E18-2002-107

M. V. Frontasyeva, L. M. Mosulishvili*,
A. I. Belokobylsky*, E. I. Kirkesali*, S. S. Pavlov

EXPERIMENTAL GROUNDS FOR DEVELOPING SELENIUM- AND IODINE-CONTAINING PHARMACEUTICALS BASED ON BLUE-GREEN ALGAE *SPIRULINA PLATENSIS*

Submitted to «Journal of Radioanalytical and Nuclear Chemistry»
(Proceedings of the International Conference
on Nuclear Analytical Methods in the Life Sciences «NAMLS-7»,
Antalya, Turkey, 16–21 June, 2002)

---

*E. L. Andronikashvili Institute of Physics, Georgian Academy of Sciences, Tbilisi, Georgia

Introduction

Investigations in the field of molecular biology show that most human illnesses are so-called «molecular» diseases stemming from lack some trace elements (Se, I, Cr, Fe, Zn, *ect.*) and their compounds. An important role of trace elements in human health is the obvious reason for the development of new progressive trends in medicine, pharmacology and biotechnology [1].

Selenium, one of the vitally meaningful elements, is a normal component of some enzymes, proteins and aminoacryl derivatives of nuclei acids.

The diverse importance of selenium for the human organism is emphasised in the proceedings of the 7th International Symposium Selenium-2000 (Venice, October 2000), where selenium was called «the element of the century». According to the data of the Institute of Nourishment of the Russian Academy of Medical Sciences (Moscow), selenium deficiency is found practically over the whole of Russia. In 1998 a programme «Selenium, Health, Man» was launched in the Russian Federation and selenization of the population is being carried out within its framework.

Selenium lowering may cause such diseases as cardiomyopathy, cancer, endemic osteoarthropathy, anaemia, *etc.* In China there is a vast area with soils low in selenium, the so-called «selenium-deficient zone», where these diseases of endemic nature occur [2]. The investigations performed in the United States showed that in areas where the selenium content of cereals is low mortality due to various kinds of cancer is higher than in other areas [3].

The function of selenium in the organism is closely related to functions of vitamin *E* and beta-carotene. According to some hypotheses, inhibition of carcinogenesis after administering selenium is due to its antioxidant property, impact on tumour metabolism, effect on the endocrine and immune systems, and inhibition of specific enzymes.

Selenium takes part in photochemical reactions related to vision. Selenium also allows detoxification of the organism by helping to bind some harmful elements, such as As, Cd, Hr, Bi, *etc*, to high-molecular proteins of blood plasma and to remove them from the organism. In the absence of selenium these elements are bound to a low-molecular protein metallothioneine and settle together with it in kidneys [4].

It has been found that selenium added to diet in particular doses decreases the cancer hazard, favours treatment of cardiological patients, reduces the acquired immunodeficiency syndrome (AIDS), slows down the ageing, *etc.* [5,6]. On the other hand, a high level of selenium causes toxicosis.

Another equally important element incorporated in all plants and animals is iodine. It is vitally important for functioning, development and growth of the organism, where it comes with food, water and air [7].

Iodine affects metabolism enhancing the oxidation-reduction processes. Iodine deficiency results in dysfunction of the thyroid, less of its hormones thyroxin and triiodothyronine are ejected into blood, which eventually leads to hypothyroidism.

Iodine content of the air strongly depends on proximity of the area to the sea. The sea air can satisfy the daily iodine demand of man (~200 μg). On the contrary, the air in the mountain areas is low in iodine, which entails iodine deficiency in the human organism and thus mass thyroid diseases.

Unfavourable environmental conditions, discharge of radioactive materials into the air during accidents, lowering of living standards have aggravated the situation both on the post-Soviet territories and in the whole world. In Russia about 70 % of the population are suffering iodine deficiency in varying degrees.

Recently the symptoms and results of iodine deficiency have been more thoroughly studied on the emotional level (irritability, memory impairment, sleepiness, *etc.*), cardiological level (arteriosclerosis, arrhythmia, deformation of vascular walls, *etc.*), immunodeficient level (susceptibility to infections and colds). The intelligence quotient (IQ) is found to be directly related to the iodine concentration in the organism.

It was found out that mental retardation of 43 million people in the world is due to iodine deficiency. Every year 100000 children suffering cretinism because of iodine deficiency are born. Therefore, elimination of diseases caused by iodine deficiency is one of UN priorities in the field of human health.

The experience of using iodinated salt to prevent iodine deficiency in the United States, Switzerland, and other countries showed that iodine excess results in such a thyroid disease as iodine-induced hyperthyroidism.

The investigations showed that only biotransformed trace elements synthesized by protein molecules are assimilated by the organism in the required amount, neither less nor more.

The ability to biotransform and endogenously add the desired elements (Se, I, Cr, *etc.*) producing complexes easily assimilated by a human organism is a distinctive feature of *Spirulina platensis.* Being a living organism, spirulina accumulates elements strictly as much as is necessary for the organism. Spirulina-based preparations contain a complex of biologically active agents and produce both therapeutic and health-improving effect. This wide use is due to its fast growth, non-toxicity, assimilability (85–95%), high protein content (60–70%), well-balanced amino acid composition, richness in vitamins, and a great variety of biologically active agents in appreciable amounts [8, 9].

All the aforesaid has led to a hypothesis that it would be reasonable to use *Spirulina platensis* biomass as a matrix for production of Se and I-containing pharmaceuticals to treat various illnesses.

To verify this hypothesis it was necessary:

- to study the dynamics of Se, I accumulation in the spirulina matrix at different loading concentrations of nutrient medium;
- to study the influence of selenium loading of the nutrient medium on the growth rate of *Spirulina platensis* and the quality of its biomass.

Experimental

Spirulina grows well in a standard alkaline mineral nutrient medium at a temperature of 30-34°C, pH 8,5-11, under sodium lamp light. Cultivation was carried out 4-5 days.

To study elements accumulation dynamics in the *Spirulina platensis* biomass cultivation was carried out in a nutrient loading with concentrations ranging:

- for selenium – selenious acid H_2SeO_3 – from 0,5 to 15 mg/L;
- for iodine – potassium iodine KI – from 10^{-8} to 10^{-4} g/L.

The growth rate, protein composition and cells conditions of spirulina biomass are basic important qualitative characteristics which must be studied at pharmaceutical production.

To examine the dependence of the *Spirulina platensis* cells growth rate on the nutrient medium loading with selenium compound H_2SeO_3, cultivation was performed at different concentrations (1–100 mg/L) introduced to the nutrient medium in the first day of cultivation ("dose–effect").

The protein composition was studied by the method of gel-electrophoresis and the cells conditions was observed in the microscope.

After cultivation the spirulina cell mass was separated from the nutrient medium, washed with distilled water three times and centrifuged. The resulting wet biomass was lyophilically dried in a adsorption-condensation lyophilizer. The native dry biomass was made into small pellets of various diameters and thickness by means of a special titanium mould.

Samples was studied by epithermal neutron activation analysis (ENAA) used at the pulsed fast reactor IBR-2 (JINR FLNP, Dubna) and described in the works [10, 11].

Results and discussion

The ENAA results of experiments with Se loading are displayed in Fig. 1.

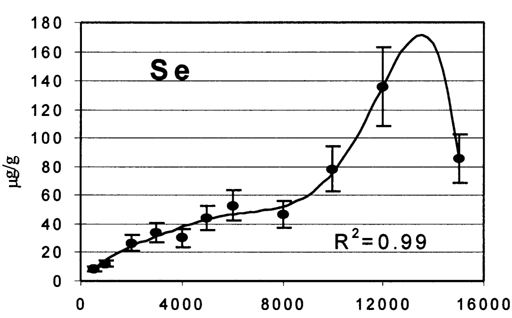

Fig. 1. Concentration of selenium in the spirulina biomass as a function of their concentration in the nutrient

The concentration dependence of selenium in spirulina biomass is well approximated by the six-order polynomial with R^2=0.99. The increase of selenium accumulation is observed with maximum in the range with 13 mg/L. This more precise new results are in good agreement with our results obtained earlier [11]. For iodine approximation is described by polynomial of the second-order.

The obtained dependence allow to determine the conditions of spirulina biomass cultivation corresponding to the apriori given doses of Se accumulation.

The concentration dependence for iodine and the methode of optimal doses determination for production of iodinated pills was early discribed in our work [12].

In the course of cultivation spirulina cells may assimilate some toxic elements, like Hg, As, Cr, Cd, Pb, *etc.*, present in the nutrient as impurity.

On the other hand, the US data on permissible doses of various elements for a human organism (see http://www.spirulina.com/SPBNutrition.html) show that our results ENAA obtained for this elements with chemically pure reagents and with reagents of pure grade do not exceed the permissible level.

The results obtained in experiments «dose–effect» are shown in Fig. 2. As follows from this figure, when the Se concentration is 50-100 mg/L, the effect of cells growth rate elimination becomes significant after 6 days of cultivation, and on the 12th day the amount of spirulina biomass reaches 25-30% of that grown without nutritional loading. At low concentration of selenium loading the effect of cells elimination is insignificant.

The results obtened with gel-electrophoresis and in microscopic observation also show that selenium loading no change the biomass quality.

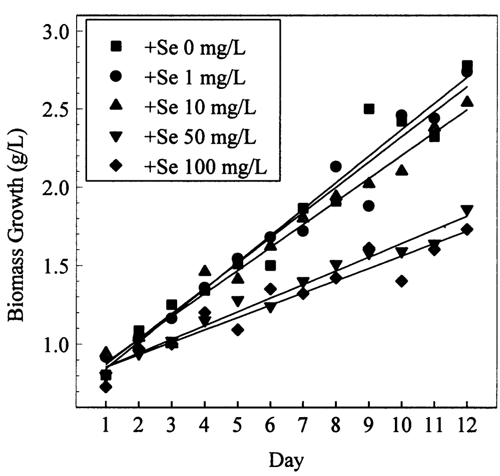

Fig. 2. Dependence of *Spirulina pl.* biomass growth on Se concentration in the nutrient medium

Conclusions

1. Target-oriented introduction of selenium and iodine in the spirulina biomass is shown to be possible during cultivation. Polynomial relationship between accumulation of selenium in the spirulina biomass and their concentration in the nutrient is found.
2. It is demonstrated that the dangerous concentration of toxic elements can be preliminarily determined in the course of drug production. It is found out that highly

pure reagents are desirable for cultivation of spirulina biomass for pharmaceutical purposes.

3. The chosen conditions of spirulina biomass cultivation with loading with the given elements provides their endogenic inclusion in Spirulina biocomplexes without deteriorating its natural features.

The present investigation was supported by the International Center for Scientific and Technical Research (grant G-408).

References

1. Trace elements in human nutrition and health. World Health Organization. Geneva. 1996, 155-160.
2. C. Chen, P. Zhang, X. Hu, Z. Che. Subcellular distribution of selenium and Se-containing proteins in human liver. *Biochemica et Biophysica Acta* 1427 (1999) 205-215.
3. L.C. Clark, K.P. Cantor, W.H. Allaway. Selenium in forage crops and cancer mortality in US countries. *Arch. Environ. Health*, 46 (1991) 37-42.
4. L.A. Daniel. Selenium metabolism and bioavailability. Biol.Trace Elem. Res. 54 (1996) 185-199.
5. G.F. Combs Jr., Chemopreventive mechanisms of selenium. *Med. Klin.*, Oct.15, 94, Suppl. 3 (1999) 18-24.
6. D.H. Holben and A. M.Smith, The diverse role of selenium within selenoproteins: a review. *J. An. Diet. Assoc.* 1999, July; 99(7) (1999) 836-843.
7. A.I. Voinar. Trace elements in Nature. M., 1962. p. 74-92 (in Russian).
8. D. Fox, Health Benefits of Spirulina. In *Spirulina, Algae of Life*, Bulletin No. 12. April 1993, Publ. by Institute of Oceanography, Monaco.
9. A. Belay, Y. Ota, K. Miyakawa, H. Shimamatsu, Current Knowledge on Potential Health Benefits of Spirulina. *J. Appl. Phycol.,* No.5 (1993) 235-241.W.
10. M.V. Frontasyeva, S.S. Pavlov: Analytical investigations at the IBR-2 reactor in Dubna. *JINR Preprint* E14-2000-177, Dubna, 2000.
11. L.M. Mosulishvili, Ye.I. Kirkesali, A.I. Belokobylsky, A.I. Khizanishvili, 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, *JINR Preprint* E14-2000-225, Dubna, 2000 (submitted to *JRNC*).
12. L.M. Mosulishvili, Ye.I. Kirkesali, A.I. Belokobylsky, A.I. Khizanishvili, M.V. Frontasyeva, S.S. Pavlov, S.F. Gundorina. Investigation of the Possibility of Developing Iodine-Containing Treatment and Prophilactic Parmaceuticals Based on Blue-Green Algae Spirulina platensis Using Neutron Activation Analysis. Particls and Nuclei, Letters. 2001. № 4 [107], 110-117.

Received on May 14, 2002.

Фронтасьева М. В. и др. E18-2002-107

Экспериментальное обоснование возможности создания селен- и йодсодержащих препаратов на основе сине-зеленой водоросли *Spirulina platensis*

Исследована возможность использования сине-зеленой водоросли *Spirulina platensis* в качестве матрицы для создания селен- и йодсодержащих фармацевтических препаратов. Определены с большой точностью зависимости аккумуляции Se и I биомассой спирулины в процессе культивации в питательной среде с нагрузкой соответствующими элементами. Изучена динамика роста биомассы спирулины в питательной среде с нагрузкой селеном. Установлено, что качество биомассы *Spirulina platensis* позволяет использовать ее в фармацевтических целях.

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

Frontasyeva M. V. et al. E18-2002-107

Experimental Grounds for Developing Selenium- and Iodine-Containing Pharmaceuticals Based on Blue-Green Algae *Spirulina platensis*

The possibility of using blue-green algae *Spirulina platensis* as a matrix for production of the selenium- and iodine-containing pharmaceuticals was studied. The dependence of Se and I accumulation in Spirulina biomass during the cultivation in a nutrient medium loading of above elements was determined more precisely. The dynamics of Spirulina biomass growth was observed with nutrient medium loading of selenium. It is found that *Spirulina platensis* biomass quality may be used for pharmaceutical purposes.

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.

Preprint of the Joint Institute for Nuclear Research. Dubna, 2002

Макет *Т. Е. Попеко*

ЛР № 020579 от 23.06.97.
Подписано в печать 18.07.2002.
Формат 60 × 90/16. Бумага офсетная. Печать офсетная.
Усл. печ. л. 0,68. Уч.-изд. л. 0,77. Тираж 270 экз. Заказ № 53432.

Издательский отдел Объединенного института ядерных исследований
141980, г. Дубна, Московская обл., ул. Жолио-Кюри, 6.