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ACCUMULATION OF SELENIUM AND CHROMIUM IN *SPIRULINA PLATENSIS* CELLS IN THE DYNAMICS OF GROWTH

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Introduction

In recent years more and more investigations in the field of biochemistry, microbiology and medicine have pointed to an exceptionally important role of trace elements in the functioning of living systems. An excess or deficiency of one or another vitally important element (such as Se, Zn, I, Cr, Cu, etc.) may often result in severe disturbance of natural processes in human or any other living organisms, including microrganisms [1,2].

It is of importance therefore to study the peculiarities of the interaction of trace elements with living systems and their endogenic inclusion into biocomplexes. For this purpose, the blue-green alga *Spirulina platensis* (*Sp. pl.*) is chosen as a model and its interaction with such vitally important elements as selenium and chromium is studied.

Being rich in content and properties *Sp. pl.* is widely used in food as a biologically active supplement (BAS) that normalizes natural processes and protective functions of the organism [3,4]. *Sp. pl.* is able to include biogenically the necessary elements as it grows in a nutrient medium and plays a role of a certain biological filter that accumulates elements in a nontoxic form in a given amount in the course of its biomass natural growth.

The peculiarities of the interaction of *Sp. pl.* with selenium and the possibility of purposeful endogenic inclusion of selenium into the biomass of *Sp. pl.* were earlier studied by the authors by neutron activation analysis (NAA) [5,6]. The NAA method was also applied to study the interaction of chromium with the *Sp. pl.* biomass. The choice of these elements is dictated by their important role in some functions of human organisms as well as by some aspects of the use of the *Sp. pl.* biomass enriched with Se or Cr for practical purposes.

This work discusses the investigation of the interaction of *Sp. pl.* with selenium and chromium together in the course of cells cultivation in a nutritient medium containing proper compounds of these elements.

It is known that in the process of interaction of living systems with several trace elements at a time there may arise the effects of synergism in one case and of antagonism in another. From this viewpoint the study of the behavior of Se and Cr in the condition of their simultaneous accumulation in the cells of *Sp. pl.* is of particular interest.

It should be taken into account that the accumulation of metals in the cells of microorganisms goes at different rates. The binding time of a metal depends on the accumulation mechanism. If a metal is bound to the cell's surface by adsorption, which does not depend on temperature and does not take energy, the process goes fast and takes from several seconds to an hour. The process of active transport of metals into the interior of the cell requires energy and depends on temperature. As a result, it goes slower and takes a period of several days or more [7].

This determines the choice of experimental conditions in which the kinetics of the accumulation of Se and Cr in the cells of *Sp. pl.* is studied.

Experiment

Cultivation of cells and preparation of samples

In the experiments the strain IPPAS B-256 of the blue-green microalgae *Spirulina platensis* from the algeological collection of the Timiriazev Institute of Plant Physiology, RAS was used.

The cultivation of *Sp. pl.*cells was carried out in the accumulation regime in Zaroukh's standard water-salt nutrient medium at a temperature of +34°C, constant light of 3000 luxe from fluorescent lamps and continuous mixing executed by the instrument УВТМ-12-250. The compounds Se(IV) and Cr(III) were introduced into the nutrient medium as sodium selenite (Na_2SeO_3) and chromium acetate ($Cr(CH_3COOH)_3$).

Since the preservation of the "natural" properties of the *Sp. pl.* biomass characteristic of pure Zarukkh's nutrient medium in the course of the experiment of the accumulation of Se(IV) and Cr(III) is one of the most important conditions, continuous control of the cells' state in the *Sp. pl.* trichoma is performed and its protein content is controlled using gel-electrophoresis in a polyacrylamide gel.

For the same reason it is necessary to determine preliminarily the concentrations of Se(IV) and Cr(III) in the nutrient medium that will enable their accumulation without destroying the natural growth and quality of the biomass. To do this, the growth dynamics of *Sp. pl.* cells in a nutrient medium that contains the compounds Se(IV) and Cr(III) in equal concentrations within the interval 1-100 mg/l was investigated individually for each compound. The investigations show that the *Sp. pl.* biomass increases linearly in both cases and an increase in the loading concentration to 10 mg/l does not virtually affect the growth and quality of *Sp. pl.* trichoma. Essential changes in the growth of the biomass are observed at concentrations of 50-100 mg/l. They include the appearance of chlorotic cells and an increase in the number of short trichomas. The chlorophyll content decreases by 10-20% in comparison with reference samples. As a result, there was chosen a concentration of 5 mg/l for selenium and of 3 mg/l for chromium.

In the first experiment *Sp. pl.* was cultivated in a nutrient medium introducing sodium selenate with a Se(IV) concentration of 5 mg/l.

In the second experiment to cultivate *Sp. pl* the nutrient medium was loaded with chromium acetate with a Cr(III) concentration of 3 mg/l.

In the third experiment the cultivation was carried out in the simultaneous presence of similar compounds of chromium and selenium with analogous concentrations. In all of the three experiments the primary concentration of the *Sp. pl.* inoculate was 0.4 g/l. The time of cultivation was 4 days. In all of the three experiments samples for analysis were taken at the end of the first, second, third and the forth day.

The biomass was harvested by centrifugation at 3000 g for 20 min at 4 °C. It was then rinsed in bidistilled water and subjected to a second centrifugation at 15000 g for 20 min.

The obtained samples were lyophilically dried in a special lyophilizer earlier designed by the authors [8]. The element content of the samples was determined by the neutron activation analysis method.

In the course of all of the three experiments microscopic control of the *Sp. pl.* state was performed.

A quantitative determination of the chlorophyll concentration in the biomass was carried out in a 85% acetone extract following the Rebbellen procedure and the protein content of the biomass was investigated by gel-electrophoresis in a polyacrylamide gel by the Leammli method [9].

Analysis

The concentration of selenium and chromium in the obtained samples was measured by epithermal neutron activation analysis (ENAA) at the fast pulsed reactor IBR-2 in FLNP JINR (Dubna) with a neutron flux of 10^{12}n/(cm^2·s).

The characteristics of the irradiation channels and the pneumatic system at IBR-2 are described in [10]. The ENAA method in application to *Sp. pl.* samples is detailed in [5,6].

Quality control of analytical measurements is ensured by the use of certified reference materials for biological sample analysis, including lichen (Lichen-336, IAEA), bottom sediments (IAEA SDM-2T) and Nordic Moss (DK-1).

The ENAA data were processed and chromium/selenium concentrations were determined by the methods and computer programs used in FLNP JINR [11].

Results and discussion

The results of the ENAA of samples in the three described experiments are illustrated in Figs. 1, 2, 3. Figure 1 shows the dynamics of the increase of Se concentration in the *Sp. pl.* biomass cultivated in a nutrient medium for a sodium selenate loading with a Se concentration of Se 5 mg/l.

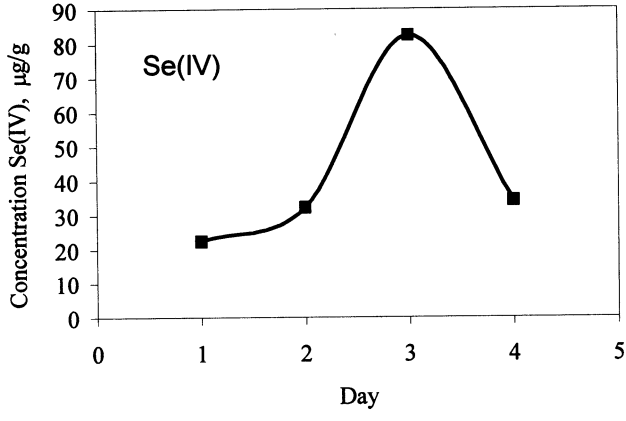

Fig. 1. The dynamics of Se(IV) accumulation in *Spirulina platensis* cells

Figure 2 shows an analogous curve for chromium in the form of chromium acetate with a Cr(III) concentration of 3 mg/l.

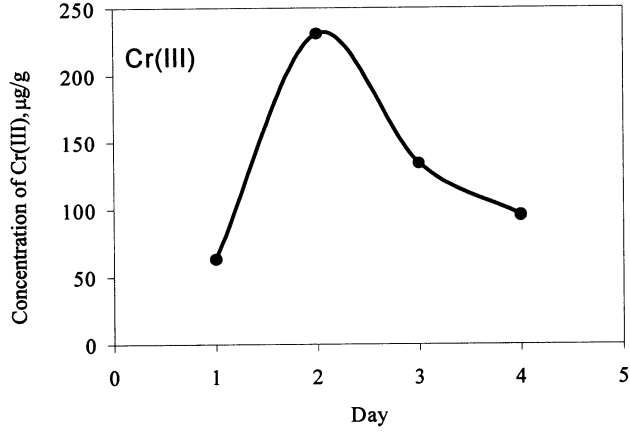

Fig. 2. The dynamics of Cr(III) accumulation in *Spirulina platensis* cells

Figure 3 presents the results obtained in the condition of simultaneous loading of the nutrient medium with Se(IV) and Cr(III) in analogous concentrations.

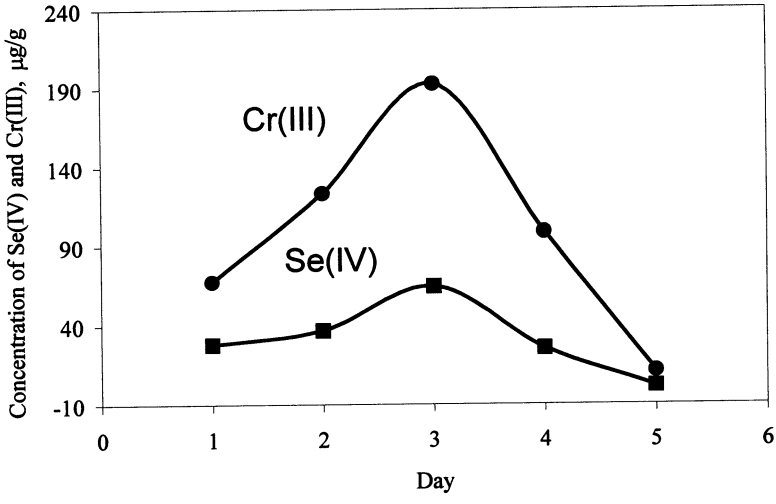

Fig. 3. The dynamics of the accumulation of Se(IV) and Cr(III) in the cells of *Spirulina platensis*

As it is seen from the obtained data the accumulation rate of Cr(III) is higher than the accumulation rate of Se(IV) if they are loaded separately (Figs. 1, 2). In the case of their combined loading, the accumulation of Cr(III) in the *Sp. pl.* biomass goes for the same period of time as of Se(IV). However, *Sp. pl.* accumulates Cr(III) faster and more effectively in both cases. This is possibly due to the fact that in the nutrient medium chromium exists as an organic compound ($Cr(CH_3COOH)_3$), which facilitates chromium penetration into cells, while selenium is present in the form of an inorganic compound (Na_2SeO_3).

The general behavior of the accumulation curves of Se(IV) and Cr(III) at their combined action on the *Sp. pl.* cells retains but there is observed a clear-cut pattern of antagonism exhibiting itself as an essential decrease in the accumulation of Se(IV) in the presence of Cr(III). Since Se(IV) in the form of SeO_3^{2-} enters *Sp. pl.* cell via anion transport channels and Cr(III) in the form of Cr^{3+} via cation transport channels, they do not compete in the process of membrane transport to cells. Therefore, the observed antagonism between chromium and selenium is most probably due to processes of intracellular metabolism.

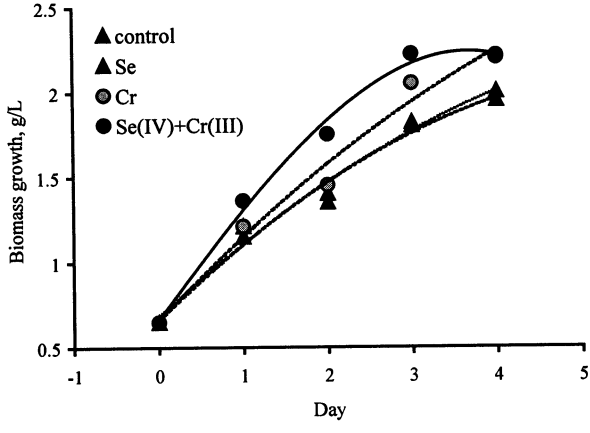

Fig. 4. The growth dynamics of the *Spirulina platensis* biomass in a nutrient medium loaded with Se(IV) and Cr(III)

The study of the *Sp. pl.* growth dynamics illustrated in Fig. 4 shows that no essential difference in the increase of the biomass is observed in the three discussed experiments. In each of then the biomass grows up to the forth day of cultivation. It should be noted that at the accumulation of heavy metals in living systems only 30-50% of cells in the population participate in the process [7].

The behavior of the accumulation curves (Figs. 1-3) obtained taking into account the growth dynamics of the biomass (Fig. 4) prompts a conclusion that intense growth of the biomass accompanied with introducing of the investigated elements into the *Sp. pl.* cells results in a decrease of their concentration in the nutrient medium and in the total biomass.

One may assume that at repeated additional introduction of Se(IV) and Cr(III) compounds into a nutrient medium the behavior of the accumulation curves will be similar to that of the biomass growth curves.

The microscopic control of *Sp. pl.* suspension samples taken daily in all the discussed experiments showed that *Sp. pl.* cells did not differ cytologically from reference cells in all the cases.

The chlorophyll and protein contents in the *Sp. pl.* biomass were also not different from reference samples.

Conclusions

1. The dynamics of the accumulation of Se(IV) and Cr(III) in *Spirulina platensis* cells in the condition of their separate or combined action in a nutrient medium during cultivation was investigated.
2. The conditions of combined introduction of selenium and chromium into the *Spirulina platensis* biomass in the course of cultivation ensuring the preservation of the natural quality and properties of *Spirulina platensis* were determined.
3. The fact of mutual antagonism between Se(IV) and Cr(III) during the cultivation of *Spirulina platensis* in a nutrient medium in the condition of its combined loading with these elements leading to suppression of Se(IV) accumulation was established.

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References

1. Trace elements in human nutrition and health. World Health Organization. Geneva. 1996.
2. H.A. Schroeder, D.V. Frost, I.I. Balassa. - Essential trace metals in man. *J. Chron. Dis.*, (1970), Vol. 23, 227-243.
3. A. Vonshak. *Spirulina platensis (Arthrospira)*: Physiology, Cell Biology and Biotechnology. Taylor&Francis, 1997.
4. A. Belay, Y. Ota, K. Miyakawa, H. Shimamatsu. - Current Knowledge on Potential Health Benefits of Spirulina. *J. Appl. Phycol.*,(1993), No. 5, 235-241.
5. L.M. Mosulishvili, Ye.I. Kirkesali, A.I. Belokobylsky, A.I. Khizanisvili, M.V. Frontasyeva, S.F. Gundorina, C.D. Oprea. Epithermal neutron activation analysis of blue-green algae *Spirulina platensis* as a matrix for selenium-containing pharmaceuticals. *JRNC* (2002), Vol.252, No. 1, 15-20.
6. L.M. Mosulishvili, E.I. Kirkesali, A.I. Belokobylsky, A.I. Khizanishvili, M.V. Frontasyeva, S.S. Pavlov, S.F. Gundorina. Experimental substantiation of the possibility of developing selenium- and iodine-containing pharmacenticals based on blue-green algae *Spirulina platensis*. JINR Preprint D14-2001-39. Dubna, 2001.
7. O.Yu.Sentsova, V.N.Maksimova. The effect of heavy metals on microorganisms. – *Uspekhi mikrobiologii*, (1985), issue 20, 227-252.
8. L.M. Mosulishvili, V.S. Nadareishvili, N.E. Kharabadze, A.I. Belokobylsky. Facility for lyophilization of biological preparatoins. Patent USSR N 779765, Bull. 42, (1980).
9. U. K. Leammli. Cleavage of structural proteins during the assembly of the hesd of bacteriophage T4. *Nature,* (1970), Vol. 227, 680-685.
10. M. V. Frontasyeva, S. S. Pavlov. Analytical investigations at the IBR-2 reactor in Dubna. JINR Preprint E14-2000-177, Dubna, 2000.
11. T. M. Ostrovnaya, L. C. Nefedyeva, V. M. Nazarov, S. B. Borrakov and L. P. Strelkova. Software for INAA on the basis of relativ and absolute methods using nuclear data base. - In Activation analysis in environmant protection. D 14-93-325, Dubna, 1993, 319-326.

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Белокобыльский А. И. и др. Д18-2002-130
Аккумуляция селена и хрома клетками *Spirulina platensis* в динамике роста

Описаны результаты исследований динамики процессов аккумуляции Se(IV) и Cr(III) клетками сине-зеленой микроводоросли с помощью эпитеплового нейтронного активационного анализа. Изучено влияние селена и хрома на некоторые биохимические свойства биомассы *Spirulina platensis*. Выявлен антагонистический характер взаимодействия Se(IV) и Cr(III) при их комбинированном использовании во время культивации *Spirulina platensis*. Этот антагонизм сопровождается подавлением процесса аккумуляции Se(IV).

Работа выполнена в Лаборатории нейтронной физики им. И. М. Франка ОИЯИ и в Институте физики им. Э. Л. Андроникашвили АН Грузии.

Препринт Объединенного института ядерных исследований. Дубна, 2002

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Accumulation of Selenium and Chromium in *Spirulina platensis* Cells in the Dynamics of Growth

The findings of the epithermal neutron activation analysis investigation of the dynamics of Se(IV) and Cr(III) accumulation in *Spirulina platensis* cells are discussed. The effects of selenium and chromium on some biochemical properties of the *Spirulina platensis* biomass are studied. An antagonistic behavior of the interaction between Se(IV) and Cr(III) accompanied with suppression of Se(IV) accumulation during the cultivation of *Spirulina platensis* in the condition of Se(IV)- and Cr(III) combined loading is established.

The investigation has been performed at the Frank Laboratory of Neutron Physics, JINR and at the E. L. Andronikashvili Institute of Physics of the Georgian Academy of Sciences.

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