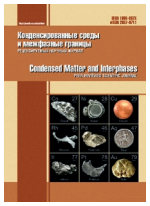

ISSN 1606-867X (Print)
ISSN 2687-0711 (Online)
Condensed Matter and Interphases
Kondensirovannye Sredy i Mezhfaznye Granitsy
https://journals.vsu.ru/kcmf/

Original articles

Research article
https://doi.org/10.17308/kcmf.2022.24/10556

Study of the proteolytic activity of ficin associates with chitosan nanoparticles

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Abstract

The purpose of the research was to develop and study biocatalysts based on ficin associates with chitosan nanoparticles. We obtained medium and high molecular weight chitosan nanoparticles with the addition of ascorbic acid and without it.The zeta potential of all types of nanoparticles was 0 mV. The associates of ficin and chitosan nanoparticles formed with the addition of ascorbic acid exhibited higher proteolytic activity. While determining the stability of the associates of chitosan and ficin nanoparticles, we noticed a decrease in the proteolytic activity of the samples within seven days. Medium and high molecular weight chitosan nanoparticles obtained using ascorbic acid differed significantly in size from the nanoparticles produced without ascorbic acid. The proposed biocatalysts have high prospects for use in cosmetology, biomedicine, and pharmacy.

Keywords: Nanoparticles, Ficin, Chitosan, Association

Funding: The study was founded by the Priority-2030 programme of Sevastopol State University, strategic project No.3 (production of chitosan nanoparticles), and by the Ministry of Science and Higher Education of the Russian Federation in the framework of the government order to higher education institutions in the sphere of scientific research for years 2020-2022, project No. FZGU-2020-0044 (complexation of nanoparticles with enzymes).

For citation: Olshannikova S. S., Redko Yu. A., Lavlinskaya M. S., Sorokin A. V., Holyavka M. G., Yudin N. E., Artyukhov V. G. Study of the proteolytic activity of ficin associates with chitosan nanoparticles. *Condensed Matter and Interphases.* 2022;24(4): 523–528. https://doi.org/10.17308/kcmf.2022.24/10556

Для цитирования: Ольшанникова С. С., Редько Ю. А., Лавлинская М. С., Сорокин А. В., Холявка М. Г., Юдин Н. Е., Артюхов В. Г. Исследование протеолитической активности ассоциатов фицина с наночастицами хитозана. *Конденсированные среды и межфазные границы*. 2022;24(4): 523–528. https://doi.org/10.17308/kcmf.2022.24/10556

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1. Introduction

Recently, nanoparticles which are highly dispersed materials smaller than 100 nm, have gained great importance in modern medicine. Their applications range from contrast imaging agents to carriers for the delivery of genes and bioactive substances to target cells. These unique nanomaterials have a number of properties due to their size distinguishing them from macromaterials of similar composition. Among these properties are high reactivity, electromagnetic energy absorption, and high biological mobility [1–3].

Nanoparticles are also known as zero-dimensional nanomaterials. They are called so because the sum of their dimensions is at the nanoscale. This feature differs them from one- and two-dimensional nanomaterials (nanowires, nanotubes, and self-assembled monolayer films, etc.) having one or two dimensions outside the nanoscale [4].

As mentioned above, nanoparticles have numerous advantages for modern medicine. First, their structural stability and ability to protect the bioactive substance against degradation, deactivation, and clearance make it possible to maintain the required substance therapeutic concentration [5–7].Moreover, it is possible to achieve the desired size and surface charge in the production of nanoparticles. The release kinetics of bioactive substances from the nanocomplexes is controllable. It occurs by diffusion, polymer swelling, or degradation, or a combination of these processes depending on the polymer type used to produce the carrier matrices [8]. Thus, nanoparticles are promising materials for the medical use. Chitosan is one of the promising polysaccharides for their production. It is a modified natural polyamino-β-glycoside combining various mechanisms of medicine release. In addition to its biodegradability and the immune response absence, chitosan is marked by antibacterial activity due to the free primary amino group presences [9–10] and high mucoadhesive properties [11].

Proteases are the first enzymes used in food biotechnologies. Today, they proteases are applied in many industrial processes such as the tanning and pharmaceutical industry, as well as in biomedicine. Ficin occupies a special place among the frequently-used plant proteases.

Ficin (EC 3.4.22.3) is a proteolytic enzyme isolated from the genus *Ficus* plant latex . It is a monomeric protein consisting of a single polypeptide chain with a molecular weight of 25–26 kDa. Ficin is a representative of cysteine papain-like proteases and it is characterised by broad substrate specificity. The maximum catalytic activity is reached in the pH range of 6.5–9.5. [12–16]. Ficin exhibits antimicrobial activity against gram-positive and gram-negative bacteria. It is also known to have anti-inflammatory, anthelmintic, antithrombotic, fibrinolytic and anticancer properties, as well as immunomodulatory effect [17–19].

However, the use of native protease solutions is limited by their low stability and autolysis resulting in the loss of practically valuable enzyme properties. One way to save them is to obtain hybrid enzyme formulations using nanoparticle carriers [20].

Therefore, the aim of this research was to study the catalytic (proteolytic) activity of ficin associates with chitosan nanoparticles.

2. Experimental

Ficin was the object of the study, while azocasein was chosen as the substrate for hydrolysis (both produced by Sigma, USA). Nanoparticles were obtained from medium molecular weight (MMWC, 200 kDa) and high molecular weight (HMWC, 350 kDa) chitosan purchased by Bioprogress, Russia.

Chitosan nanoparticles with ascorbic acid and without it, and their associates with ficin were prepared using the method described in [20–22].

Protease activity of the immobilized ficin was measured by the common method described in [24]. The research was carried out using 0.5% azocasein solution in 50 mM Tris-HCl buffer with pH 7.5 for 2 h at 37 °C. The amount of ficin (in mg of protein) that hydrolysed 1 μmol of substrate in 1 min was taken as a unit of catalytic activity.

Nano Zetasizer ZS (Malvern Instruments, USA) equipped with 4 mW He/Ne-laser with $\lambda = 632.8$ nm and scattering angle 173 °C was used to measure the nanoparticle and ficin associates size and surface charge.

3. Results and discussion

In the first series of experiments, we determined the size and zeta potential of chitosan nanoparticles. The nanoparticle parameters are presented in Table 1. It was found that medium and high molecular weight chitosan nanoparticles obtained using ascorbic acid differed significantly in size from the ones produced without ascorbic acid. The median zeta potential of all nanoparticles was 0 mV.

When ficin was associated with medium and high molecular weight chitosan nanoparticles formed without ascorbic acid, the proteolytic activity of the associated enzyme was 84 and 88 % of native enzyme. When ficin was associated with nanoparticles obtained with ascorbic acid, its catalytic activity increased by 15 % for medium molecular weight chitosan and by 18 % for high-molecular-weight chitosan (Fig. 1). The better preserving enzyme activity in the complex with chitosan nanoparticles obtained with ascorbic acid is probably due to the antioxidant effects of additive on the biocatalyst[25, 26].

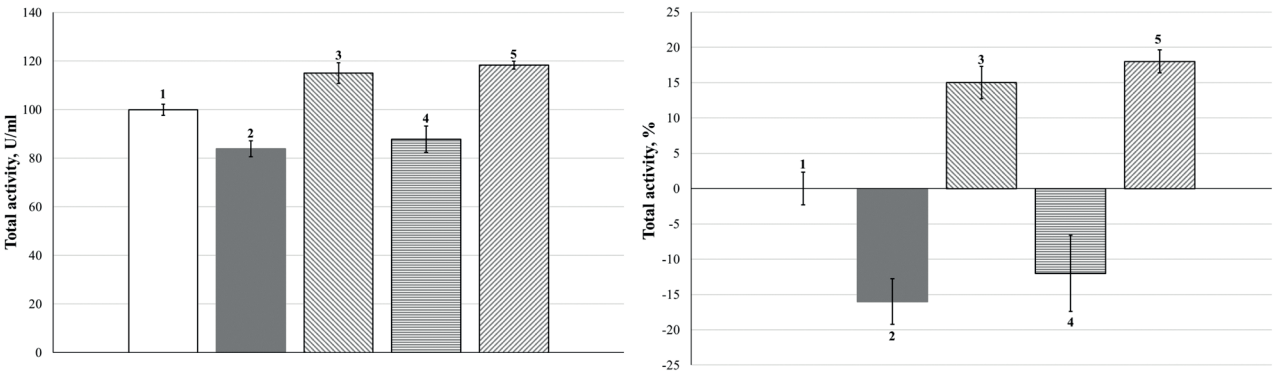
We carried out experiments to determine the residual proteolytic activity of native and associated ficin at 37 °C and pH 7.5 in 0.05 M Tris-HCl buffer. All samples showed a decrease in their activity within 7 days.

After incubation for 168 hours, native ficin retained 8 % of its initial catalytic activity. Its associates with medium and high molecular weight chitosan nanoparticles obtained without ascorbic acid retained 27 and 16 % of their activity, respectively. The ficin associates with medium and high molecular weight chitosan nanoparticles with ascorbic acid retained 39 and 18 % of their proteolytic activity, respectively (Fig. 2).

Starting from 4 hours of incubation at 37 C in 0.05 M Tris-HCl buffer at pH 7.5, the ficin associates with nanoparticles were more stable than the free enzyme.

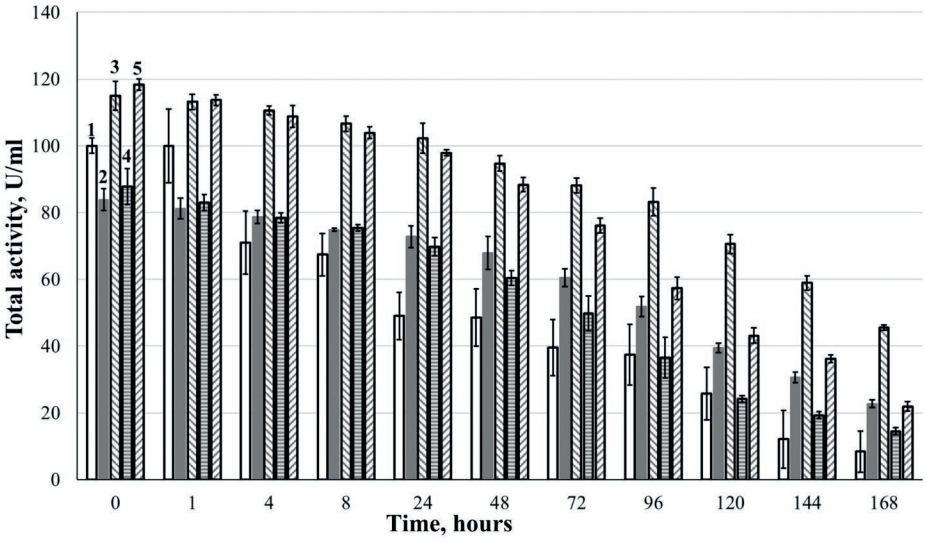
Table 1. Parameters of medium and high molecular weight chitosan nanoparticles

Chitosan nanoparticles	Average size, nm	Size range, nm	Median zeta-potential, mV	Zeta-potential range, mV
Medium molecular weight chitosan	12	7–21	0	0
Medium molecular weight chitosan with ascorbic acid	21	14–59	0	0
High molecular weight chitosan	33	18–79	0	0
High molecular weight chitosan with ascorbic acid	38	28–79	0	0



a b

Fig. 1. Catalytic activity of ficin, units/ml (a) and its change, % (b): soluble ficin (*1*); ficin associated with medium molecular weight chitosan nanoparticles (*2*); ficin associated with medium molecular weight chitosan nanoparticles with ascorbic acid (*3*); ficin associated with high molecular weight chitosan nanoparticles (*4*); and ficin associated with high molecular weight chitosan nanoparticles with ascorbic acid (*5*). The activity of free ficin under optimum hydrolysis conditions was taken as 100 %.



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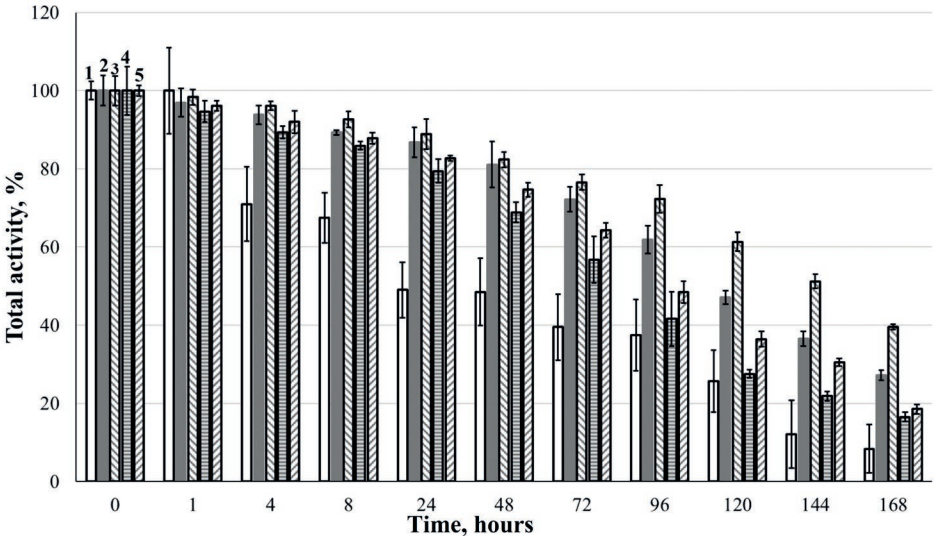

b

Fig. 2. Residual catalytic activity of ficin after incubation of the samples at 37 °C (a: in units/ml of solution or suspension, b: % of the original value): *1* – soluble ficin; *2* – ficin associated with medium molecular weight chitosan nanoparticles; *3* – ficin associated with medium molecular weight chitosan nanoparticles with ascorbic acid; *4* – ficin associated with high molecular weight chitosan nanoparticles; and 5 – ficin associated with high molecular weight chitosan nanoparticles with ascorbic acid. The catalytic activity of the obtained samples under optimum hydrolysis conditions was taken as 100 %.

4. Conclusions

Thus, we obtained medium and high molecular weight chitosan nanoparticles with or without ascorbic acid. The ficin associates and chitosan nanoparticles obtained with ascorbic acid exhibited higher proteolytic activity. While determining the stability of the ficin and chitosan nanoparticles, we noticed a decrease in the proteolytic activity of the samples within seven days.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author contributions

All authors made an equivalent contribution to the preparation of the publication.

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Received 06.05.2022; approved after reviewing 30.06.2022; accepted for publication 15.09.2022; published online 25.12.2022.

Translated by Anastasiia Ananeva
Edited and proofread by Simon Cox