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PRODUCTION OF INVERTASE IMMOBILIZED ON POLYAMIDE IN A MEDIUM WITH A HIGH SUCROSE CONCENTRATION

D. T. Mirzarakhmetova and S. Kh. Abdurazakova

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A preparation of invertase immobilized on polyamide through glutaraldehyde has been obtained in a medium with a high concentration of substrate (60% sucrose). The optimum concentrations of glutaraldehyde and enzyme have been selected and the substrate dependences of the native and immobilized enzymes have been determined. It has been shown that the covalent addition of invertase to polyamide carried out in the presence of 60% sucrose leads to the most stable immobilized preparation.

Invertase is one of the most important enzymes used in industry. For effective use, it must be immobilized. Reports are found in the literature of the immobilization of invertase on aminopolystyrene through glutaraldehyde [1] and on siliceous sorbents modified with an organosilane and activated by glutaraldehyde [2]. These immobilized preparations are not distinguished by high activity, as is confirmed in the literature. It is assumed that the active center of invertase contains an imidazole group. According to this, the enzyme is bound to the glutaraldehyde-modified support through the functional groups of its catalytic center [3]. We have therefore conducted immobilization in the presence of a substrate blocking the catalytic center, which gave a considerable increase in enzyme activity (485 units/h).

The immobilization of invertase was carried out on polyamide through the amino groups of the invertase and the amino groups of activated polyamide in a substrate – sucrose. Glutaraldehyde was used as the bifunctional agent.

The addition reaction was carried out at room temperature by the principle of Schiff's base formation. The number of enzyme molecules attached to the support was found by determining the loss of protein from the initial solution in the binding process and taking into account the quantity of enzyme desorbed from the support by washing [4]. The activity of the enzyme was determined for glucose [5]. It was established that on immobilization in the absence of a substrate, the specific activity amounted to 122 units/h at a relative concentration of the binding agent of 0.1%.

A determination of the dependence of the enzyme activity of the immobilized preparation on the initial weight ratio of enzyme to support showed that the maximum specific activity was obtained with the use of 7 mg of protein per 1 g of support. Binding of the enzyme was carried out at pH 7.5-9.0, in steps of 0.5 unit. The greatest activity of the enzyme was noted in the region with an immobilization Ph of 8.5.

Figure 1 shows the dependence of the initial rate of hydrolysis of sucrose on its concentration. The maximum activity of the enzyme was observed on the addition of a 0.75 M solution of sucrose to the medium, while for the immobilized enzyme it was observed for a 1.0 M solution. Both a decrease and an increase in the amount of sucrose in the solution led to a fall in the initial rate of the reaction. The decrease in the initial rate at low concentrations of sucrose of hydrolysis is explained by the law of mass action. The fall in the initial rate of hydrolysis at sucrose concentrations above 0.15 M can be explained by the phenomenon of substrate inhibition. The experimental results at low concentrations of substrate can be linearized in the Lineweaver–Burk coordinates (Fig. 2). The "effective" Michaelis constant obtained in this way for the immobilized preparation was 6 times smaller than for the native enzyme. It was established that in the case of the substrate sucrose the apparent constant K_M was 1.25 for the native enzyme and 0.24 for the bound enzyme. The reason for the change in the Michaelis constant may be assumed to be a conformational change in the enzyme taking place as consequence of the interaction of the support and the enzyme. The experimental results permit the conclusion that a covalent bond between the enzyme and the support and other

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TABLE 1. Dependence of the Enzyme Activity of Invertase on the Concentration of Glutaraldehyde in the Immobilization Medium

Concentration of glutaraldehyde in the medium, %	Protein bound per 1 g of sorbent, mg	Total activity per 1 g of sorbent, μMR/min	Specific activity, μM/min·mg
0.05	11.9	1201	1016
0.10	11.8	1470	124.6
0.12	21.2	1470	69.3
0.24	25.8	1643.2	63.7

TABLE 2. Dependence of the Enzyme Activity of Invertase on the pH in the Immobilization Process

pH	Protein bound per 1 g of sorbent, mg	Binding of the protein, %	Total activity per 1 g of sorbent, μM/min	Specific activity, μM/min·mg
7.5	60.52	42.98	2210	36.5
8.0	51.04	46.40	3300	64.6
8.5	22.36	29.40	2600	116.3
9.0	27.32	25.50	2200	80.5

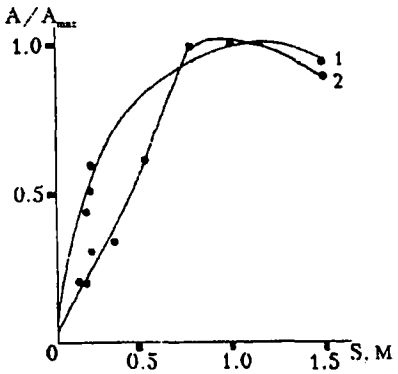

Fig. 1

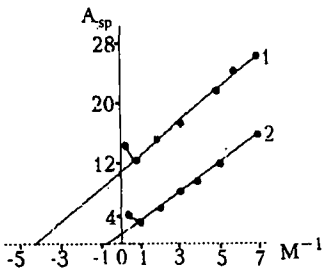

Fig. 2

Fig. 1. Dependence of the rate of hydrolysis of sucrose on the concentration of the substrate added to the medium.

Fig. 2. Dependence of the rate of hydrolysis of the enzyme [sic] in the Lineweaver–Burk coordinates: *1*) immobilized enzyme; *2*) native enzyme.

secondary interactions destabilize the structure of the β-fructofuranosidase. It is assumed that not only tertiary but also quaternary structural changes of the enzyme play a role in these phenomena.

EXPERIMENTAL

A G10x invertase preparation, polyamide, glutaraldehyde (Merck), sucrose, and salts for the preparation of buffer solutions were used.

Immobilization of Invertase. A suspension of 1 g of polyamide in 7 ml of 3.6 N HCl was stirred at 45°C for 2 h. After this, the polyamide was washed with a 40% solution of acetone (20 ml) and with distilled water to pH 5.6. Then 5 ml of 0.1 M borate buffer, pH 8.5, and, with vigorous stirring, 20 μl of 25% glutaraldehyde were added and the mixture was left at room temperature for 2 h. The excess of glutaraldehyde was eliminated with distilled water and the sorbent was washed with borate buffer, pH 8.5.

Addition of the Enzyme. At the selected optimum concentration of protein, the enzyme was dissolved in borate buffer, pH 8.5, already containing sucrose (60% in the medium), and the mixture was left in the refrigerator with constant stirring

for a day. To eliminate the noncovalently bound enzyme the sorbent was washed with distilled water, 0.1 M NaCl, and water again until the wash waters were completely free from protein. The protein contents of the fractions were determined by Lowry's method [4], the enzyme activity of the invertase by the method of [5], and the amount of product formed by that of [6].

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