Chemistry Proceedings

MDPI

Proceedings

Covalent Immobilization of Thiol Proteinases on Chitosan †

Svetlana Olshannikova [1], Victoria Koroleva [2,*], Marina Holyavka [1], Alexander Pashkov [2] and Valeriy Artyukhov [1]

[1] Department of Biophysics and Biotechnology, Voronezh State University, Voronezh 394036, Russia; Olshannikovas@gmail.com (S.O.); marinaholyavka@yahoo.com (M.H.); artyukhov@bio.vsu.ru (V.A.);
[2] Department of Biology, Voronezh State Medical University named after N.N. Burdenko, Voronezh 394036, Russia; vgma-pashkov@yandex.ru (A.P.)
* Correspondence: koroleva_victoria@bk.ru
† Presented at 1st International Electronic Conference on Catalysis Sciences, 10–30 November 2020; Available online: https://eccs2020.sciforum.net.

Published: 10 November 2020

Abstract: Plant enzymes such as ficin (EC 3.4.22.3), papain (EC 3.4.22.2) and bromelain (EC 3.4.22.4) are obtained from tropical plants. These biocatalysts belong to thiol proteases, in the active center of which cysteine is contained. Ficin, papain and bromelain have a wide substrate specificity, which provides a demand for their use in various industries. Enzymes in the free state are less commonly used; immobilized biocatalysts are the preferred form. The aim of this work was to determine the optimal concentration of a crosslinking agent in the covalent immobilization of ficin, papain and bromelain on a chitosan matrix. Ficin, papain, bromelain (Sigma) were chosen as objects of study. An acid-soluble chitosan (350 kDa, Bioprogress CJSC) was used as an immobilization carrier. The concentration range of glutaraldehyde (crosslinking agent) ranged from 1 to 25%. Suitable concentration of glutaraldehyde for covalent immobilization were identified by the optimal ratio of protein content (mg per g of carrier), total activity (in units per ml of solution) and specific activity (in units per mg of protein). It was shown that for covalent immobilization of ficin and bromelain on a chitosan matrix, it is most promising to use 10 % glutaraldehyde. For immobilization of papain on chitosan by covalent means, the concentration of glutaraldehyde equal to 20 % is optimal.

Keywords: ficin; papain; bromelain; chitosan; covalent immobilization

1. Introduction

Ficin (EC 3.4.22.3) is a proteolytic enzyme isolated from fig tree latex. Bromelain (EC 3.4.22.32) is a proteolytic plant enzyme derived from pineapple. The largest amount of the enzyme is found in the lower part of the pith of the mature plant stem. Papain (EC 3.4.22.2) is isolated from *Carica papaya* latex (content in latex is 5–8%) [1,2].

The use of this enzymes in medicine is widely known, however, in addition, they can be used in the leather industry to soften leather products, when purifying waste water, when removing rust from metals [3,4].

It is known that as a result of immobilization, enzymes acquire the advantages of heterogeneous catalysts: they can be removed from the reaction mixture by simple filtration, it becomes possible to transfer many periodic enzymatic processes to a continuous mode using columns or flow-through apparatus with immobilized enzymes. Immobilized enzymes, on the whole, turned out to be much more resistant to external influences than native (soluble) enzymes. They are more durable and thousands and tens of thousands of times more stable than free enzymes [5].

Chitosan is an aminopolysaccharide composed of glucosamine and N-acetylglucosamine polymers. Chitosan has the following properties important for practical use: biocompatibility, film formation, bioadhesiveness, polyfunctionality, hydrophilicity, most of which are associated with their cationic nature, which is unique among polysaccharides and natural polymers [6].

The aim of this work was to determine the optimal concentration of a crosslinking agent in the covalent immobilization of ficin, papain and bromelain on a chitosan matrix.

2. Methods

Ficin, papain, bromelain (Sigma) were chosen as objects of study. Azocasein (Sigma-Aldrich) was used as a substrate for hydrolysis. Highmolecular weight chitosan (350 kDa, Bioprogress CJSC) was used as a carrier for immobilization.

To 900 mg of chitosan were added 18 ml of enzyme (ficin, papain, bromelain) solution and 10 ml of glutaraldehyde (1, 2.5, 5, 10, 15, 20 and 25%), incubated with periodic stirring for 1 h. The suspension was centrifuged at 1.500 g for 10 min. After the end of the incubation, the formed precipitate was washed with 50 mM Tris-HCl buffer (pH 7.5) until there was no protein in the washings (control was carried out on a spectrophotometer at λ = 280 nm).

Protein content in immobilized enzymes was determined by the Lowry method [7].

3. Results and Discussion

The largest amount of protein in immobilized samples (in mg per g of carrier) was observed during covalent immobilization of ficin and papain on a chitosan matrix using glutaraldehyde with a 25% concentration, while binding bromelain—at a concentration of 5%, 10%, 25% (Figure 1).

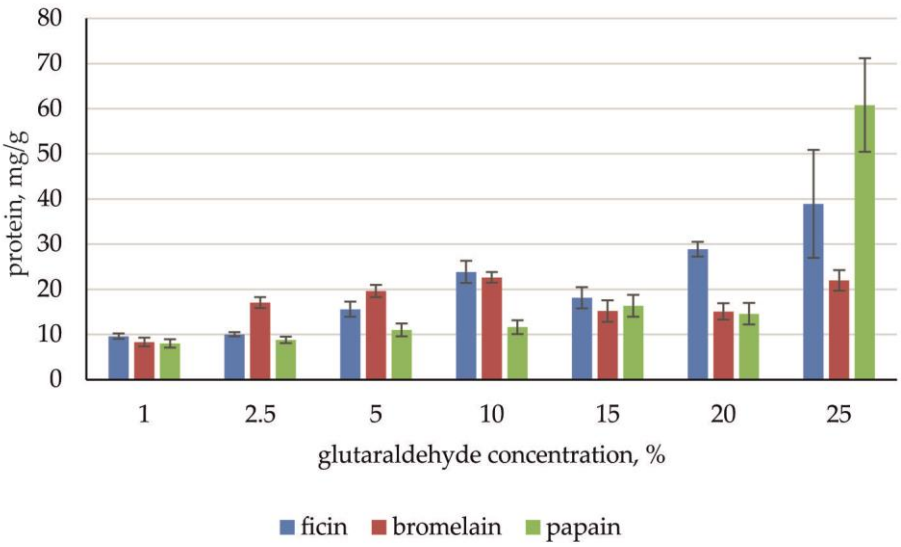

Figure 1. Protein content in immobilized enzymes (in mg per g of carrier).

High values of the total activity (in units per ml of solution) of ficin were observed during its immobilization on chitosan using glutaraldehyde with a 10% concentration. When creating immobilized enzymes based on papain and chitosan, the highest activity was detected applying 20% glutaraldehyde. High activity of bromelain immobilized on a chitosan matrix was detected when using glutaraldehyde with 5%, 10%, 20% concentration (Figure 2).

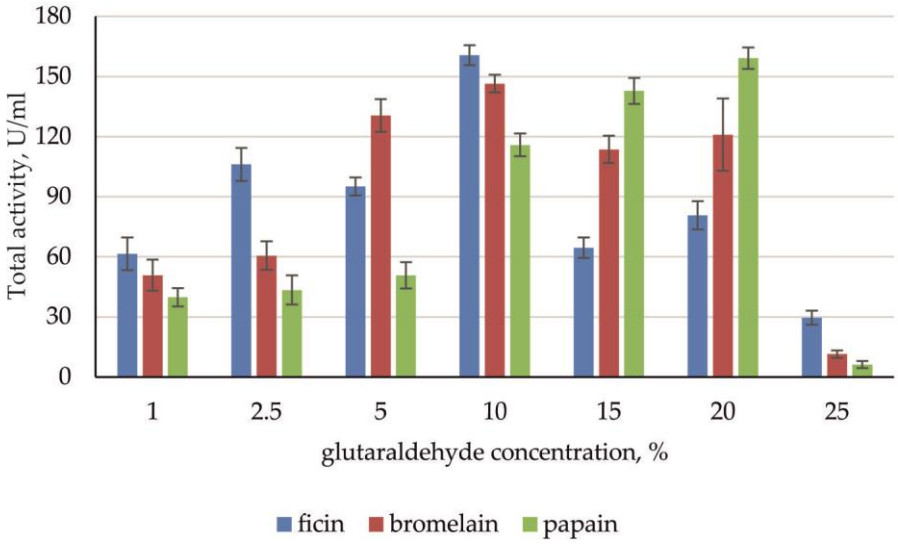

Figure 2. Total activity of immobilized enzymes (in units per ml of solution).

The highest specific activity was shown by ficin and bromelain, immobilized by covalent binding on a chitosan matrix, using glutaraldehyde with a 10% concentration. When developing biocatalysts based on papain and chitosan, the highest specific activity was observed when 20% glutaraldehyde was applied (Figure 3).

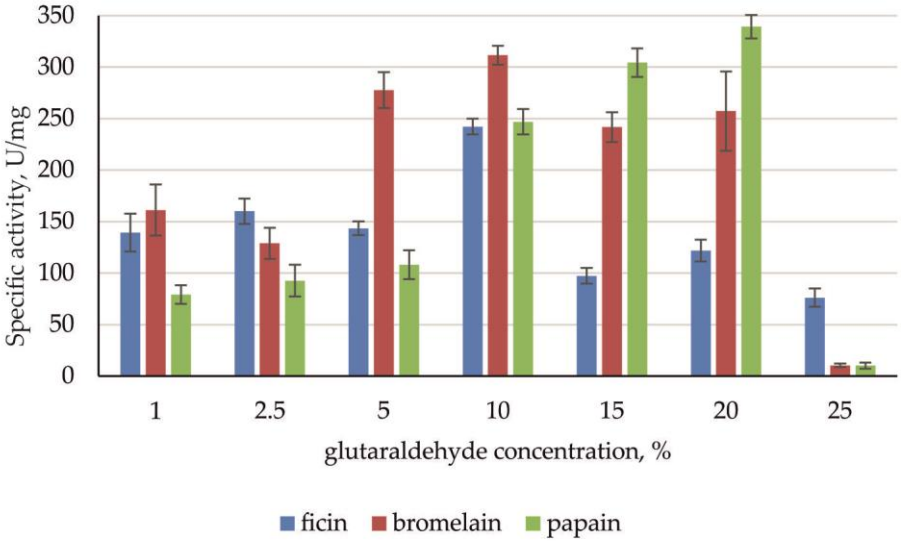

Figure 3. Specific activity of immobilized enzymes (in units per mg of protein).

4. Conclusions

It was shown that for covalent immobilization of ficin and bromelain on a chitosan matrix, it is most promising to use 10 % glutaraldehyde. For immobilization of papain on chitosan by covalent means, the concentration of glutaraldehyde equal to 20 % is optimal.

5. Patents

Holyavka, M., Artyukhov, V., Olshannikova, S. Method for producing a heterogeneous bromeline preparation which is covalently bound to a chitosan matrix. RU 2711786 C1. Date of publication: 22.01.2020 Bull. № 3

Author Contributions: Authors have equal contributions in the preparation and writing of the article.

Funding: This work was financially supported in the form of a grant from the President of the Russian Federation for state support to young Russian scientists - doctors of sciences (MD-1982.2020.4. Agreement 075-15-2020-325).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Manosroi, A., Chankhampan, C., Manosroi, W., Manosroi, J. Transdermal absorption enhancement of papain loaded in elastic niosomes incorporated in gel for scar treatment. *Eur J Pharm Sci* **2013**, *48*, 474–83.
2. Feijoo-Siota, L., Villa, T.G. Native and biotechnologically engineered plant proteases with industrial applications. *Food Bioprocess Technol*. **2011**, *4*, 1066–1088.
3. Tavano, O.L., Berenguer-Murcia, A., Secundo, F., Fernandez-Lafuente, R. Biotechnological applications of proteases in food technology. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 412–436.
4. Taussiga, S.J., Batkin, S. Bromelain, the enzyme complex of pineapple (*Ananas comosus*) and its clinical application. An update. *J. Ethnopharmacol*. **1988,** *22,* 191–203.
5. Efremenko, E.N., Stepanov, N.A., Senko, O.V., Spiricheva, O.V., Varfolomeev, S.D., Nikolskaya, A.B. Biocatalysts based on immobilized cells of microorganisms in the production of bioethanol and biobutanol. *Catal. Ind.* **2011,** *3*, 41–46.
6. Crini, G., Badot, P. Application of chitosan, a natural aminopolysaccharide, for dye removal from aqueous solutions by adsorption processes using batch studies: A review of recent literature. *Prog. Polym. Sci.* **2008**, *33*, 399–447.
7. Lowry, O.H., Rosebrough, N.J., Faar, A.L., Randall, R.J. Protein measurement with folin-phenol reagent. *J Biol Chem* **1951**, *193,* 265–275.

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.