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Isolation and Characterization of Water-Soluble Chromoproteins from *Arthrospira platensis* Cyanobacteria: C-Phycocyanin, Allophycocyanin, and Carotenoid- and Chlorophyll-Binding Proteins

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Abstract—A method using chromatography on DEAE-Toyopearl 650M was developed for the simultaneous extraction of water-soluble chromoproteins from *Arthrospira platensis* cyanobacteria cells. These chromoproteins were C-phycocyanin, allophycocyanin, and carotenoid- and chlorophyll-binding proteins. The purity of the isolated C-phycocyanin was 4.42 (A_{620}/A_{280}). The allophycocyanin purity was 3.40 (A_{652}/A_{280}). The phycocyanin purity was confirmed by the presence of only two bands obtained during SDS-PAGE: α-subunit (17 kDa) and β-subunit (18 kDa). The isolated carotenoid- and chlorophyll-binding proteins were analyzed by high-performance exclusion chromatography on TSK-GEL 2000SW (XL) with detection at three wavelengths (280, 480, and 678 nm). The spectral, chromatographic, and electrophoretic analyses of chromoproteins, as well as pigment analysis, made it possible to conclude that the carotenoid—chlorophyll a binding protein was a xanthophyll-chlorophyll a protein complex, and the chlorophyll a binding protein was a chlorophyll a protein complex. The molecular weight of the proteins was determined by high-performance exclusion chromatography and SDS-PAGE to be 57 and 16 kDa, respectively. The photoprotective properties of these proteins and their possible functioning as part of evolutionary precursors of photosynthetic systems are discussed.

Keywords: spirulina, *Arthrospira platensis*, allophycocyanin, C-phycocyanin, water-soluble carotenoid–chlorophyll a binding protein, water-soluble chlorophyll a binding protein, evolution of photosynthesis

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INTRODUCTION

Arthrospira platensis cyanobacterium belongs to the class of oxygenic photosynthetic bacteria (*Oxyphotobacteria*) and the group of cyanobacteria (*Cyanobacteria*) containing phycobiliproteins (**PBP**s). The *A. platensis* species belongs to the genus *Arthrospira* and to the oscillatory order (*Oscillatoriales*). At present, the algological classification system is taken into account, along with the modern microbiological classification system, and the double name of cyanobacteria or blue-green algae is often used. Two species are primarily edible for humans and animals: *A. platensis* and *A. maxima* (which have the commercial name spirulina) [1–3].

Spirulina inhabits lakes with a high concentration of carbonates and bicarbonates, and the ambient pH optimum for its growth and development is within 8–10. An increased temperature and illumination of the reservoir are also vital factors [4, 5]. It survives at a temperature of up to 60°C, and some of its species survive by switching to the state of anhydrobiosis in a dried water reservoir on stones at a temperature of 70°C. Naturally grown spirulina is currently found only in Lakes Chad (Africa) and Qinghai (China), since the Mexican Texcoco Lake disappeared (https://ru.wikipedia.org/wiki/Arthrospira). Spirulina is cultivated in many countries: the United States, China, India, Pakistan, Thailand, Spain, Italy, Greece, Chile, etc., and also Russia [2].

Spirulina is called superfood because of its high content of easily digestible protein, as well as other nutritional and biologically active substances. The possible use of spirulina biomass obtained during its cultivation during long space flights has been examined [6, 7].

The increasing practical interest in spirulina is determined by its biochemical composition. Dried spirulina is about 60% protein, including all essential amino acids and lipids (approximately 7%), among which valuable polyunsaturated fatty acids (gamma-linolenic and linoleic acids, as well as arachidonic and eicosapentaenoic acids) are present. Spirulina contains a complex of vitamins B, C, D, A, and E vitamins, as well as macro- and microelements (potassium, calcium, chromium, copper, iron, magnesium, manganese, zinc, and phosphorus) in bioavailable organic form [1, 2, 8].

The pigments present in significant amounts in spirulina are also bioavailable and have a therapeutic effect. Of these pigments, phycobilins and carotenoids, along with chlorophyll, are the most important. For example, C-phycocyanin isolated from spirulina has anti-inflammatory, antioxidant, antitumor, hepatoprotective, and immunostimulating properties, which makes it possible to use it both as a medicine and as a functional food product [9–12].

There is constantly great interest in the biology of this cyanobacterium due to the practical importance of spirulina. The PBPs of spirulina (phycocyanin and allophycocyanin) are products that are important in biotechnology and biomedicine. Thus, the study of characteristics of the functioning spirulina cells under various cultivation conditions and its adaptive capabilities is of current importance. Today, the nature of tolerance to unfavorable factors, e.g., high-intensity light, dehydration, nutrient depletion, and other factors, remains to be elucidated.

Many studies have been devoted to the isolation and purification of PBP from cyanobacteria and red algae [13, 14]. Phycocyanin and allophycocyanin are isolated preliminarily from cyanobacteria [15–17], while phycoerythrin is extracted from red algae [18]. Cyanobacterial cells are destroyed by freezing-thawing and/or ultrasound [15, 19–21] or passed through a French press [16, 22], or the cell walls are sometimes destroyed by lysing enzymes [14, 23]. The protein content is then determined in the aqueous extract. At this stage, the phycocyanin content is usually ~0.8 by the A_{620}/A_{280} ratio. PBP precipitation with ammonium sulfate ($(NH_4)_2SO_4$) with different degrees of saturation is subsequently carried out [15, 20–22]. At this stage, the A_{620}/A_{280} ratio is approximately 1.8–2.0. The obtained mixture of water-soluble proteins is separated by anion exchange chromatography on DEAE-cellulose [24] or DEAE-Sepharose in the NaCl gradient; the purity of the obtained phycocyanin is from 4.0 to 4.9 in this case [15, 22]. The purity of phycocyanin within 3.00–4.52 was achieved with hydrophobic chromatography on Phenyl Sepharose 6FF with $(NH_4)_2SO_4$ gradient [21, 22]. Song et al. achieved a very high phycocyanin purity with an A_{620}/A_{280} ratio of 5.32 using hydrophobic and anion exchange chromatography, followed by a third type of chromatographic purification, i.e., gel filtration on Sephacryl S-100 HR [22]. A different approach to the isolation and purification of phycocyanin was also used [25]. The extract was first treated with chitosan, and a column with activated carbon and subsequent aqueous two-phase extraction with PEG was used, during which a phycocyanin purity of 5.22 (A_{620}/A_{280} ratio) was achieved. Subsequent chromatography on DEAE-Sephadex increased the phycocyanin purity to 6.69.

No research on the extraction of water-soluble chlorophyll- and carotenoid-binding chromoproteins, together with PBP, was reported in the literature.

Spirulina, along with other cyanobacteria, has a complete photosynthetic apparatus that is typical of oxygen-releasing photosynthetic organisms. The electron transport chain includes PS II, b_6f cytochrome complex, and PS I. Light-harvesting complexes consist of PBP assembled into phycobilisomes (**PBS**s), which are protein microbodies adjacent to thylakoids (intracellular system of photosynthetic membranes). The current state of the research on the properties and functions of PBP and PBS was analyzed in a review by Stadnichuk and Tropin. The molecular mechanisms of light adaptations associated with PBS were shown to be not fully understood [13].

Cyanobacteria are members of the most ancient oxygenic photosynthetic organisms. The study of the early evolution of photosynthetic systems and the development of simple photoconverters associated with this are of significant interest. According to the above, the study of the complex of water-soluble photoactive chromoproteins, i.e., C-phycocyanin, allophycocyanin, and chlorophyll-binding and carotenoid-binding proteins, is an important task.

The goal of the study was to develop a method for the simultaneous isolation of a wide range of water-soluble chromoproteins from *A. platensis* cyanobacteria and to study their characteristics.

EXPERIMENTAL

Spirulina cultivation. Spirulina (*Arthrospira platensis*) biomass was provided by the Department of Biotechnology and Phytoresources of the Kovalevsky Institute of Marine Biological Research of the Russian Academy of Sciences (Sevastopol, Russia), which is headed by R. P. Trenkenshu. Algologically pure spirulina culture was grown in plane-parallel, glass photobioreactors with a depth of the illuminated layer of 5 cm on Zarrook mineral medium at a pH of 9.0–9.5 in concentration mode with constant stirring under constant illumination by DRL-700 fluorescent lamps (mean surface irradiation of 50 W/m^2, Russia) at a temperature of 29–32°C [26].

Chromoprotein isolation. Cell disruption was carried out via two passages of biomass suspended in 0.05 M Tris-HCl buffer (pH 7.8) with 0.1 M NaCl, 0.05 M EDTA, and 10^{-4} M phenylmethylsulfonyl fluoride through a French press. In the subsequent purification steps, 0.05 M Na-phosphate buffer (pH 7.0) containing 0.001 M sodium azide was used. All further procedures were performed at 2–8°C. Undisturbed cells were separated by centrifugation at 3000 *g* for 10 min. The supernatant obtained by repeated centrifugation at 21000 *g* for 60 min to remove the thylakoid membranes was used as a source of water-soluble chromoproteins. Dry ammonium sulfate was added to the obtained supernatant until 20–25% saturation with constant stirring. After 1 h, the solution was centrifuged at 12000 *g* for 20 min and the precipitate was

discarded. Ammonium sulfate was added to the supernatant until 25–50% saturation, which resulted in precipitation of the main part of the proteins. A precipitate was obtained after centrifugation at 12000 *g* for 20 min. It was dissolved in 50 mM phosphate buffer (pH 7.0) and dialyzed in dialysis tubes with a molecular weight boundary of 11473 overnight against phosphate buffer to release $(NH_4)_2SO_4$. The dialysate was concentrated over PEG with a molecular weight of 20000 or in Amicon Ultra 10K device centricones (Millipore, United States).

Chromoprotein separation. Chromoprotein separation was carried out with anion exchange chromatography on a DEAE-Toyopearl 650M (2.5 × 10 cm). The column was equilibrated with 0.05 M Na-phosphate buffer and a pH of 7.0 (flow rate of 0.7 mL/min). After the initial mixture was applied, the column was washed with the same phosphate buffer. The fractions of the yellow-green proteins were collected and subsequently eluted with a linear gradient via the addition of NaCl (0–0.2 M), and the blue ficobiliprotein fractions were collected. An index that expresses the ratio of optical densities at two wavelengths (A_{620}/A_{280} for phycocyanin and A_{652}/A_{280} for allophycocyanin) was used to determine the degree of phycobiliprotein purity [15].

Additional phycocyanin purification with PEG. A fraction containing phycocyanin with an A_{620}/A_{280} ratio of 4.42 was mixed in an 1 : 1 ratio with a 14% PEG solution with a molecular weight of 4000 in 1.2 M NaCl solution, and the mixture was shaken. It was maintained for phase separation overnight, and the upper phase was subsequently analyzed for phycocyanin content.

Pigment separation and analysis by krause. The sum of the fractions of yellow-green proteins was freeze-dried, and the pigments were extracted from the dry residue with ethanol. An equal volume of hexane and a few drops of water were then added to the alcohol extract (2 mL); the tube contents were thoroughly shaken and left to separate the layers. The upper hexane layer became green, and the lower ethanol layer became yellow. The spectra of the two layers were subsequently measured.

Separation of chlorophyll and carotenoid-binding proteins with high-performance exclusion chromatography. Chromatographic separation of the mixture of chlorophyll- and carotenoid-binding proteins was carried out in an Agilent 1100 HPLC system (Agilent, United States) on a 5-μm TSK-GEL G2000SW XL column (30 cm × 7.8 mm, Tosoh Corporation, Japan) with a diode array spectrograph. Separation was carried out by elution in isocratic mode with 100 mM Na-phosphate buffer (pH 7.0) and 0.1% SDS (a rate of 0.5 mL/min). The sample (400 μL) was preliminarily equilibrated with the eluting buffer and concentrated to 100 μL on a centricone (Amicon Ultra 10K device, Millipore, United States). The sample (50 μL) was then applied to the column, and the elution results were recorded and analyzed with the Agilent Chemstation software program. The molecular weights were determined according to the calibration curve plotted with the use of native globular proteins and blue dextran to determine the zero volume. The calculations were performed with the MS Excel 2010 (Microsoft).

Spectrophotometry. The absorption spectra of the fractions were obtained on a SF 2000 spectrophotometer (Spectrum, Russia) and the fluorescence spectra were obtained on a Flyuorat-02-Panorama spectrophotometer (Lyumeks, Russia) for analysis of the chromoproteins.

Electrophoresis. Electrophoresis was performed according to the method of graduated electrophoresis in PAGE with SDS according to Laemmli [27]. The separating (15%) and concentrating (5%) gels were prepared in a standard way. A sample containing 1% SDS, 10% glycerol, and dithiothreitol was heated at 95°C for 5 min and applied to the wells of the concentration gel after cooling. The separation was carried out in a midi camera (LKB broma, Sweden). The results of this electrophoresis and determination of the molecular masses of proteins were processed in the GelAnalyzer software package (gelanalyzer.com) with the construction of the R_f calibration dependence according to the standards: a mixture of PageRuler recombinant uncolored proteins (ThermoFisher scientific, United States).

RESULTS AND DISCUSSION

The task of the study was to identify both water-soluble carotenoid- and chlorophyll-binding proteins along with phycobiliproteins. Disintegration of cyanobacterial cells with the French press was chosen to disrupt cell walls and preserve the native properties of the isolated proteins. It was necessary to avoid the destruction of thylakoid membranes and chlorophyll complexes aggregated in membranes. After cell disintegration with the French press and the precipitation of thylakoid membranes by centrifugation, the supernatant was subjected to a graduated salting with ammonium sulfate to precipitate water-soluble proteins. The fraction obtained at 25–50% saturation with ammonium sulfate was dissolved in 50 mM phosphate buffer (pH 7.0) and dialyzed against the same buffer to remove ammonium sulfate. As a result, the fraction of phycocyanin with an A_{620}/A_{280} ratio of 1.45 was obtained.

Separation of the water-soluble protein mixture was carried out by column chromatography on DEAE-Toyopearl 650M. The mixture of water-soluble proteins was applied to the column and fractions of yellow-green proteins (Fig. 1a) were collected. A linear gradient of NaCl (0–0.2 M) was subsequently added to the eluting phosphate buffer, and the blue phycocyanin (Fig. 1c, *1*) and allophycocyanin (Fig. 1c, *2*) fractions were collected. The purity of the phycocyanin fractions esti-

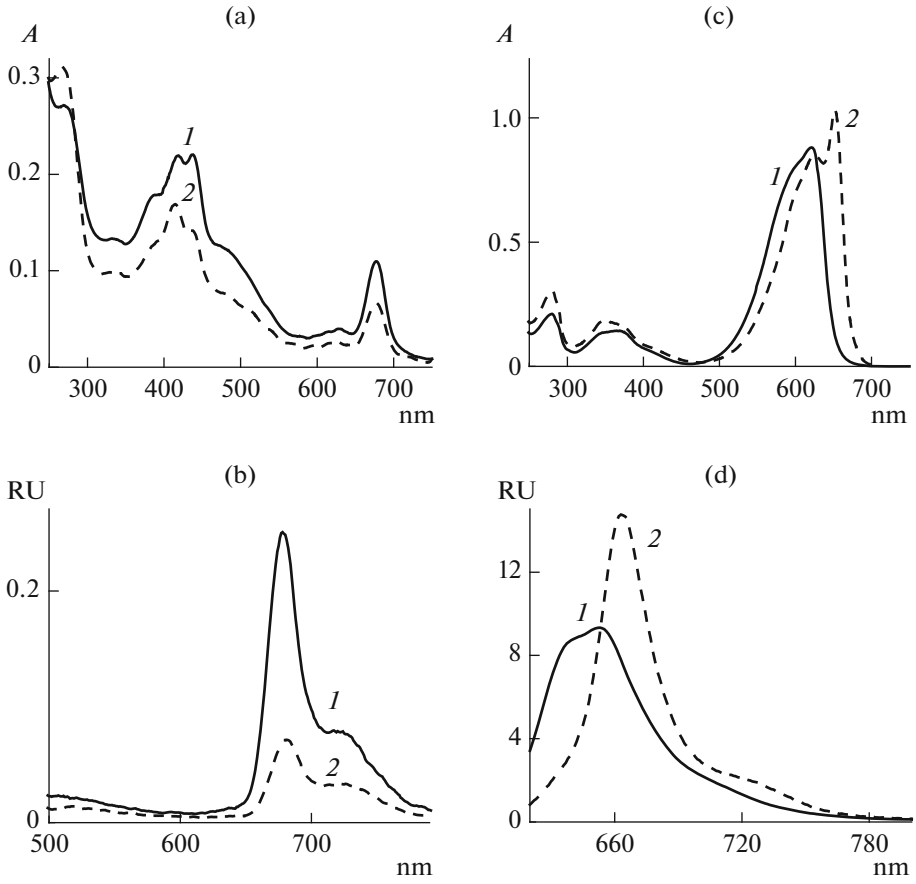

Fig. 1. Absorption and fluorescence spectra of major fractions of chromoproteins after separation by anion exchange chromatography on DEAE-Toyopearl 650M. Absorption spectra of fractions (a) containing *1*, carotenoid–chlorophyll a protein complexes and *2*, chlorophyll a protein complexes; the fluorescence spectra of carotenoid–chlorophyll a protein complexes (b): *1*, at λ_{excit} 415 nm and *2*, at λ_{excit} 438 nm; the absorption spectra of fractions (c) containing: *1*, C-phycocyanin and *2*, allophycocyanin; the fluorescence spectra at λ_{excit} 610 nm (d): *1*, phycocyanin and *2,* allophycocyanin.

mated by the A_{620}/A_{280} ratio reached 4.42. For allophycocyanin fractions, the purity, as calculated from the A_{652}/A_{280} ratio, reached 3.40. Figure 1d shows the fluorescence spectra of the fractions of phycocyanin and allophycocyanin upon an excitation of λ 610 nm. Additional phycocyanin purification was carried out by two-phase aqueous extraction [25] with PEG with a molecular mass of 4000. With this purification, the A_{652}/A_{280} ratio in the upper aqueous layer became 5.01, but the loss of phycocyanin was due to its partial presence in the lower layer with PEG.

The high purity of the isolated phycocyanin was confirmed by the PAGE method (Fig. 2). Electrophoresis in denaturing conditions with SDS according to Laemmli showed the presence of only two bands in the sample of phycocyanin (Fig. 2, *2*), which belong to two subunits of phycocyanin. The molecular weights of the subunits were estimated from their relative mobility (R_f) on gel calibrated with marker proteins (Fig. 2, *3*) and were calculated from the calibration graph. A mass of 17 kDa was obtained for the α subunit, and a 18 kDa weight was obtained for the β subunit; this agreed with the literature data [13]. Thus, the DEAE-Toyopearl 650M anion exchanger used by us made it possible to separate phycocyanin from allophycocyanin and to obtain them in the form of analytically pure preparations.

An important result of the use of anion exchange chromatography on DEAE-Toyopearl 650M was also the fact that it was possible to isolate water-soluble chlorophyll and carotenoid-binding proteins with this anion exchanger. The carotenoid- and chlorophyll-binding proteins isolated in two fractions were spectrally characterized and subjected to solvent treatment. The first fraction had absorption maxima in the region of 280, 415, 438, and 678 nm and a wide arm in the region of 480–520 nm (Fig. 1a, *1*), which may indicate the presence of chlorophyll and carotenoids in the composition of this protein. Pheophytin may be responsible for absorption in the 415-nm region. The second fraction has absorption in the region of 280, 415, 438, and 678 nm (Fig. 1a, *2*) and very little

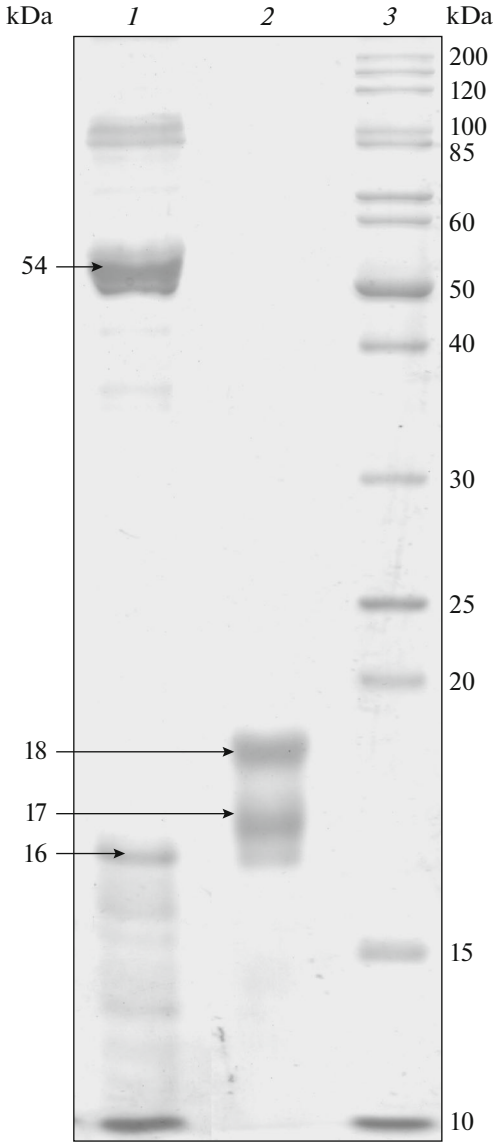

Fig. 2. Electrophoresis of fractions (obtained after separation of chromoproteins on DEAE-Toyopearl) in polyacrylamide gel with SDS according to Laemmli. *1*, a mixture of fractions containing carotenoid and chlorophyll a protein complexes; *2*, fraction of phycocyanin; and *3*, marker proteins.

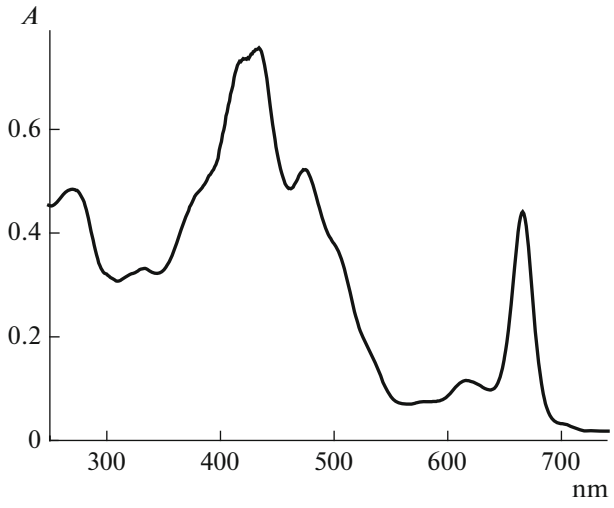

Fig. 3. Absorption spectrum of yellow substances soluble in diluted ethanol, which were obtained by pigment separation according to Krause from fractions containing carotenoid–chlorophyll a protein complexes.

absorption in the 480–520-nm region, which may indicate the presence of primarily only chlorines in this protein. The ratios of absorption at these wavelengths in these two fractions differed. In the fluorescence spectra, only the fluorescence bands at 678 and 720 nm, which appeared upon excitation from 415 and 438 nm, which corresponds to the absorption of chlorophyll (438 nm) and presumably pheophytin (415 nm), were visible (Fig. 1b). Fluorescence was absent when excited in the absorption region of carotenoids (480–520 nm). This indicated the absence of energy transfer from carotenoid to chlorophyll, which was probably due to their spatial separation and the absence of close contact.

Electrophoresis of the sum of fractions 1 and 2 (Fig. 2, *1*) in Laemmli denaturing conditions, in which the proteins should dissociate into subunits, showed the presence of proteins with molecular weights of approximately 16 and 54 kDa.

The pigments composing the sum of carotenoid- and chlorophyll-binding proteins were subsequently analyzed. The freeze-dried amount of chromoproteins was poured with ethanol; hexane and some water were further added to the ethanol extract. They were thoroughly mixed and left to separate the layers. Chlorophyll is a complex ester. When the alcohol was diluted with water, chlorophyll moved to the hexane layer, which turned green. Spectral analysis indicated that chlorophyll a was present in the hexane layer. Figure 3 shows the spectrum of soluble yellow-colored substances in diluted ethanol. Based on the obtained spectrum, we suggest that xanthophylls, which could be dissolved in alcohol in the presence of two layers (hexane-ethanol), were in the composition of the yellow chromoproteins, along with chlorophyll [28].

The fractions containing carotenoid- and chlorophyll a-binding proteins were analyzed by high-performance column chromatography on a TSK-GEL G2000SW (XL) column to determine the exact molecular masses and subsequently characterize the isolated proteins. Detection of the separated substances was carried out at three wavelengths (Fig. 4): at 280 nm (protein determination by absorbtion of their aromatic amino acids); at 480 nm (the absorption of carotenoids); and at 678 nm (the absorption of chlorophyll a). After separation, two major fractions with absorbance at 280 and 678 nm emerged; the latter

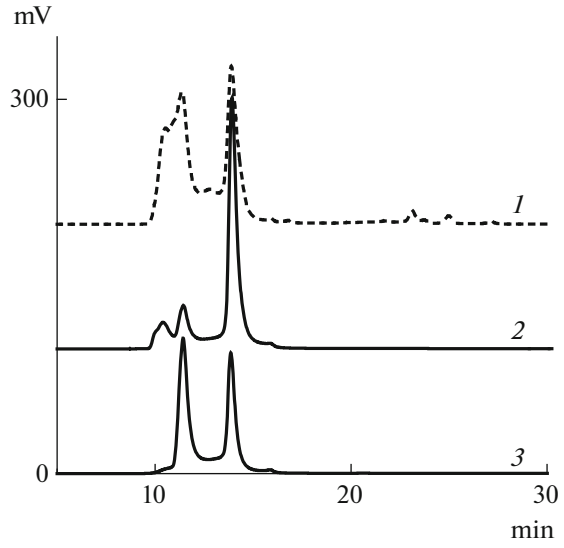

Fig. 4. Highly effective exclusion chromatography of the sum of fractions containing carotenoid–chlorophyll a protein complexes and chlorophyll a protein complexes during registration at different wavelengths: *1*, 280; *2*, 480; and *3*, 678 nm.

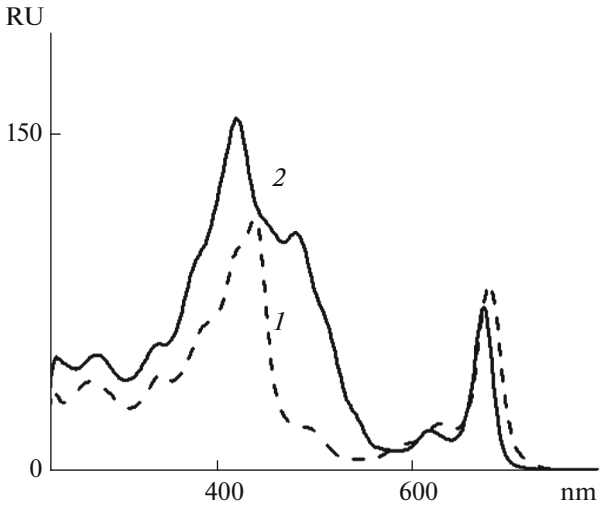

Fig. 5. Absorption spectra of fractions obtained after separation by high-performance exclusion chromatography: *1*, chlorophyll a protein complexes (57 kDa), *2*, carotenoid–chlorophyll a protein complexes (16 kDa).

fraction had also strong absorption at 480 nm, which may correspond to carotenoids. The first major fraction had very low absorption at 480 nm, and the protein, which did not separate from the first main fraction, also weekly absorbed in the carotenoid absorption region (480 nm) but did not absorb in the absorption region of chlorophyll (678 nm). This may suggest that this protein is a carotenoid-binding protein. The absorption spectra of the two main fractions were recorded in the maximal yield from the column (Fig. 5). The obtained absorption spectra of fractions are somewhat similar to the absorption spectra of two fractions extracted on a DEAE-Toyopearl 650M column (Fig. 1a). It should be noted that the yield of the fractions from these two columns was the opposite. Chlorophyll protein complexes with a mass of over 50 kDa were released first from the gel filtration column (TSK-GEL), and carotenoid—chlorophyll a protein complexes with a mass of 16 kDa were in the second fraction; carotenoid–chlorophyll protein complexes were eluted from a DEAE-Toyopearl 650M anion-exchange column first, and the second fraction contained chlorophyll-protein complexes.

To calculate the molecular weights of the studied proteins, the column was preliminarily calibrated with protein markers. According to the calibration graph, the molecular weight of the chlorophyll protein complexes was 57 kD and the molecular weight of the carotenoid–chlorophyll protein complexes was 16 kDa.

The pattern of the absorption spectra of the isolated proteins (Fig. 5) may indicate that the protein with a molecular mass of 54–57 kDa was a chlorophyll a protein complex with an absorption in the 280-nm region, which is associated with the aromatic amino acids of the proteins, while the region of 438 and 678 nm was associated with chlorophyll a. The protein with a molecular mass of 16 kDa was obviously a carotenoid–chlorophyll a protein complex, since it contained a protein component (absorption at 280 nm), a xanthophyll component (a broad absorption band with a maximum in the 480-nm region and a shoulder in the range of 500–520 nm), and a chlorophyll component (absorption in the 678-nm region).

The water-soluble carotenoid- and chlorophyll-binding proteins isolated from spirulina are of considerable interest. Free chlorophyll and its metabolic intermediates are known to be photochemically active compounds that cause the formation of reactive oxygen species, leading to oxidative stress and the destruction of cellular structures. When the photosynthetic apparatus of cyanobacteria is damaged under conditions of excessive illumination, under the influence of ionizing radiation [29], or a lack of nitrogen [14], it is necessary to remove the photochemically active chlorophyll; this photo- and radioprotective function probably belongs to water-soluble chlorophyll-binding proteins. It was shown that, when the *Synechocystis* sp. PCC 6803 cyanobacterium was irradiated with helium nuclei with energy of 30 MeV, the intensity and lifetime of fluorescence in the region of 660 nm increased, which was caused by a violation of phycobilisome integrity. In addition, the inactivation of PS II and induction of PS II chlorophyll fluorescence also occurred. Similar effects were obtained in the cells that were in space orbit around the Earth on the Foton M4 satellite. Subsequent cultivation of these cells under normal conditions led to a complete recovery of the cell parameters, which indicated a high viability of cyanobacteria [29].

It is possible that the water-soluble chlorophyll-binding and carotenoid—chlorophyll binding proteins contributed to the survival of cyanobacteria under the conditions of destroyed membrane complexes of PS II.

In the future, comparison of the properties and photochemical activity of the isolated water-soluble chlorophyll-binding protein with a molecular weight of 54–57 kDa and water-soluble chlorophyll-binding proteins (WSCP) [30, 31] is of interest. The subsequent study of carotenoid—chlorophyll a binding protein with a molecular weight of 16 kDa is also interesting, since its absorption spectrum is very wide (from 380 to 550 nm) and it can be a protector from excess radiation in the blue and green regions of the spectrum.

In our study, a DRL 700 high-pressure arc lamp was used to grow the spirulina. The phosphor covering the outer bulb radiated visible light from inside; the spectrum was dominated by blue and green components. Carotenoid, which is part of the isolated protein, probably determined its photochemical properties siginificantly, making it similar to the water-soluble orange carotenoid protein [32, 33]. It is also known that water-soluble carotenoid–chlorophyll a binding proteins are part of the main light-harvesting complexes in photosynthetic dinoflagellates [34]. Comparative analysis of the carotenoid–chlorophyll a binding proteins isolated from spirulina with those of dinoflagellates is of interest. It is important to note that cyanobacteria are the most ancient representatives of oxygen photosynthesizers. A paper [13] suggests that the appearance of phycobilins in evolution occurred later than the appearance of chlorophylls and hems and that oxygen, which is necessary for phycobilin formation from hemes, could be formed photosynthetically in the proto-cyanobacteria thylakoids in the presence of chlorophyll and without the participation of PBP.

CONCLUSIONS

Thus, we believe that the subsequent study of the structure and photochemical properties of water-soluble carotenoid- and chlorophyll-binding proteins is important from the evolutionary point of view as a possible model of a primitive photosystem functioning in the absence of hydrophobic membranes under conditions of irradiation, which includes the UV region.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. This article does not contain any studies involving animals performed by any of the authors.

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