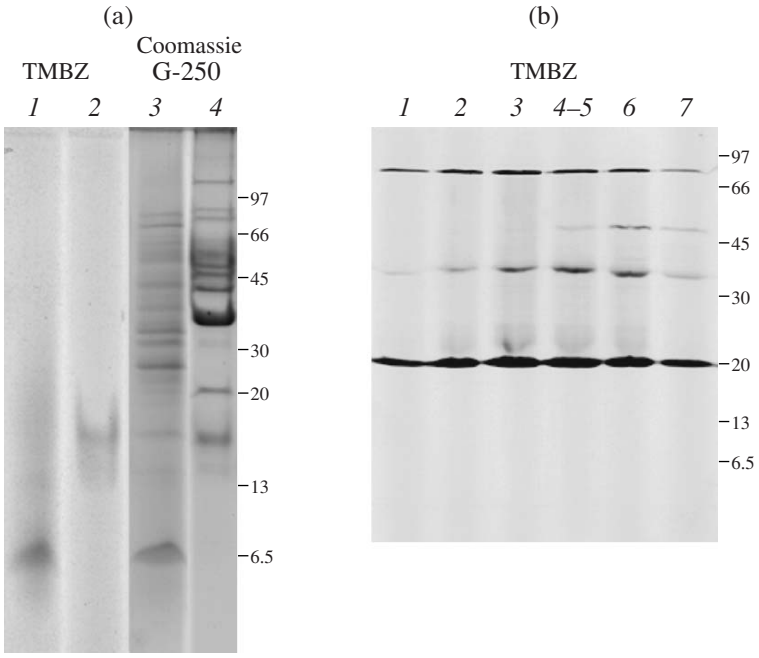

Fig. 6. Electrophoresis of *Rna. thooxidans* water-soluble cytochromes *c*: a, electrophoresis of low-molecular cytochromes in the Schagger–von Jagow system; on the left (*1, 2*), staining with TMBZ; (*3, 4*), staining of the same preparations with Coomassie G-250; *1, 3*, 6-kDa cytochrome from the 20% AS fraction; *2, 4*, 15-kDa cytochrome unadsorbed on the column from the 35% AS fraction; vertically at the right: molecular weights of markers; b, electrophoresis in the Laemmli system of cytochrome fractions eluted from the butyl-TSK-fractogel chromatographic column: detection of cytochrome zones using TMBZ; horizontally on top: fraction numbers; vertically on the right: molecular weights of markers.

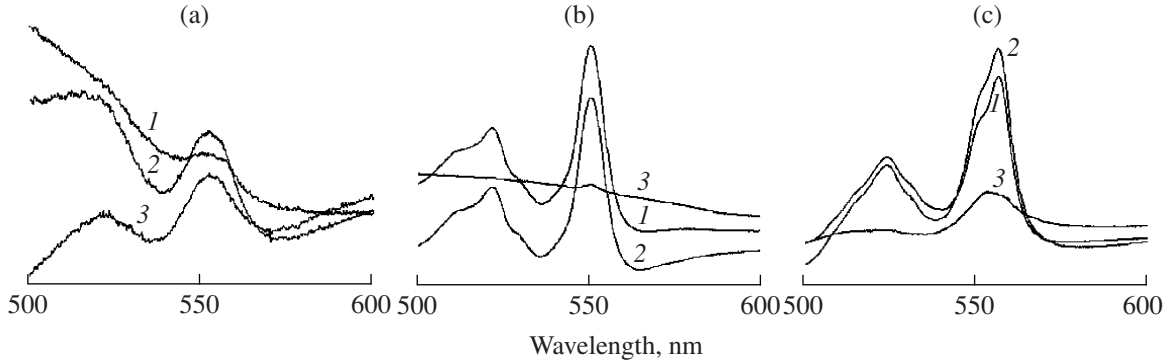

Fig. 7. Differential "reduced minus oxidized" absorption spectra for the fractions of *Rna. thooxidans* water-soluble cytochromes *c*: a, low-molecular, 6 kDa, low-potential cytochrome from the 20% AS fraction; b, high-potential cytochrome, 15 kDa, from the 35% AS fraction not adsorbed on the chromatographic column; c, total material of the cytochromes eluted from the column with molecular masses of 20–80 kDa; the spectra: *1*, "ascorbate minus ferricyanide"; *2*, "dithionite minus ferricyanide"; *3*, "dithionite minus ascorbate".

complex, its study will be presented in a separate publication.

Thus, the data obtained in this work concern the participants of energy metabolism in the cells of an ABC bacterium *Rna. thiooxidans*. The functioning of the photosynthetic apparatus and the presence of two terminal cytochrome oxidases of the cbb_3 and aa_3 types, bc_1 complex, water-soluble cytochrome c_{551}, and a few periplasmic high- and low-potential cytochromes (probable participants in two systems of oxidation of sulfur compounds) have been established. The low redox potential of some of the found periplasmic cytochromes of *Rna. thiooxidans* conforms with the data on the low potentials of soluble cytochromes in alkaliphilic bacteria [31]. Even though these data are fragmentary, due to the previous lack of information about the relevant species and insufficient case study for ABC bacteria as a whole, it is possible to make some prelim-

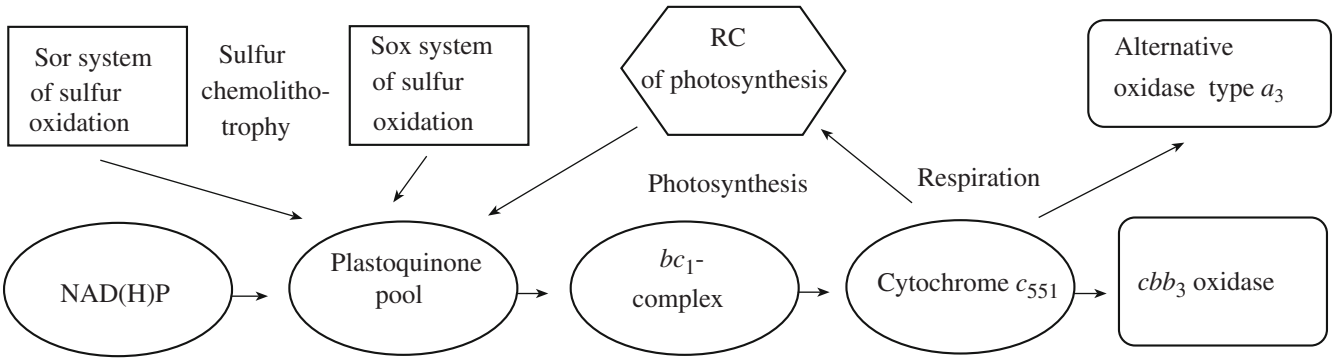

Fig. 8. Probable scheme of electron transfer pathways of *Rna. thooxidans* in accordance with the data on the composition of cell cytochromes.

inary conclusions. The cyclic electron transport in the photosynthetic chain and the respiratory ETC in ABC bacteria are considered as quite similar or coinciding with the pathways of electron transfer in facultatively aerobic purple bacteria [2]. By analogy, sulfur oxidation pathways may be considered as coinciding with the known pathways in sulfur-oxidizing prokaryotes. The metabolic pool of sulfur compounds, as has been shown recently [34], is connected with the terminal respiratory oxidases via the electron transport chain. These data, the known pathways of electron transport in facultatively aerobic purple bacteria, including the data for the sequenced species of ABC bacteria [14, 28], and the results of this work obtained for the molecular masses, composition, and estimated redox potential of the cytochromes, and the data on the pigment apparatus and capacity for the oxidation of sulfur compounds make it possible to suggest the general scheme of electron transfer pathways in *Rna. thiooxidans* (Fig. 8). We expect to obtain a more precise probable scheme for the individual participants of electron transport and their role in the cell by varying the conditions of *Rna. thiooxidans* cultivation in the light or in the dark, at macro- and microaerophilic conditions, in the presence or absence of mineral sulfur (thiosulfate), and at different concentrations of organic compounds.

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