Investigation of Photosynthetic Pigments of Symbiotic Algae of the Baikal Sponges

OLGA YU. GLYZINA and GRIGORY I. BARAM

Limnological Institute, Siberian Branch of the Russian Academy of Sciences, Ul. Ulan-Batorskaya 3, Irkutsk 664033 (Russia)

E-mail: bioin@sifibr.irk.ru

(Received August 18, 2001; in revised form October 24, 2001)

Abstract

The data on *a* and *b* chlorophyll content of unicellular algae incorporated into the symbiotic association of the Baikal sponges *Lubomirskia baicalensis* and *Baikalospongia bacillifera* are obtained by means of microcolumn reverse-phase high-performance liquid chromatography with UV detection. A connection between the number of chlorophylls and the depth of sponge habitat is demonstrated. A stronger decrease of chlorophyll content with depth increase is observed in *Lubomirskia baicalensis*.

INTRODUCTION

In the present study we consider problems connected with interrelations between the plant and animal components in a complex symbiotic organism of the Baikal sponge. Investigations of this type are urgent in the area of physiology of these organisms and in the ecology of biocenosis based on sponge. These problems may be important in understanding the mechanisms of adaptation of complex symbiotic associations and in estimating their stability in the lake ecosystem.

Several adaptation reactions of sponges to light have been discovered and investigated by present. For example, the adaptation to decreased light intensity involves the increase of chlorophyll content in symbiotic algae and increase of the content of these algae per unit surface of sponge [1].

The Baikal sponge forms complicated symbiotic associations with the participation of bacteria and unicellular green algae, i.e. zoo chlorella. The latter do not occur freely living in the open Baikal [2]. The relations of the sponge and algae are a typical intracellular symbiosis with mutual profit. The sponge utilizes oxygen and metabolites released by algae. In the cell of the sponge, the algae receive protection, mineral substances and carbon dioxide released by the sponge when breathing. This intracellular mutualism involves regular mechanisms bringing the growth of the endo-symbiotic in agreement with the host organism [3]. Thanks to the presence of such a symbiotic association, the Baikal sponges are able to both heterotrophic and autotrophic nutrition due to the photosynthetic activity of intracellular symbiotic algae. On well-illuminated regions of bottom, the autotrophic nutrition is prevailing in sponges.

The goal of the present work is to determine the *a* and *b* chlorophyll content of the zoochlorellas of the Baikal sponge, *Lubomirskia baicalensis* and *Baikalospongia bacillifera*, depending on the habitat depth, and to investigate the features of the adaptation of sponges and their symbionts to light.

EXPERIMENTAL

Identification and quantitative determination of chlorophylls were carried out by means of high-performance liquid chromatography

(HPLC) which is widely used for these purposes [2–8].

When transporting samples to the laboratory, the rules were followed: sponge samples were transported in semitransparent containers with the Baikal water (the ratio of sponge mass to water mass was 1 : 25, temperature of water was 10–15 °C); transportation time did not exceed 2 h. Each sample was separated into two parts. One part of the sample was homogenized in a porcelain mortar; the homogenate was extracted according to the procedure [4]. The extract was filtered and immediately analyzed by means of HPLC.

The second part of the sample was extracted after separating the symbionts in the phycol density gradient [5] at which the algae fraction is separated from bacteria and from the cells of the sponge itself. This provides the possibility to estimate quantitatively their mass ratio and to determine the chlorophyll content directly in symbiont algae.

Determination of *a* and *b* chlorophyll content was carried out by HPLC with Milikhrom A-02 chromatograph (JSC EkoNova, Novosibirsk) [8] under the conditions: column, 2 × 75 mm filled with Nucleosil 100-5 C18 (Machery-Nagel, Germany); mobile phases, A – water : methanol (5 : 95), B – methanol; linear gradient: 25 min from 0 to 100 % B; flow rate, 100 μl/min; column temperature, 35 °C; UV detector wavelength, 330 and 360 nm. Acetone extracts from sponge obtained according to the technique described in [2–5] were evaporated till dry, the residue was dissolved in methanol; 2–20 μl of solution was injected into the column. To identify pigments and perform their quantitative determination, we used the series of *a* and *b* chlorophyll solutions (Sigma, USA) in methanol with concentration 2 to 20 ng/ml. The intensity of illumination, or photosynthetically active radiation (PAR) was measured by Yanishevsky's pyranometer [2].

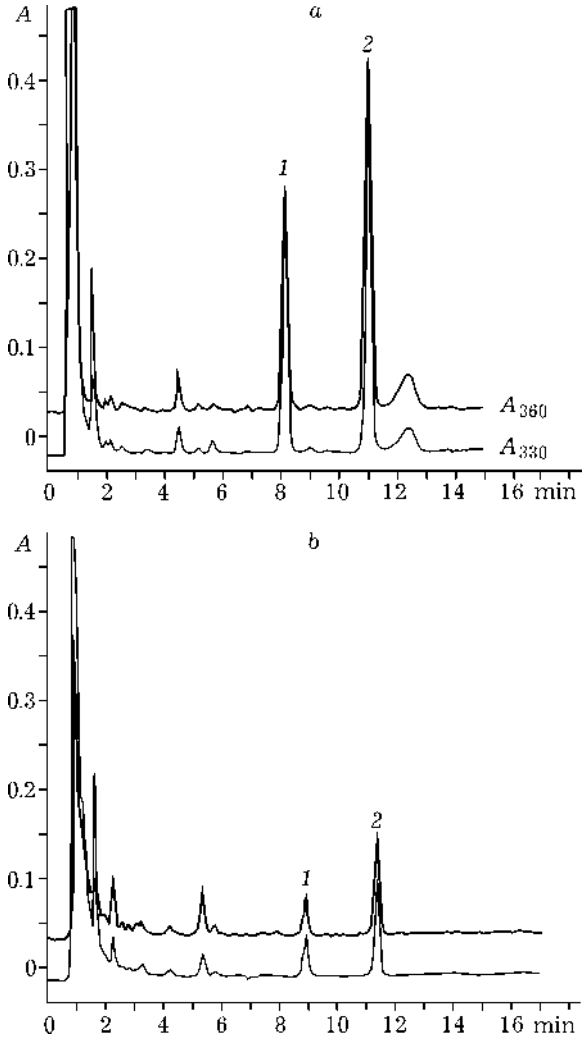

Fig. 1. Chromatograms of *a* (*1*) and *b* (*2*) chlorophylls in the endosymbiotic algae of the Baikal sponges *Lubomirskia baicalensis* (*a*) and *Baikalospongia bacillifera* (*b*).

RESULTS AND DISCUSSION

During investigations, it is necessary to pay special attention to sampling and storage because the correctness of the obtained results is strongly dependent on these operations. Because of this, it is reasonable to perform investigations of this type directly at sampling site. A special chromatographic equipment intended for operation in a field (ship) laboratory is necessary for this purpose. We used a portable chromatograph Milikhrom A-02 and the microcolumn HPLC method. The resolution of a short column (microcolumn) is quite sufficient for the chlorophylls a and b to be separated, as one can see in Fig. 1.

Practically in al the studies into the HPLC of chlorophylls, detection is performed in the visible spectral region at the wavelength of 410–670 nm, since these phytopigments have characteristic absorption maxima in this region. However, chlorophylls have also substan-

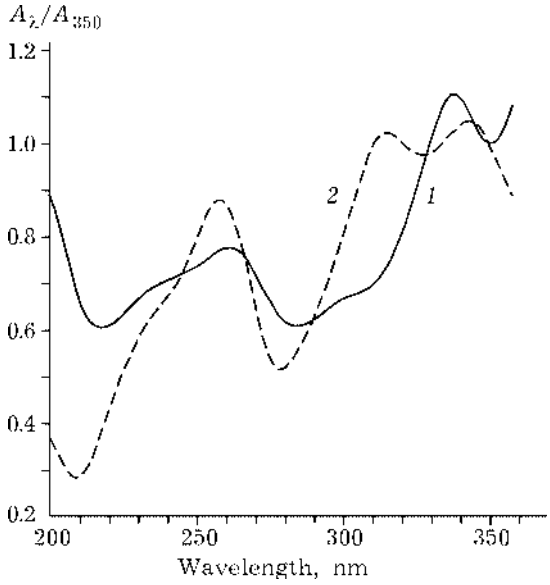

Fig. 2. Normalized UV spectra of *a* (*1*) and *b* (*2*) chlorophylls in methanol.

tial absorption in the UV spectral region which allows us to use detectors more widely spread in HPLC, namely, UV photometers. The spectra of *a* and *b* chlorophylls within the range 200–360 nm are shown in Fig. 2.

The application of multiwave detection increases the reliability of identification because we also use spectral relations along with retention times. For *a* and *b* chlorophylls, the ratios of the areas of chromatographic peaks S_{360}/S_{330} are 1.100 ± 0.04 and 0.972 ± 0.03, respectively.

The chlorophyll content of sponges and of algae isolated from sponges is shown in Tables 1 and 2. The content and ratio of the chlorophylls in the investigated sponges changed with depth (Fig. 3). The averaged change is 50 % for chlorophyll *a* in *Lubomirskia baicalensis*. The largest difference in chlorophyll content was detected in *Baikalospongia bacillifera* at the depth of 40 m.

The obtained results showed that the higher concentration of chlorophyll *a* is observed at a depth of 5 m in *Lubomirskia baicalensis*. Along the vertical in the layer from 0 to 40 m, the *a* chlorophyll content decreased to a larger extent than that for the chlorophyll *b*. The decrease of illumination causes the decrease of *a* chlorophyll content per unit surface of the sponge. This change of the amount of chlorophyll in shadowed sponges occurs due to the decrease of the amount of algae and increase of the number of bacteria cells in the sponge. In the sponges living at the depth of 30–40 m,

TABLE 1

Concentrations of *a* and *b* chlorophylls in the *Lubomirskia baicalensis* sponge

Depth, m	Year	Number of samples	Concentration, μg/g of sponge			Mass of algae in 1 g of wet, mg
			a	*b*	*a*/*b*	
5	1999	7	0.34±0.04	0.10±0.01	3.4	0.12±0.02
	2000	4	0.42±0.04	0.10±0.01	4.2	0.12±0.01
	2001	2	0.32±0.04	0.10±0.01	3.2	0.10±0.01
10	1999	4	0.43±0.05	0.15±0.03	2.7	0.25±0.01
	2000	8	0.40±0.04	0.14±0.04	2.8	0.25±0.02
	2001	4	0.46±0.04	0.18±0.04	2.5	0.23±0.01
20	1999	4	0.44±0.03	0.28±0.01	1.6	0.41±0.05
	2000	3	0.43±0.04	0.27±0.04	1.6	0.41±0.05
	2001	3	0.44±0.02	0.28±0.04	1.6	0.41±0.05
30	1999	3	0.09±0.02	0.01±0.3	2.0	0.13±0.02
	2000	5	0.06±0.01	0.03±0.01	2.0	0.13±0.01
	2001	2	0.08±0.02	0.03±0.01	2.6	0.12±0.02
40	1999	3	0.09±0.02	0.04±0.02	2.3	0.14±0.04
	2000	5	0.09±0.02	0.05±0.01	1.8	0.11±0.04
	2001	2	0.09±0.02	0.05±0.01	1.8	0.12±0.02

TABLE 2

Concentrations of *a* and *b* chlorophylls in the *Baikalospongia bacillifera* sponge

Depth, m	Year	Number of samples	Concentration, μg/g of sponge			Mass of algae in 1 g of wet, mg
			a	*b*	*a*/*b*	
5	1999	6	0.25±0.01	0.11±0.01	2.3	0.14±0.01
	2000	6	0.24±0.04	0.09±0.01	2.6	0.14±0.03
	2001	2	0.21±0.03	0.08±0.01	2.6	0.14±0.01
10	1999	4	0.22±0.04	0.08±0.01	2.8	0.18±0.05
	2000	4	0.21±0.06	0.08±0.01	2.6	0.18±0.03
	2001	2	0.20±0.03	0.08±0.01	2.5	0.19±0.05
20	1999	6	0.27±0.01	0.12±0.02	2.3	0.26±0.02
	2000	5	0.22±0.01	0.12±0.02	1.8	0.26±0.02
	2001	1	0.25±0.01	0.12±0.02	2.1	0.26±0.02
30	1999	5	0.05±0.01	0.03±0.01	2.0	0.25±0.05
	2000	4	0.05±0.02	0.03±0.01	1.7	0.22±0.03
	2001	2	0.05±0.01	0.03±0.01	1.7	0.27±0.01
40	1999	2	0.10±0.02	0.05±0.01	2.0	0.14±0.02
	2000	2	0.09±0.01	0.05±0.01	1.8	0.14±0.03
	2001	2	>0.01	>0.01	–	0.10±0.03

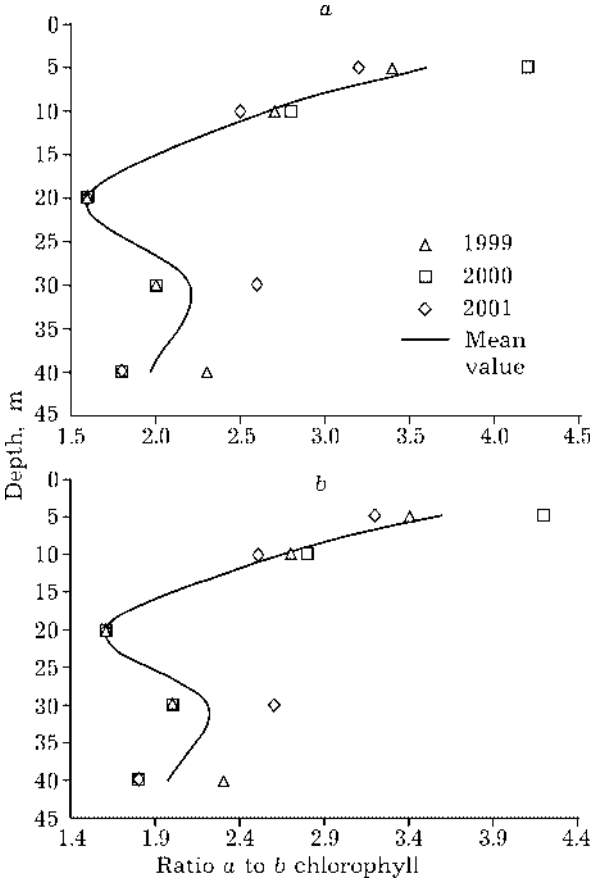

Fig. 3. The ratio of *a* to *b* chlorophyll in *Lubomirskia baicalensis* (*a*) and *Baikalospongia bacillifera* (*b*) depending on the habitat depth.

relative content of the algae cells decreases by a factor of 2 in comparison with the sponges living at the depth of 5–10 m. The decrease of illumination causes the decrease of the total *a* and *b* chlorophyll content.

It is known that the adaptive reaction common in all the sea sponge species is the accumulation of chlorophyll in algae, which corresponds to the conclusions of the authors [2]. We discovered that the Baikal sponges accumulate chlorophyll only in the case of rather good illumination (30–70 % PAR).

CONCLUSIONS

1. At a depth of 5 to 20 m, the accumulation of chlorophyll is observed in different species of Baikal sponges.

2. Chlorophyll concentrations decrease at a depth of 30–40 m; the ratio of *a* to *b* chlorophyll also changes.

3. The change of the amount of chlorophylls is connected with the changes of the mass of algae.

4. Different species of the Baikal sponges have quantitatively different adaptive reaction to the change of illumination.

REFERENCES

1 L. V. Garibov, Yu. K. Dundin, T. F. Koptyaeva, V. R. Filin, Vodorosli, lishayniki i mokhoobraznye SSSR, Mysl', Moscow, 1978, pp. 15–40.
2 Ekologicheskiye aspekty fotosinteza morskikh makrovodorosley, in E. A. Titlyanov, V. I. Zavalinsky (Eds.), Vladivostok, 1978, pp. 38–54.
3 T. N. Kropacheva, I. A. Mamleeva, L. I. Nekrasova, *Zhurn. fiz. khimii*, 58, 3 (1984) 692.
4 L. E. Grishina, E. E. Lazareva, G. D. Brykina, *Vestn. MGU. Ser. 2, Khimiya*, 38, 1 (1997) 73.
5 G. D. Brykina, L. E. Grishina, M. I. Uvarova, O. A. Shpigun, *Zhurn analit. khimii*, 52, 5 (1997) 501.
6 Yu. A. Vladimirov, A. Ya. Potapenko, Fiziko-khimicheskiye osnovy fotobiologicheskikh protsessov, Vysshaya shkola, Moscow, 1989, pp. 108–115.
7 N. N. Vsevolodov, Biopigmenty – fotoregistratory, Nauka, Moscow, 1988, pp. 58–59.
8 G. I. Baram, *J. Chomatogr. A*, 728, 1–2 (1996) 387.