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IMMOBILIZATION OF INVERTASE VIA ITS CARBOHYDRATE MOIETY ON MACROPOROUS GLYCIDYL METHACRYLATE

Radivoje M. Prodanović, Slobodan M. Jovanović, Zoran M. Vujčić

Immobilization via carbohydrate moiety is suitable for immobilization of glycoenzymes because it has little effect on enzyme active site. Macroporous glycidyl methacrylate is better support for enzyme immobilization from the much more used polystyrene because of its lesser hydrophobicity.

We found optimal conditions for invertase immobilization via its carbohydrate moiety on macroporous glycidyl methacrylate by varying enzyme concentration. We obtained immobilized enzyme with specific activity of 5500 IU/g, which is the highest activity reported in the literature. Immobilized enzyme has Km=43 mmol/l, temperature optimum of 60°C, and pH optimum between 3.5 and 5.5.

KEY WORDS: invertase, periodate, immobilization, macroporous, glycidyl methacrylate

INTRODUCTION

The main goal of immobilization is to prepare highly active immobilized enzyme, with good yield and stability for industrial use. Therefore, method of immobilization should be gentle, in order not to inactivate enzyme, and to bind as much as possible of enzyme on the support. One of such methods is enzyme immobilization via its carbohydrate moiety after periodate oxidation of its vicinal diol groups of its carbohydrate component (1). In previous reports that deal with this method of invertase immobilization (1,2), little attention has been paid to the influence of enzyme concentration on specific activity of the immobilized enzyme (IME).

Macroporous polymers like polystyrene and glycidyl methacrylate have large surface area and good mechanical and chemical stability under industrial condition. Macroporous polystyrene has been used in industrial conditions for invertase immobilization (3). Glycidyl methacrylate is less hydrophobic than polystyrene, which could lead to higher enzyme activity of the immobilized enzyme.

We found the optimal conditions for invertase immobilization on macroporous glycidyl methacrylate prepared by a new procedure and characterized the immobilized enzyme.

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EXPERIMENTAL

Polymer was obtained by the method previously described (4) and named GE 10/12. Particle size distribution was determined by sieve analysis. A commercial mercury porosimeter, Model Carlo Erba, was used to determine of specific pore volume, specific area, and pore size distribution. The morphology of macroporous copolymer was studied by scanning electron microscopy (DSM-962, Zeiss). The polymer was activated with 1 M 1,2-diaminoethane at 60°C for 4 h at pH 10, and the concentration of amino groups, determined by the titration with 0.01 mmol/l HCl in 0.5 mmol/l KCl, was 1.2 mmol/g.

Invertase was obtained from bakers yeast by purification with DEAE chromatography and gel filtration onto the Sephadex G-200. The activity of the lyophilized enzyme was 250 IU/mg of solid.

Activity was determined in 5 ml reactor at 1000 rpm in 0.5 mmol/l acetate buffer pH 4.7 in 100g/l sucrose at 25°C. One unit of enzyme activity was the amount of enzyme which catalyses the hydrolysis of 1 μmol of sucrose per minute under the test conditions.

Glutaraldehyde immobilization was performed by the activation of modified polymer with 2.5 % glutaraldehyde at pH 7.0 in phosphate buffer for 2 hours. After that the polymer was rinsed and incubated with enzyme in acetate buffer pH 4.7 for two day at 4°C.

Invertase was oxidized by incubation 20 mg/ml of enzyme with 2 mmol/l sodium metaperiodate in acetate buffer pH 5.0 for 6 hours in dark at 4°C. The unreacted $NaIO_4$ was then removed with 10 mmol/l ethylene glycol for 30 minutes. The oxidized invertase was then dialyzed against 50 mmol/l acetate buffer pH 5.0 for 18 h. After that the enzyme is incubated with the modified polymer for two days at 4°C.

Immobilized enzyme was rinsed five times with 1.0 M NaCl and stored in 50 mM acetate buffer pH 4.7 until used.

RESULTS AND DISCUSION

Particle diameters of the polymer named GE 10/12 were in the range of 150-500 μm. Specific pore volume was 0.610 ml/g, and specific area 50 m^2/g. The mean pore diameter of the support was 51 nm. The concentration of epoxy groups was 2.1 mmol/g. The particles surface morphology is presented in Fig 1.

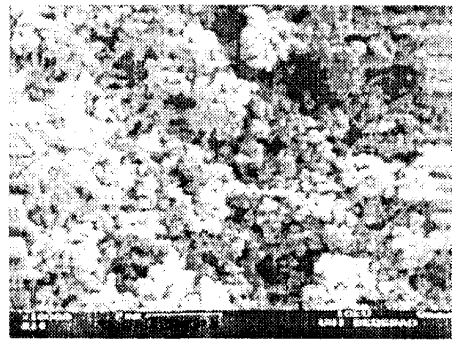

Fig. 1. Scanning electron micrograph of the particle surface of macroporous glycidyl methacrylate

Specific activity of IME was increased and yield of immobilization was decreased with increase of the amount of enzyme added. During this experiment it was observed that all the enzyme was bound to the polymer regardless of how much enzyme was added (Table 1).

Table 1. Dependence of specific IME activity and percentage of unbound enzyme on the amount of added enzyme in periodate immobilization

Amount of added enzyme IU/g	Specific activity IU/g	Nonbound enzyme %
720	490	0
1440	1250	0
2880	2200	0
5760	4100	0.35
28800	5700	6.25

Glutaraldehyde immobilization gave less specific activity of IME and could not bind all of the added enzyme activity (Table 2).

Table 2. Dependence of specific IME activity and percentage of unbound enzyme on the amount of added enzyme in glutaraldehyde immobilization

Amount of added enzyme IU/g	Specific activity IU/g	Nonbound enzyme %
720	500	11
1440	1100	11
2880	1550	25
5760	2300	29
28800	2850	60

Therefore we could obtain desired specific activity by varying the amount of added enzyme and leaving it in contact with the polymer long enough during immobilization. In earlier reports little attention has been paid to the effect of time of immobilization on enzyme binding to the polymer.

Km was determined for the IME obtained with periodate and having a specific activity of 5500 IU/g dry polymer by Linewiver-Burke linearization (Fig. 2).

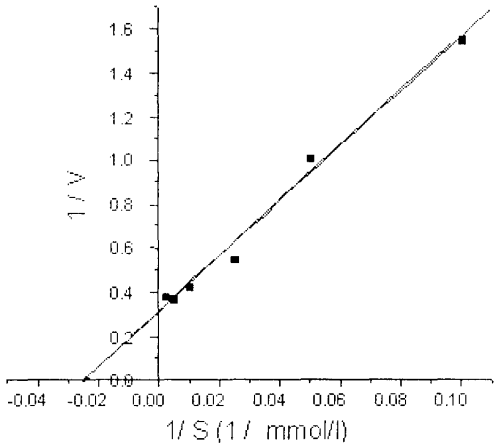

Fig. 2. Linewiver-Burke graph for immobilized invertase

The determined Km for the immobilized enzyme (43 mmol/l) was higher with respect to the native enzyme (29 mmol/l). This could be explained in terms of diffusional effects, because sucrose must penetrate to the interior of the particle, so that local concentration of sucrose inside the particle is decreased.

As can be seen from Fig. 3, pH optimum of IME was between 4 and 5.

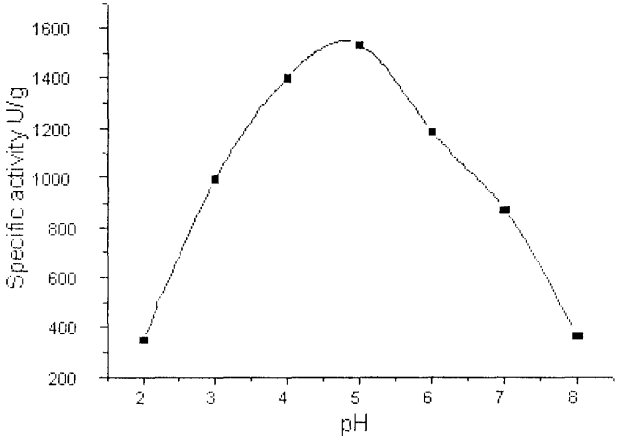

Fig. 3. Dependence of immobilized enzyme activity on pH

Temperature optimum for IME in 10% sucrose, was at 60°C (Fig. 4).

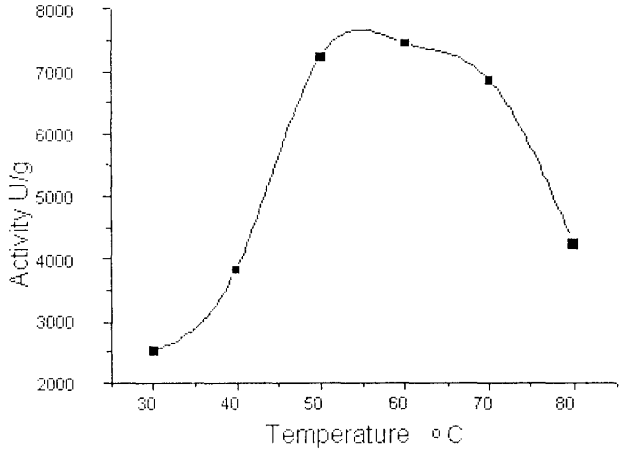

Fig. 4. Dependence of immobilized enzyme activity on temperature

CONCLUSION

Immobilization via carbohydrate moiety is a mild method, more suitable for glycoenzyme immobilization than glutaraldehyde. By varying the amount of added enzyme we obtained IME with a specific activity 10 times higher than previously described (1). This is also 4 times higher specific activity than the best one described in the literature on macroporous polymers (3). The obtained IME has higher Km and temperature optimum than the soluble one, while pH optimum is not change significantly.

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ИМОБИЛИЗАЦИЈА ИНВЕРТАЗЕ ПРЕКО УГЉЕНОХИДРАТНОГ ДЕЛА НА МАКРОПОРОЗНИ ГЛИЦИДИЛ МЕТАКРИЛАТ

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Имобилизација преко шећерног дела је погодна за гликоензиме зато што има мало утицаја на активно место ензима. Макропорозни глицидил метакрилат је бољи носач за имобилизацију ензима од више коришћеног полистирена због своје хидрофилности.

Утврђени су оптимални услови за имобилизацију инвертазе преко шећерног дела на макропорозни глицидил метакрилат варирањем концентрације ензима. Добијен је имобилизовани ензим специфичне активности 5000 IU/g, што је највећа активност до сада описана у литератури. Имобилизовани ензим је имао Км 43 mmol/l, температурни оптимум на 60°C и pH оптимум између 4 и 5.

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