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The Stability of β-galactosidase *(Aspergillus oryzae)* Immobilized on Eupergit C

Stabilnost β-galaktozidaze *(Aspergillus oryzae)* imobilizirane na Eupergit C

D. Huzjak, Jasna Huzjak** and J. Križanić***

*Belupo d.o.o., Sektor razvoja proizvoda, Koprivnica, Croatia
** Podravka d.d., FC-istraživanja i razvoj, Koprivnica, Croatia

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Summary

*The stability of β-galactosidase (**A. oryzae**) covalently immobilized on epoxy-activated acrylic beads (Eupergit C) was examined and compared with the stability of the native enzyme. Inmmoblization of β-galactosidase to Eupergit C was carried out in 1 M potassium phosphate buffer, pH = 7.5 at room temperature for 24 h.*

*Comparative investigations have shown that immobilization had no significant effect on the pH stability of β-galactosidase from **A. oryzae**. Also, immobilization had no effect on the stability of enzyme preparations toward ionic strength of storage buffer, or to proteolytic activity of proteinases from **Streptomyces griseus** (»pronase P«). On the other hand, it was found that the immobilization on Eupergit C results in an increased thermal stability of β-galactosidase. Immobilized β-galactosidase from **A. oryzae** was also much more resistant to denaturation at high urea concentration than a native enzyme.*

Sažetak

*Ispitana je stabilnost β-galaktozidaze (**A. oryzae**) kovalentno imobilizirane na epoksi-aktivirana akrilna zrnca (Eupergit C) i uspoređena sa stabilnošću nativnog enzima. Imobilizacija β-galaktozidaze na Eupergit C provedena je na sobnoj temperaturi preko noći u 1 M fosfatnom puferu, pH = 7.5.*

*Usporednim ispitivanjem imobilizirane β-galaktozidaze iz **A. oryzae** utvrđeno je da imobilizacija ne utječe bitno na pH stabilnost enzima, kao ni na njegovu stabilnost prema ionskoj jakosti pufera u kojem se on čuva, a imobilizirani enzim nije stabilan niti prema djelovanju proteinaza iz **S. griseus** (pronaza P).*

Nasuprot tome, imobilizacijom na Eupergit C povećava se termostabilnost, a imobilizirana β-galaktozidaza znatno je otpornija prema denaturirajućem djelovanju velikih koncentracija mokraćevine od nativnog enzima.

Introduction

Considerable attention has been focused on the use of β-galactosidase (lactase, EC 3.2.1.23) for the hydrolysis of lactose in dairy products, from the view-points of the full utilization of whey and the removal of lactose from milk due to its low solubility and the physiological problem for the lactose intolerant *(1)*. Although several sources of β-galactosidase are available, only those from yeasts *(Kluyveromyces lactis, Kluyveromyces fragilis)* and fungi *(Aspergillus niger, Aspergillus oryzea)* have been used for this purpose *(2)*. Fungal β-galactosidases have acid pH optima and are therefore recommended for acid whey processing. Yeast β-galactosidases are neutral and are of choice for milk and sweet whey hydrolysis *(3)*. For efficient use of the enzyme in industrial hydrolysis of lactose in milk and whey, immobilization of the enzyme is superior to the soluble enzyme. A number of papers on the immobilization of β-galactosidase by various methods have appeared in the last twenty-five years *(for review see 1,2,4,5)*.

Recently, we have prepared a new immobilized β-galactosidase preparation consisting of β-galactosidase from *A. oryzae* covalently linked to Eupergit C (epoxy-activated acrylic beads) *(6)*. This support is very stable and has good chemical and mechanical properties (simple immobilization procedure, high binding capacity, low water uptake, high flow rate in column procedures, excellent performance in stirred batch reactors etc.) *(7,8)*. In this study the stability of β-galactosidase *(A. oryzae)* immobilized to Eupergit C was examined and compared with the stability of the native enzyme.

Materials and Methods

All chemicals used in this work were analytical grade and were obtained either from Sigma Chemical Co. (St. Louis, USA) or Kemika (Zagreb, Croatia). Immobilization of β-galactosidase *(A. oryzae)* to Eupergit C was carried out in 1 M potassium phosphate buffer of pH = 7.5 at room temperature for 24 h *(6)*. The activity of β-galactosidase was determined with o-nitrophenyl-β-D-galactopyranoside (ONGP) as the substrate. A mixture containing 2.5 mL of a solution of ONGP in 0.1 M Na-acetate buffer pH = 5.0 (γ_{ONGP} = 0.68 mg/mL) and 0.5 mL of native or 100 mg of immobilized enzyme was incubated 10 min at 30 °C. The reaction was stopped by adding 2.0 mL of 1.0 M Na_2CO_3 to the reaction mixture. The amount of released o-nitrophenol was determined by measuring the absorbance at 420 nm using a Pye Unicam spectrophotometer SP 8-100.

Results and Discussion

As shown in Fig. 1, the immobilization on Eupergit C had no significant effect on pH stability of β-galactosidase from *A. oryzae*. Thus, both enzyme preparations remained fully active in the pH range 3.5–8.0 after the overnight storage at room temperature.

The effect of ionic strength of storage buffer on the stability of native and immobilized β-galactosidase from *A. oryzae* was also examined. The enzyme preparations were incubated overnight at room temperature in Na-acetate buffers pH = 5.0 of various ionic strength and the remaining activity was assayed with ONGP as substrate by the method described above. The results show that the immobilization had no effect on the stability of β-galactosidase from *A. oryzae* toward ionic strength of storage buffer. Thus, both enzyme preparations retained their full activities after the overnight storage at room temperature in Na-acetate buffers pH = 5.0 of ionic strength from 0.1 to 1.0.

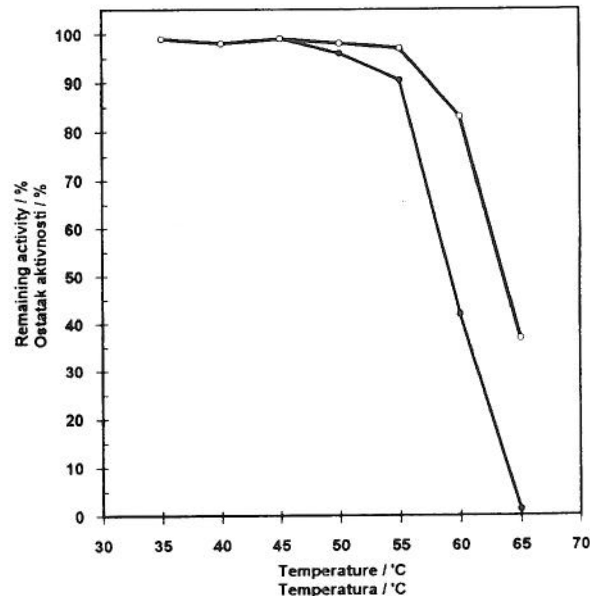

Fig. 2. The thermal stability of native (•) and immobilized (o) β-galactosidase from *A. oryzae*. The enzyme preparations were incubated in 0.1 M Na-acetate buffer pH = 5.0 at various temperature for 60 min and the remaining activity was assayed with ONGP as substrate.

Slika 2. Utjecaj temperature na stabilnost nativne (•) i imobilizirane (o) β-galaktozidaze iz *A. oryzae*. Uzorci enzima inkubirani su u 0,1 M Na-acetatnom puferu, pH = 5,0 na različitim temperaturama tijekom 60 min, a zatim im je određivan ostatak aktivnosti standardnom metodom s ONGP kao supstratom.

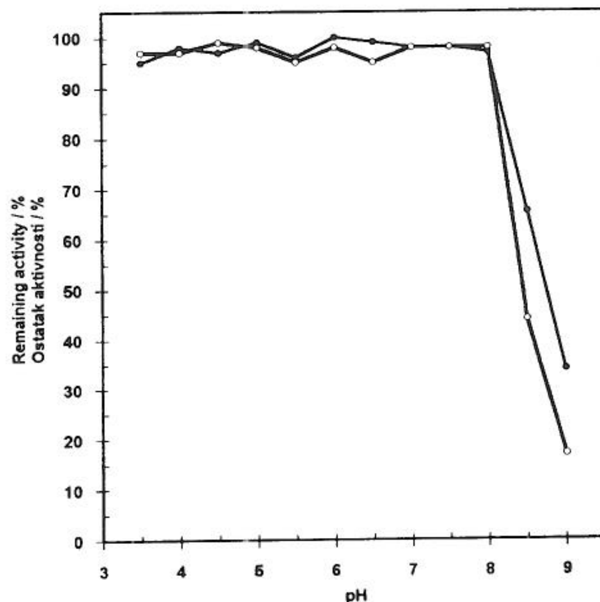

Fig. 1. The pH stability of native (•) and immobilized (o) β-galactosidase from *A. oryzae*. The enzyme preparations were incubated overnight at room temperature in an appropriate buffer and the remaining activity was assayed with ONGP as substrate by the method described in Materials and Methods.

Slika 1. Utjecaj pH na stabilnost nativne (•) i imobilizirane (o) β-galaktozidaze iz *A. oryzae*. Uzorci enzima inkubirani su preko noći na sobnoj temperaturi u odgovarajućem puferu, a zatim im je određivan ostatak aktivnosti postupkom opisanim u Material and Methods s ONGP kao supstratom.

The thermal stability curves of β-galactosidase are shown in Fig. 2. The native enzyme lost about 60 % of its original activity when incubated at 60 °C for 60 min. After 60 min at 65 °C the native enzyme almost completely lost its activity. In comparison, the immobilized enzyme lost about 20 and 65 % of its activity at 60 °C and 65 °C, respectively. These results show that the immobilization on Eupergit C results in an increased thermal stability of β-galactosidase from *A. oryzae*. The increased thermostability of immobilized β-galactosidase is a very important property because the enzyme can be used in processes at elevated temperatures to protect both substrates and enzymatic products from microbial contamination and deterioration.

The results of the effect of urea on β-galactosidase stability are shown in Fig. 3. It was found that the immobilized enzyme was much more resistant to denaturation at high urea concentration than the native β-galactosidase. Thus, the immobilized enzyme retained almost 70 % of its original activity after 120 min of incubation at 30 °C in 8 M urea, while the native enzyme, under the same conditions, retained only 10 % of its initial activity.

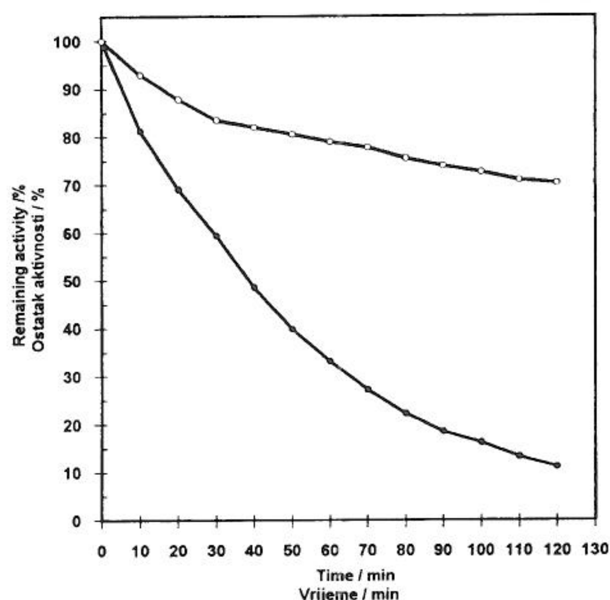

Fig. 3. The effect of urea on the stability of native (•) and immobilized (o) β-galactosidase from *A. oryzae*. The enzyme preparations were incubated in 8 M urea at 30 °C, aliquots of the preparation were withdrawn from the incubation mixtures at the indicated time, diluted with 0.1 M Na-acetate buffer pH = 5.0 and the remaining activity was assayed with ONGP as substrate.

Slika 3. Utjecaj ureje na stabilnost nativne (•) i imobilizirane (o) β-galaktozidaze iz *A. oryzae*. Uzorci su enzima inkubirani u 8 M ureji na 30 °C, alikvoti uzoraka uzimani iz inkubacijske smjese u pravilnim vremenskim intervalima, razrjeđivani s 0,1 M Na-acetatnim puferom, pH = 5,0, a zatim im je određivan ostatak aktivnosti standardnom metodom s ONGP kao supstratom.

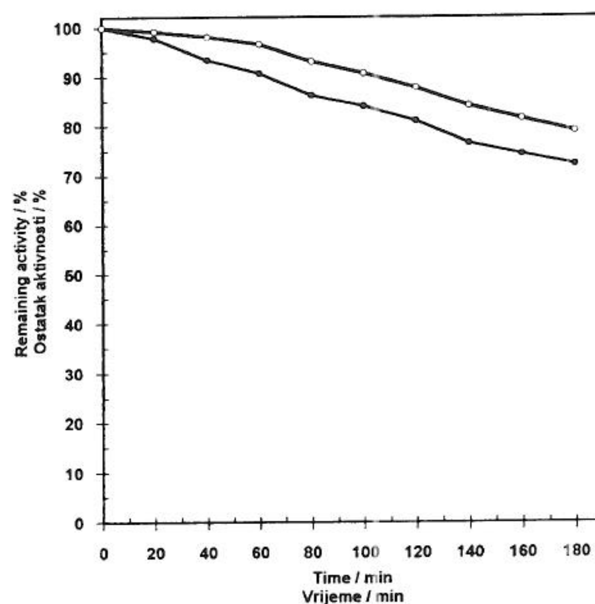

Fig. 4. The influence of »pronase P« (proteinases from *Streptomyces griseus*) on the stability of native (•) and immobilized (o) β-galactosidase from *A. oryzae*. The β-galactosidase preparations were mixed with proteinases from *Streptomyces griseus* in 0.1 M Tris/ /HCl buffer pH = 7.5 and incubated at 30 °C. The concentration of proteinases in the incubation mixture was 0.5 mg/mL. At the indicated time, aliquots of the preparation were withdrawn from the incubation mixtures, diluted with 0.1 M Na-acetate buffer pH = 5.0 and assayed with ONGP as substrate.

Slika 4. Utjecaj »pronaze P« (proteinaze iz *Streptomyces griseus*) na stabilnost nativne (•) i imobilizirane (o) β-galaktozidaze iz *A. oryzae*. Uzorci β-galaktozidaze pomiješani su s proteinazom iz *Streptomyces griseus* u 0,1 M Tris/HCl puferu, pH = 7,5 i inkubirani na 30 °C. Koncentracija proteinaze u reakcijskoj smjesi iznosila je 0,5 mg/mL. U određenim vremenskim intervalima iz inkubacijske smjese uzimani su alikvoti uzorka, razrjeđivani s 0,1 M Na-acetatnim puferom, pH = 5,0, a zatim im je određivan ostatak aktivnosti standardnom metodom s ONGP kao supstratom.

The stability of β-galactosidase to proteolysis was also examined. As shown in Fig. 4, the immobilization on Eupergit C had no significant effect on stability of β-galactosidase to proteolysis. The immobilized enzyme retained approximately 80 % of its original activity after 180 min of incubation at 30 °C. Under the same conditions, the native enzyme retained about 70 % of its original activity.

Conclusion

All results presented in this work suggest that β-galactosidase from *A. oryzae*, immobilized on Eupergit C, could be usefully applied as recoverable and reusable form of this enzyme for the commercial hydrolysis of lactose in dairy products.

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