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Immobilization of β-D-galactosidase from *Lactobacillus bulgaricus* on polyacrylamide in the presence of protective agents and properties of the immobilized enzyme

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Abstract. β-D-Galactosidase (EC 3.2.1.23) from *Lactobacillus bulgaricus* (1373) was immobilized in a Polyacrylamide gel lattice in the presence of dithiothreitol, glutathione, cysteine, bovine serum albumin, casein, lactose and glucono-δ-lactone. Cysteine, bovine serum albumin, and lactose were found very effective in preserving the activity. With cysteine, bovine serum albumin and lactose, the activity yields were 61, 60 and 66% respectively, as compared to 31% without protective agents. The yield improved upto 85% when all the three protective agents, cysteine, bovine serum albumin and lactose were added during immobilization. The addition of protective agents did not have any effect on optimum pH, optimum temperature, kinetic constants and pH stability when compared with β-galactosidase immobilized without the use of protective agents; however the heat and storage stabilities were found to increase.

Keywords. Immobilized β-galactosidase; *Lactobacillus bulgaricus;* Polyacrylamide gel entrapment; protective agents; kinetic constants.

Introduction

In our previous study (Makkar *et al*., 1981) β-D-galactosidase from *Lactobacillus bulgaricus* was immobilized in a Polyacrylamide gel lattice, and the properties of the immobilized and native enzymes were compared. However, the maximum activity yield of immobilized β-galactosidase preparation was very low (31%). A low activity yield during entrapment in Polyacrylamide was reported for a number of enzymes (Miyamoto *et al*., 1977; Ohmiya *et al*., 1975; Kobayashi *et al*., 1975; Chibata, 1978). The loss in activity may be due to: a) denaturation by acrylamide in a manner similar to urea and b) oxidation by potassium persulphate (Digani and Miro, 1970; Miyamoto *et al*., 1977). In the present study efforts have been made to decrease the activity losses by using protective agents (substrate, inhibitor and thiols) during immobilization.

Materials and methods

The materials are the same as those employed earlier (Makkar *et al*., 1981).

The immobilized β-galactosidase was prepared essentially as described earlier (Makkar *et al.*, 1981) with incorporation of different protective agents in the enzyme solution before the addition of enzyme to monomers. The immobilized β-galactosidase preparation obtained by the addition of bovine serum albumin, lactose and cysteine (2.5, 5 and 10 mg respectively per 8 ml) as protective agents was used for studying the properties.

The native and immobilized β-galactosidases were assayed as described previously (Makkar *et al.,* 1981).

Results

Effect of protective agents on activity yield

β-Galactosidase of *L. bulgaricus* requires sulfhydryl groups for activity (unpublished observation). In order to protect—SH groups from oxidation by potassium persulphate, dithiothreitol, glutathione and cysteine were added (table 1). Cysteine

Table 1. Effect of protective agents during polymerization on the activity of immobilized β-galactosidase.

Protective agents	Activity yield* (%) Concentrations of protective agents (mg)		
	2.5	5	10
Dithiothreitol	38	53	55
Glutathione	37	49	50
Cysteine	40	55	61
Bovine serum albumin	60	60	—
Casein	37	37	—
Lactose	66	66	—
Glucono-δ-lactone	—	33	33

Without protective agent the activity yield was 31%
With nitrophenylgalactopyranoside substrate.
—Not determined.

was found to be the most effective protecting agent, enhancing the yield from 31% to 61%. Among the additives tried; bovine serum albumin and lactose were very effective, in preventing loss of activity but casein and glucone-δ-lactone were ineffective (table 1).

The activity yields of combinations (bovine serum albumin+lactose) and (cysteine+lactose) were comparable but greater than that obtained by individual protective agents (tables 1 and 2). A mixture of bovine serum albumin and

Table 2. Effect of different combinations of bovine serum albumin, lactose and cysteine on the activity of immobilized β-galactosidase.

Protective agents	Activity yield* (%)
Bovine serum albumin+lactose	74
Bovine serum albumin+cysteine	65
Lactose+cysteine	73
Bovine serum albumin+lactose+cysteine	85

The concentration of bovine serum albumin, lactose and cysteine used was 2.5, 5 and 10 mg respectively.

* Using nitrophenylgalactopyranoside as substrate.

cysteine gave a 65% activity yield while the addition of all the three, bovine serum albumin, lactose and cysteine yielded the maximum activity (85%). The properties of the immobilized β-galactosidase obtained by the addition of these three protective agents were studied.

Properties of the enzyme immobilized in the presence of protective agents

Immobilized β-galactosidase had pH and temperature optima at 6.5 and 42°C with both lactose and nitrophenyl-galactopyranoside as substrate in 50 mM phosphate buffer. The K_m values calculated from Lineweaver—Burk plots drawn with lactose and nitrophenyl-galactopyranoside were 9.4 mM and 0.90 mM respectively. Immobilized β-galactosidase started losing lactivity above 45°C when heated for 10 min. The preparation was more stable above 60°C than the native enzyme or the β-galactosidase immobilized without using protective agents. The pH stability of the immobilized β-galactosidase preparation with a protective agent was studied as reported earlier (Makkar *et al.*, 1981). The immobilized β-galactosidase prepared without protective agents showed maximum stability at pH 6.5, and the residual activity obtained at all the pH values was almost the same as that obtained for immobilized β-galactosidase prepared without the use of protective agents (Makkar *et al*., 1981). Immobilized β-galactosidase was stored at 4 and 25°C. No loss in activity was found even after 30 days of storage at 4°C and after 60 days of storage 80% of the initial activity was found. At 25°C no decrease in activity was recorded upto 20 days of storage. No loss in activity was found in the immobilized preparation after using it 15 times. At 2.5 and 5 h of incubation 45 and 60% respectively of lactose were hydrolysed (Makkar *et al.,* 1981).

Discussion

As anticipated, dithiothreitol, glutathione and cysteine markedly increased the activity yield of immobilized β-galactosidase as sulfhydryl groups are required for the catalytic activity of β-galactosidase from *L. bulgaricus* (unpublished data). The protection of activity in the presence of dithiothreitol, glutathione and

cysteine might be due to the protection of essential sulfhydryl groups of β-galactosidase of *L. bulgaricus* during immobilization. Dithiothreitol has also been reported to be effective during immobilization of β-galactosidase from *Aspergillus oryzae, Kluyveromyces lactis* and *Escherichia coli* K12. However, glutathione was effective only for *A. oryzae* and not for *K. lactis* and *E. coli* β-galactosidase (Kobayashi *et al.*, 1975; Ohmiya *et al.,* 1975). Bovine serum albumin was found to be very effective in the present study (activity yield 60%). However, in the case of β-galactosidase from *A. oryzae,* it is reported to stabilize the immobilized activity only moderately (40%), as against 28% without it (Ohmiya *et al*., 1975). In the case of *E. coli* β-galactosidase, bovine serum albumin increased the activity yield only marginally and for *K. lactis* β-galactosidase, it was not effective (Kobayashi *et al*., 1975). The presence of substrate (lactose) during immobilization too was very effective. This suggests that substrate binding protected the enzyme against inactivation. The presence of substrate during immobilization of hexokinase (Miyamoto *et al*., 1977) and aspartase (Tosa *et al.*, 1973) was found to be effective. Glucono-δ-lactone, an inhibitor of β-galactosidase, did not increase the activity yield (33%). Similar results have been obtained by Kobayashi *et al*., (1975) for β-galactosidase of *K. lactis* and *E. coli.* However, for *A. oryzae* β-galactosidase glucono-δ-lactone was found to be the best amongst all the studied protective agents (Ohmiya *et al.*, 1975). The difference in the degree of protection by different protective agents appears to depend upon the origin of β-galactosidase.

Table 3. Comparison of properties of native β-galactosidase, immobilized β-galactosidase prepared without the use of protective agents and immobilized β-galactosidase prepared with protective agents.

Properties	Native	Immobilized β-galactosidase (without protective agents)	Immobilized β-galactosidase (with protective agents)
Optimum pH	6.5	6.5	6.5
Optimum temperature	42°C	42°C	42°C
K_m (lactose)	10 mM	9.7 mM	9.4 mM
K_m (nitrophenylgalactopyranoside)	0.94 mM	0.88 mM	0.90 mM
Heat stability (remaining activity %):			
45°C, 10 min	97	97	100
65°C, 10 min	10	19	28
pH stability (maximum stability at pH)	6.5	6.5	6.5
Storage stability (remaining activity %):			
4°C (60 days)	40	70	80
25°C (20 days)	73	100	100
Lactose hydrolysis (%):			
2.5 h	—	20	45
5.0 h	—	30	60

Data for native and immobilized β-galactosidase (without protective agents) taken from earlier : paper (Makkar et al., 1981).

The addition of cysteine, bovine serum albumin and lactose gave an excellent activity yield (85%). It is quite likely, in other preparations too the activity yield would increase when different combinations of protective agents were used.

No difference was observed in the optimum pH, optimum temperature, kinetic constants and pH stability between immobilized β-galactosidase prepared with and without protective agents and the native β-galactosidase. However heat stability and storage stability of the preparation made by using protective agents were found to be greater (table 3). The extent of hydrolysis of lactose was greater with the protected immobilized enzyme (table 3). The greater hydrolysis of lactose was obviously due to greater activity yield of the preparation.

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References

Chibata, I. (1978) *Immobilized Enzymes: Research and Development* (New York: John Wiley and Sons) p.71.

Degani, Y. and Miro, T. (1970) *Biochim. Biophys. Acta,* **212,** 362.

Kobayashi, T., Ohmiya, K. and Shimizu, S. (1975) *Immobilized Enzyme Technology: Research and Applications,* ed. H. H. Weetall and S. Suzaki (New York: Plenum Press) p. 169.

Makkar, H. P. S., Sharma O. P. and Negi, S. S. (1981)*J. Biosci.*, **3,** 7.

Miyamoto, K., Ohyabu, M., Okazaki, M. and Mitura, Y. (1977)*J. Ferment. Technol.,* **55,** 63.

Ohmiya, K., Terao, C, Shimizu, S. and Kobayashi, T. (1975) *Agri. Biol. Chem.,* **39,** 491.

Tosa, T., Sato, T., Mori, T., Matuo, Y. and Chibata, I. (1973) *Biotechnol. Bioeng.,* **15,** 69.