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# KINETICS OF IMMOBILIZED AND NATIVE INVERTASES

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*A comparative study has been made of the kinetic characteristics of native invertase and the enzyme immobilized on polyamide in the presence of a substrate (60% sucrose). The thermostability, pH optimum, temperature optimum, the hydrolysis of sucrose, and the dependences of the initial rate of hydrolysis on the concentrations of enzyme and substrate were investigated. It was shown that the covalent addition of invertase to polyamide through glutaraldehyde performed in the presence of 60% sucrose leads to the most stable preparation of the immobilized enzyme.*

Invertase is one of the most widely used industrial enzymes. The immobilization of invertase broadens the field of its application, since it prevents the crystallization of sugar in food products and the assimilation of alcohol in fortified wines [1] and provides the possibility of regulating the composition of the volatile components of wine, brandy, and aqueous liqueurs [2-4]. Characteristics of enzymes important for their practical use are their dependences on the pH and the temperature. Moreover, the study of the influence of the pH of chemical reagents on the rate of an enzyme reaction gives definite information on the ionogenic groupings of the active center of the enzyme.

In the investigation of the pH optima of native and immobilized invertases, as the substrate we used a 0.5 M solution of sucrose in the pH range from 3.5 to 6 in acetate buffer. It can be seen from Fig. 1 that the pH optimum of native invertase was in the neighborhood of pH 4, with a profile extended to the right. According to the literature, purified preparations of yeast invertase are heterogeneous with respect to their isoelectric points. The presence of a shoulder on the pH-optimum curve in the pH range from 4.5 to 6 may indicate the presence of two or more isoenzymes having pH optima in this region [5].

In the investigations performed for immobilized invertase, we obtained a pH optimum in the 4.5-5.0 region and a narrower symmetrical profile. The shift of the pH optimum into the neutral region is probably due to a change in the local concentration of hydrogen ions in the microenvironment of the enzyme through the introduction of amino groups during the modification of the support. The observed narrowing of the pH profile of the immobilized invertase may be a consequence of the selective binding of the more neutral forms of the enzyme with the modified support in the immobilization process.

The temperature optima were determined in the temperature interval of 20 to 70°C, using as substrate 0.5 M sucrose in 0.1 M acetate buffer at the pH optima. The results, which are given in Fig. 2, reveal a shift of the temperature optimum of the immobilized invertase towards higher temperatures (55°C) as compared with the native enzyme (50°C). This shift is apparently a consequence of the action of two factors: an increase in the resistance of immobilized enzymes to heat denaturation, and an increase in the activation energy of the formation of the enzyme-substrate complex at 60 and 70°C followed by determination of activity in the presence of 0.5 M sucrose [8].

The thermostability curves are shown in Fig. 3. The native invertase preparation was inactivated practically completely during preincubation in 0.1 M acetate buffer, pH 5, at 60-70°C for 0.5-1 h. Characteristic for the immobilized enzyme was a rapid loss of 50% of the activity during the first 1.5 h of preincubation at 55 and 70°C.

The course of the hydrolysis of sucrose was followed from the accumulation of glucose in the reaction mixture. Figure 4 shows the dependence of the concentration of reaction products on the time for the immobilized and native enzymes. The activity of the immobilized enzyme was stable for 1 h, while the native enzyme was inactivated after 15 min. In the interval of invertase concentrations from 0 to 0.3 mg/ml the dependence of the initial rate of hydrolysis on the enzyme concentration,

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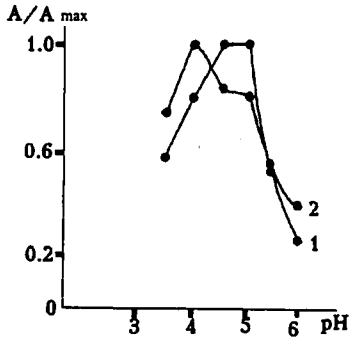


Fig. 1. Dependence of the rate of hydrolysis of immobilized (*1*) and native (*2*) invertases on the pH of the medium.

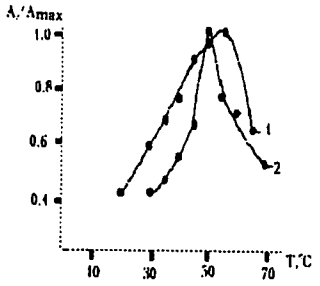


Fig. 2

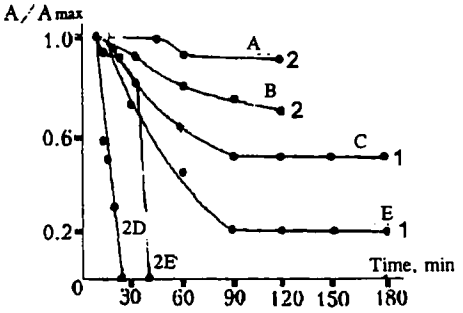


Fig. 3

Fig. 2. Determination of the temperature optima for immobilized (*1*) and native (*2*) invertases.

Fig. 3. Thermostabilities of immobilized (*1*) and native (*2*) invertases: *A*) 30°C; *B*) 50°C; *C*) 55°C; *D*) 60°C; *E*) 70°C.

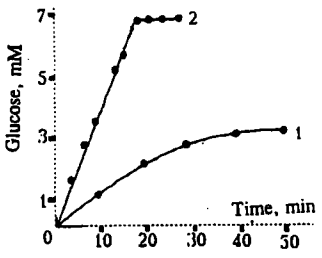


Fig. 4

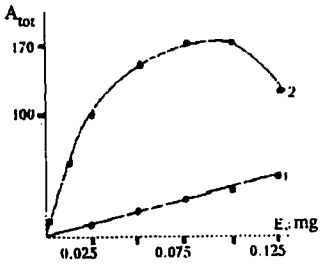


Fig. 5

Fig. 4. Kinetics of the formation of the products of the enzymatic hydrolysis of sucrose: *1*) immobilized enzyme; *2*) native enzyme.

Fig. 5. Dependence of the initial rate of hydrolysis of sucrose on the concentration of enzyme: *1*) immobilized enzyme; *2*) native enzyme.

$V = f([E])$, had a bell-shaped form for the native enzyme (Fig. 5). This is explained by the aggregation of the enzyme when its concentration in the medium is increased, which interferes with the access of the substrate to the enzyme and, as a result, decreases the activity of the enzyme. On immobilization, the aggregation effect is eliminated by the fixed state of the enzyme, and therefore the dependence of the initial rate of hydrolysis on the enzyme concentration is rectilinear.

Thus, the investigations performed have shown that on the immobilization of invertase there is a narrowing of the profile of the pH curve, a slight shift of the pH optimum of the immobilized invertase to a more neutral region, and abroadening of the zone of pH stability and also a shift of the temperature optimum in the direction of higher temperatures and a rise in the thermostability of water-insoluble invertase preparations.

## EXPERIMENTAL

We used a G10x invertase preparation and sugar and salts for the preparation of buffer solutions of ch.d.a [pure for analysis] grade. The kinetic characteristics of the immobilized invertase preparations were studied in glass bottles (15 ml) with the immobilized invertase preparation in 0.1 M acetate buffer, pH 5, the suspension being maintained with constant stirring for 30 min. Then the enzyme activities of the native and the immobilized invertases were determined by the method of [6, 7], and the protein content by the Lowry method [8].

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