Indicators of Life Conditions of the Baikal Sponge When Keeping It in Aquariums with the Use of Mass Spectrometry and Liquid Chromatography

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Abstract

Methods of chromatography mass spectrometry and microcolumn high performance liquid chromatography have been used to study complex biological systems. Potentialities to apply these methods have been disclosed by the example of biochemical research of symbiotic community of the endemic Baikal sponge *Lubomirskia baicalensis* (Dybowsky, 1874) when it is kept both under natural and under simulated conditions. It has been demonstrated that the use of a complex of modern methods of biochemical analysis allows effective, rapid determination, high sensitivity detecting of changes in biologically active compounds in living organisms.

INTRODUCTION

To estimate life conditions of any organism and its response to a change in the environment is possible by simply observing it. However, external manifestations reflect internal changes in an organism that have already occurred, and first of all at the biochemical level. In this relation, monitoring at the biochemical level makes it possible to reveal a tendency in changes of life conditions of organisms at an earlier stage.

This work considers monitoring at the level of biochemical indices such as fatty acids and chlorophyll. This is related to the fact that lipids take one of the leading places in the metabolism of organisms. They control stability of cellular and endocellular membranes, deposition of lipid ingredients; they serve as regulators for transportation of water and salts, for enzymatic activity and for alternative important functions for an organism. The variety of complex lipids is controlled in many respects by a spectrum of fatty acids (FA) that depends in many respects in complex symbiotic communities on photosynthesising pigments of their autotrophic ingredient and, consequently, the spectrum exerts a huge effect on the vital activity of the whole community.

From all modern-day methods of biochemical analysis, it is good practice to use methods of analytical chemistry for the like purposes, namely, thin layer chromatography (TLC), gas liquid chromatography (GLC), high-performance liquid chromatography (HPLC) and mass spectrometry (MS) [1]. These methods get more and more popular both for Russian, and foreign researchers.

EXPERIMENTAL

The most bulk kind of a large branchy sponge *Lubomirskia baicalensis* (Pallas) has been chosen as the subject of the investigation. It is most promising to keep it under simulated conditions, both from the point of view of a scientific research and under live museum expositions.

The Baikal sponges are complex symbiotic communities [2]. Among endosymbionts we have determined single-celled seaweed, yeast, and bacteria living inside sponge cells [3–5]. All of them are intimately involved into physiological processes of the sponge and they define the way of its nutrition (that of hetero- or autotrophic nature) [6–8]. It was found in the previous research [8] that the consequent tropic interactions that can be readily observed with marker FA exist in the community of Baikal sponges: bacteria ↔ of sponge ↔ seaweed. This is related to the fact that FAs are partially synthesized by the sponge, and partially they enter from symbionts. Fatty acids of the sponges themselves considerably differ from lipids of more developed invertebrate animals, primarily, it terms of the availability of demospongic acids, the biosynthesis of which has been revealed only within organisms of sponges [4, 5, 8]. Fatty acids that the sponge receives from symbiotic seaweed are of short-chain nature

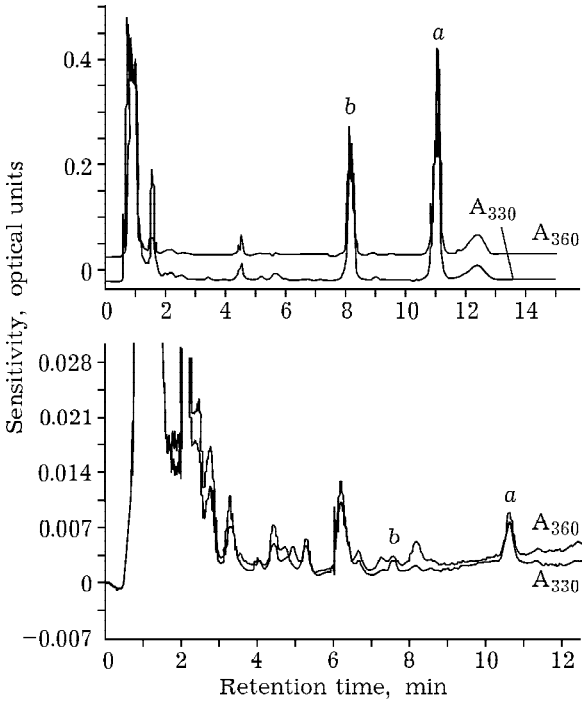

Fig. 1. Chromatograms of chlorophylls *a* and *b* for endosymbiotic seaweed of Baikal sponges *Lubomirskia baicalensis* before the incubation (above) and after the incubation (below).

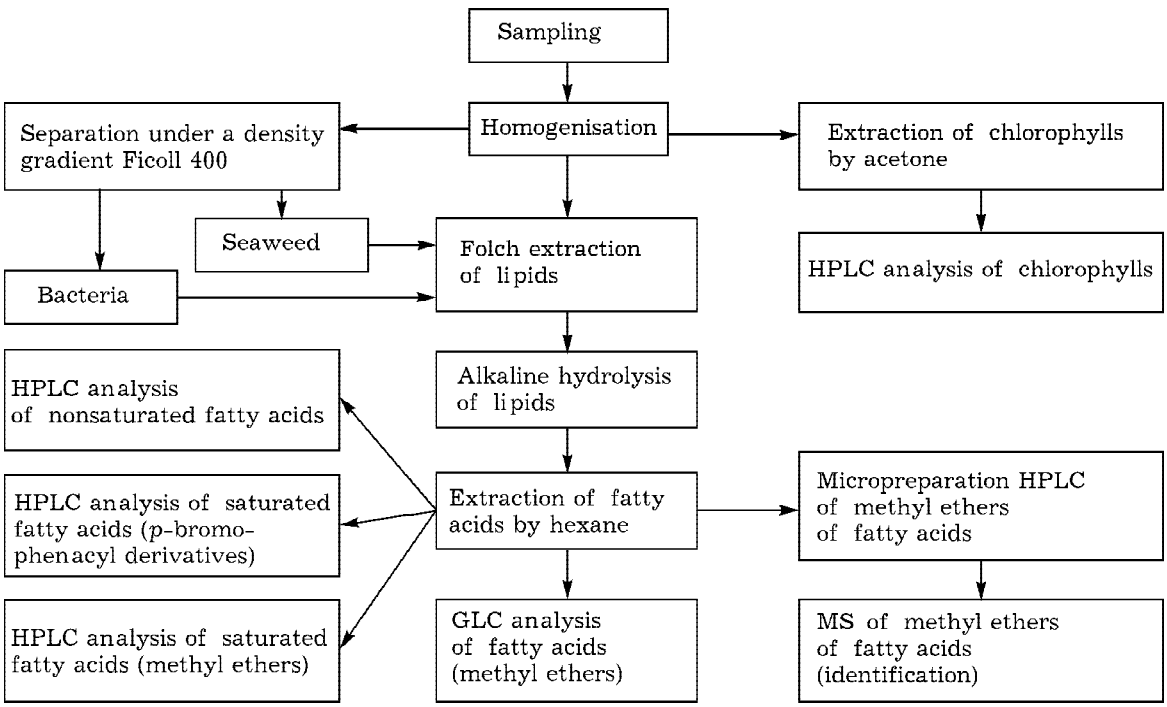

Fig. 2. Schematic diagram of the basic experiments.

and they are synthesized mostly by seaweed only; and other organisms receive them only through food chain [8]. Chlorophyll, the main pigment of symbiotic seaweed, constitutes a significant fraction in lipide extract of sponges [9]. The content of pigments varies in significant range depending on the environment of the seaweed and the symbiotic community as a whole.

Chromatography mass spectrometer analysis was conducted with an Agilent 5793N-GC6890 unit. Column Ultra was 2.5 m in length, the inner diameter of 0.32 mm. The temperature of the injector was 280 °C, that of an ionic source – 230 °C, that of a quadrupole – 150 °C. Temperature schedule of the thermostat: the gradient of 200–300 °C at the rate of 2 °C/min, exposition over the course of 25 min at 300 °C. Identification was made using NIST computer database.

Analysis of methyl ethers of fatty acids (MEFA) was conducted on a gas liquid chromatograph of Shimadzu corporation: 1) of GC-9A specification with a flame ionisation detector, with Chromatopac data processing base; 2) of C-R4AX specification, on a glass capillary column of 45 m in length and of the inner diameter of 0.27 mm with the Carbowax-20M stationary phase of moderate polarity. A number of samples has been analysed on a column with Supelcowax 10TM phase. The gas carrier was helium. Parameters of the analysis: peripheral speed of gas carrier was 20 cm/s; the temperature of the evaporator was 240 °C, that of a thermostat – 210 °C, that of a detector – 240 °C; the flow rate of gas carrier was 45 mL/min; the pressure of gas carrier was 1.8 kg/cm [5, 8].

Quantitative and qualitative analysis of *a* and *b* chlorophylls was conducted by the HPLC method in a Milikhrom A-02 chromatograph (EcoNova Co., Novosibirsk) [9] under the following conditions: a column 2 × 75 mm with Nucleosil 100-5 C18 (Machery-Nagel, Germany); mobile phases: A – water : methanol (5 : 95), B – methanol; the linear gradient 25 min was from 0 to 100 % B; the flow rate was 100 μL/min; the column temperature of 35 °C; the UV detector wavelengths of 330 and 360 nm. To identify pigments and to determine them quantitatively, series of solutions of chlorophylls *a* and *b* (Sigma, the USA) were used (Fig. 1).

TABLE 1

Change in the composition of fatty acids (FA) in the sponge *Lubomirskia baicalensis* when keeping it in an aquarium

FA code	Content, % from the total of lipids	
	before the incubation	after 30 days of the incubation
	Saturated FA	
14:0	2.1	1.5
15:0	0.1	0.3
16:0	7.0	12.0
17:0	1.5	0.4
18:0	5.4	3.4
19:0	0.2	0.1
20:0	0.5	0.5
Iso 15:0	0.3	0.8
Aiso15:0	0.3	0.1
Iso 16:0	0.2	0.1
Aiso 16:0	0.1	0.2
Iso 17:0	1.0	0.8
Aiso 17:0	1.1	0.2
Iso 18:0	1.4	0.1
Aiso 18:0	0.2	0.1
Total	21.4	20.7
	Nonsaturated FA	
16:1 n-9	Traces	0.5
16:1 n-7	1.6	1.1
16:1 n-5	0.1	Traces
17:1 n-9	2.2	0.2
18:1 n-9	16.2	26.4
18:1 n-7	1.8	0.8
19:1	Traces	Traces
20:1 n-11	0.7	Traces
20:1 n-9	0.3	1.8
20:1 n-7	Traces	0.2
16:2 n-4	Traces	0.7
16:3 n-3	0.9	1.0
18:2 n-6	2.2	2.2
18:3 n-6	0.2	0.4
18:3 n-3	7.1	8.0
18:3 Δ5.9.12	0.4	Traces
18:4 n-3	3.5	1.9
20:3 n-3	Traces	0.1
22:1 n-9	0.3	0.2
20:4 n-3	0.7	0.2
20:5 n-3	12.2	7.2
22:4 n-3	0.1	0.2
22:5 n-3	0.5	1.2
22:6 n-3	0.2	1.2
24:1 n-9	3.7	Traces
24:4	0.2	Traces
26:3 Δ5.9.1	17.6	13.9
Total	74.7	69.7
Grand total	96.1	90.4

TABLE 2

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

Content, μg/g of a crude sponge			Biomass of seaweed,
a	*b*	*a*/*b*	g/g of a crude sponge
0.34/0.12	0.10/0.05	3.40/2.40	0.12/0.04
0.42/0.10	0.10/0.04	4.20/2.50	0.12/0.02
0.32/0.11	0.10/0.04	3.20/2.75	0.10/0.02

Note. The first value – before the incubation, the second – after incubation.

RESULTS AND DISCUSSION

Figure 2 presents the schematic diagram of experiments in biochemical analysis of lipid ingredients of the Baikal sponge before and after incubation.

It has been found as the result of the performed biochemical analysis that the Baikal sponge that is kept in an aquarium and that is of externally healthy appearance shows a change in its composition of FA (Table 1) and a decrease in the quantity of chlorophylls (Table 2).

Based on the analysis of the data acquired, an assumption can be made that the following sponge changes develop under conditions of the incubation:

i) The mass fraction of seaweed in the total mass of endosymbionts decreases fairly quickly, but their species composition is unaffected, as evidenced by the chlorophylls *a* and *b* available.

ii) The total content of biologically active FA shows a decrease, including 20:5 n-3, which leads to disbolism and to a decrease in viability of the whole symbiotic community of the sponge.

iii) Cells of the sponge itself show dysfunction (the synthesis of demospongic acid 26:3 D5.9.19 tapers off).

CONCLUSION

The performed research testifies that application of a complex of modern-day methods of biochemical analysis (thin layer chromatography, gas liquid chromatography, high performance liquid chromatography, and mass spectrometry) allows effective, rapid determination, high sensitivity detecting of changes in biologically active compounds in living organisms. This complex of methods makes it possible also to assess interactions within complex symbiotic communities. The use of a similar instrument complex is also promising when monitoring a variety of biotechnological processes.

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