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DOES THE TEMPERATURE OF –80 °C PRESERVE THE ACTIVITY OF ANTIOXIDANT ENZYMES EMBEDDED INTO ALGINATE MICROCAPSULES?

Key words: *catalase, superoxide dismutase, low temperature storage, alginate microcapsules.*

Superoxide dismutase (SOD) and catalase (CAT) are among a wide class of enzymes of practical importance in medicine and biotechnology, being the most important factors of antioxidant defense [1, 10]. In a recent paper of S. Anwar *et al.* [1], an insufficient stability, low bioavailability and specificity of this enzyme delivery to target tissues are emphasized as significant obstacles to more widespread use of catalase in practical medicine. Increasing /improving these parameters, as well as the catalase "longevity", will reveal its full therapeutic potential. One of the ways to solve this issue is to incorporate the enzyme into various microcarriers, including alginate-based ones. All the above also applies to the SOD enzyme [10], which additionally has therapeutic properties.

A widespread use of the enzymes embedded into hydrogel carriers in biomedicine and biotechnology requires the development of techniques for their long-term storage with enzymatic activity preservation. There are the reported data on storage of hydrogel carriers with embedded enzymes at 4 °C [9], but studies on storing these complexes at low temperatures are currently scarce.

Much attention is paid to studies on freezing and storage of various biological objects at –80 °C, including monoclonal antibodies, various enzymes, extracellular vesicles, *etc.* [4—6, 8].

The totality of findings show the prospects of storing biological substances at –80 °C with preserving their properties for further use in medical practice and biotechnology. However, the issue of the possibility of direct application of the results obtained to other biological objects has still remained unresolved.

To change the characteristics of sodium alginate-based hydrogels, they are usually modified with laponite, a biocompatible nanomaterial that is easily biodegradable and can improve protein incorporation and mechanical properties of hydrogels. When changing the content of laponite in hydrogels, the kinetics of protein release may be adjusted [2].

The research aim was to explore the impact of storage at –80 °C on the activity of enzyme proteins: superoxide dismutase and catalase, embed-

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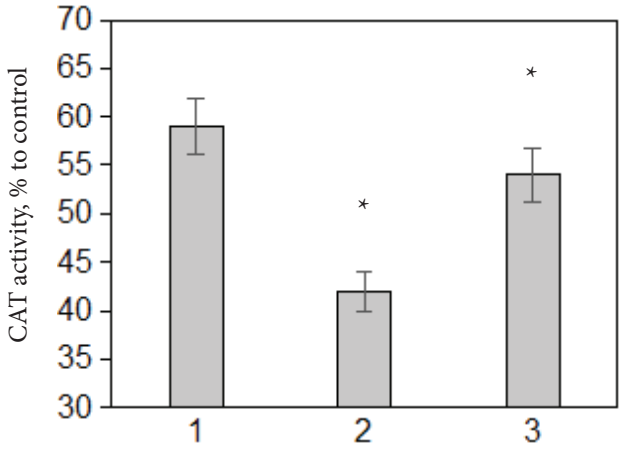

Fig. 1. Impact of storage at –80°C for 30 days on catalase activity. * — differences are significant as compared to CAT activity in alginate capsules, $p < 0.05$

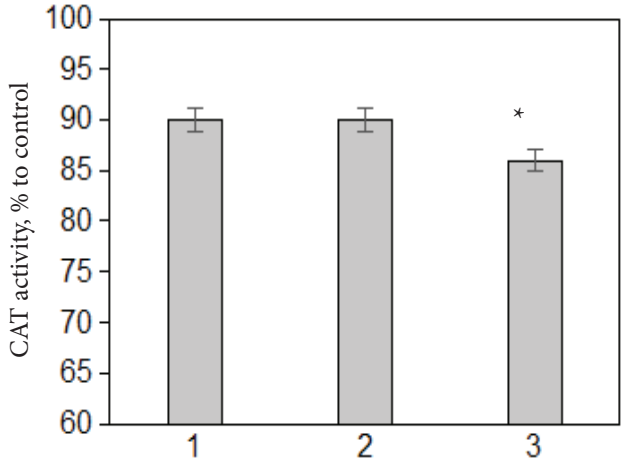

Fig. 2. Impact of storage at –80°C for 30 days on superoxide dismutase activity. * — differences are significant as compared to SOD activity in alginate capsules, $p < 0.05$

ded into alginate microcapsules and those modified with laponite nanoparticles.

Here we used sodium alginate (ChemCruz, USA) of low viscosity (2-4 cP); laponite RD (GMW, Germany), SOD (Sigma-Aldrich Chemical, Germany), CAT (Sigma-Aldrich Chemical) and CaCl2 (UkrChemGroup, Ukraine). Alginate microcapsules and those modified with laponite nanoparticles with embedded enzymes were prepared according to the method of I.P. Khala *et al.* with some modification [7]. The solution of sodium alginate with enzymes was prepared in a following way: 2 ml of the initial enzyme solution (10 mg/mL for SOD and 20 mg/mL for CAT) were mixed with 300 μL of 2% sodium alginate solution, brought to 5 ml with Tris-HCl buffer (pH = 7.8), and added dropwise to a bath with 2% $CaCl_2$ in the same buffer, *i. e.* by forcing the drop to detach from the sprayer tip using an air stream. The COD and CAT activities were assessed according to the above method [7]. The enzyme activity in the initial solution was considered as the control. The activity was further calculated taking into account the enzyme dilution. To evaluate the efficiency of enzyme embedding into hydrogels and to control their leakage, the enzyme concentration was measured spectrophotometrically (280 nm) in microcapsules and supernatant after their precipitation. Absorption spectra were recorded using a Pye Unicam SP 8000 spectrophotometer (Pye Unicam Ltd, UK).

To study the impact of –80 °C on the enzyme activity within microcapsules, the latter were frozen and stored in an ultra-low temperature freezer Haier DW-86W100J (Haier Biomedical Co., China) at –80 °C for 30 days. Then, the samples were thawed in a water bath at 37 °C.

The data in Figs. 1 and 2 are presented as mean ± standard deviation. Experimental results were statistically processed using the 'Statistica 6.0' (StatSoft Inc., USA) software. The Mann-Whitney test was used to assess statistical differences in the studied numerical indices. Differences were considered significant at $p \leq 0.05$. The number of experiments in each series of trials was at least five.

Using ionotropic gelation, there were formed the alginate microcapsules, including those modified with laponite nanoparticles, with 0.4—0.8 mm diameter and encapsulated enzymes, which retained sufficient activity as compared to the initial solution.

After a 30-day storage of CAT embedded into hydrogel microcapsules at –80 °C, the enzyme activity decreased *vs.* the control (activity in initial solution). At the same time, as shown in Fig. 1, the activity of CAT stored within alginate microcapsules was 59%, that was slightly higher than that of CAT after storage in buffered saline (54%). Storage for 30 days at –80 °C affected the activity of CAT embedded into the alginate microcapsules modified with laponite nanoparticles the most. The activity decreased down to 42%, which was much lower as compared to non-immobilized CAT (Fig. 1).

The research has shown a 30-day storage of SOD at –80 °C not to virtually affect the activity of the enzyme embedded in both alginate microcapsules

and those modified with laponite nanoparticles. In both cases, the SOD activity was about 90% of that in the initial solution (Fig. 2) and exceeded the activity of SOD preserved in buffer solution (86%).

It has been demonstrated that storage at –80 °C affects the properties of antioxidant enzymes such as catalase and superoxide dismutase, that correlates with findings of J. Hartmann and F. Asch [4], who, using the example of antioxidant enzymes from rice tissues, *i. e.* ascorbate peroxidase, glutathione reductase and superoxide dismutase, have revealed different impacts of the environment in which freezing was done (tissue extracts, buffer solutions), the storage time and temperature itself (–20 and –80 °C) on enzymatic activity preservation. In addition, the enzymes under study differ significantly in molecular weight and conformation (CAT is a tetramer of four polypeptide chains (250 kDa) and SOD is a homodimer (32.5 kDa)). This fact may also explain the different effects of storage at –80 °C on the activity of enzymes embedded in alginate microcarriers.

The results of study of freezing and storage of monoclonal antibodies showed their long-term storage in phosphate-buffered saline in a frozen state at –80 °C not to result in any additional aggregation as compared to a single freeze-thawing cycle [5, 8]. This suggests that protein degradation occurs most likely during freeze-thawing rather than after long-term storage. Findings of O.I. Gordiyenko *et al.* [3] indicate the disorder in structure of biological objects under slow freezing down to –80 °C as well.

Thus, storage at –80 °C promoted to preserve the activity of SOD embedded into alginate microcapsules, but reduced the CAT activity. Notably, that modification of alginate hydrogels with laponite nanoparticles has different impacts on SOD and CAT preservation, which may result from both direct effect of laponite on activity of embedded enzyme, and the structure of hydrogel itself, changed by laponite.

To clarify this difference, further comparative studies on storage of enzymes embedded into alginate carriers at –40 °C are required.

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ЧИ ЗБЕРІГАЄ ТЕМПЕРАТУРА −80 °С АКТИВНІСТЬ ВБУДОВАНИХ
У АЛЬГІНАТНІ МІКРОКАПСУЛИ АНТИОКСИДАНТНИХ ФЕРМЕНТІВ?

Ключові слова: каталаза, супероксиддисмутаза, низькотемпературне зберігання, альгінатні мікрокапсули.