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Note

Biospecific sorption of cyclodextrin glucosyltransferase on physically modified starch

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For the isolation of cyclodextrin glucosyltransferase (EC 2.4.1.19), non-specific precipitation methods are most frequently used, such as precipitation with acetone [1] or ammonium sulphate [2], or non-specific sorption methods, such as sorption on a strongly acidic cation exchanger, e.g., Sephadex SE [2]. Biospecific sorption methods have also been described, viz., sorption on native starch [3] and on α-cyclodextrin covalently bound to agarose [4]. The purification effect of precipitation is generally very low and the achievement of a sufficiently high specific activity necessitates several repetitions of the precipitation process. Sorption on ion exchangers results in a relatively good enzyme purification, but its application is limited to enzyme solutions of low ionic strength. Consequently, for both the laboratory and the industrial isolation of cyclodextrin glucosyltransferase, biospecific sorption is most suitable. Sorption on native starch has the disadvantage that it requires the use of a batch method, and biospecific sorption on bound cyclodextrins has the disadvantage of being laborious and costly.

For these reasons, in this work we utilized biospecific sorption on physically modified starch as described previously [5] and concentrated primarily on the determination of optimal conditions.

EXPERIMENTAL

The cultivation of *Bacillus macerans* for the preparation of cyclodextrin glucosyltransferase has been described elsewhere [6]. The cultivation broth was centrifuged and the clear supernatant thus obtained was used for all experiments. The activity of cyclodextrin glucosyltransferase was determined by the method of Kitahata et al. [7].

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All other reagents were of "chemically pure" grade from Lachema (Brno, Czechoslovakia).

Modified starch was prepared from maize starch that had been gelatinized, frozen at −20 °C and kept at this temperature for 24 h, then allowed to thaw.

All photometric measurements were carried out using a Spekol Model 11 photometer.

RESULTS AND DISCUSSION

The dependence of the sorption of cyclodextrin glucosyltransferase on temperature was determined in the temperature range 5–40 °C as follows: 0.5 g of modified starch was suspended in 50 ml of enzyme solution of activity 160 U/ml and the suspension was stirred overnight at various temperatures. The sorbent was then removed by centrifugation and the residual enzymic activity in the clear supernatant was determined. The results are shown in Fig. 1. The effect of temperature is very small in the range 5–15 °C. At temperatures above 15 °C, the sorption efficiency decreases rapidly and at 25 °C only 50% of the enzymic activity is adsorbed. At temperatures above 35 °C the sorption of enzyme no longer takes place. The relationship is approximately exponential until zero sorption is reached.

This dependence suggests the probable formation of an enzyme–substrate complex which at low temperatures dissociates into the enzyme and the product to only a limited extent or not at all. With this assumption, the process is a biospecific affinity sorption. The sorption is probably also favourably influenced by the fact that non-liquefied starch is a less favourable substrate than liquefied starch. The differences in reactivity are kinetic rather than thermodynamic.

For the practical utilization of this sorption for enzyme isolation, the sorption

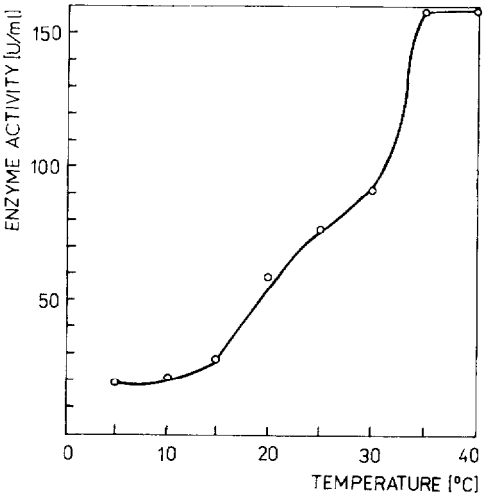

Fig. 1. Temperature dependence of the sorption of cyclodextrin glucosyltransferase on modified starch. Mixtures of 0.5 g of modified starch and 50 ml of enzyme solution were stirred overnight at 5–40 °C.

capacity of the sorbent is decisive. We determined it by the frontal analysis of an enzyme specimen, in which a cultivation broth of activity 206 U/ml was pumped at 0.2 ml/min through a column of volume 0.9 ml. Fractions were taken at 15-min intervals, i.e., 3-ml volumes, and the residual enzymic activity was determined in the individual fractions. Fig. 2 shows the results of these measurements. The capacity calculated from these results was 17 166 U per gram of wet sorbent (292 mg of protein per gram of sorbent). This capacity is more than satisfactory for the macropreparative isolation. The sorption capacity depends on the initial concentration of the enzyme in solution. For practical reasons, however, we determined the sorption capacity only at that enzyme concentration which existed in the cultivation broth. For a good preparative yield, the capacity of the sorbent cannot be utilized up to the point of saturation because the yield of the enzyme is then markedly reduced. For this reason, we considered as the practical capacity the maximum amount of the enzyme solution that does not result in an increase in the enzyme concentration in the eluate. The purity of the enzyme prepared in this way is more than sufficient for any use.

Column sorption is generally more advantageous than batch sorption. In spite of this, even the application of the latter may be advantageous in some instances. In such instances the sorption equilibrium is established only once, and the rate of the establishment of this equilibrium acquires increased importance. For this reason, we monitored the time course of the enzyme concentration in solution at 5 °C in the presence of excess of sorbent. The relationship is shown in Fig. 3. It is obvious that for batch sorption a period of at least 4 h is necessary.

The sorption equilibrium and its dependence on the enzyme/sorbent ratio were determined with a sorbent excess by three independent measurements at twelve different concentrations. The results were evaluated by Student's t-test It was proved that at $p = 0.95$ the sorption equilibrium does not depend on enzyme concentration in the presence of excess of sorbent. The solution/sorbent distribution

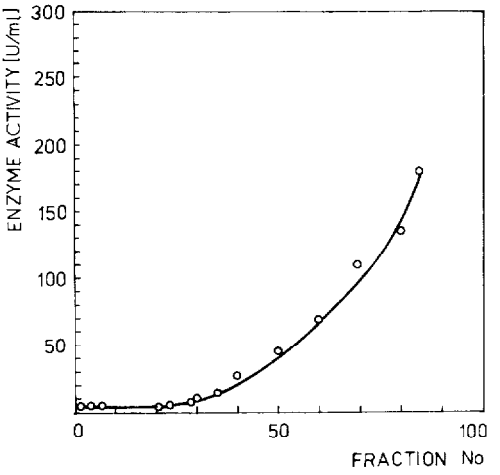

Fig. 2. Determination of the capacity of modified starch for the sorption of cyclodextrin glucosyltransferase. Amount of sorbent, 0.9 g; temperature, 10 °C; column length, 1.3 cm.

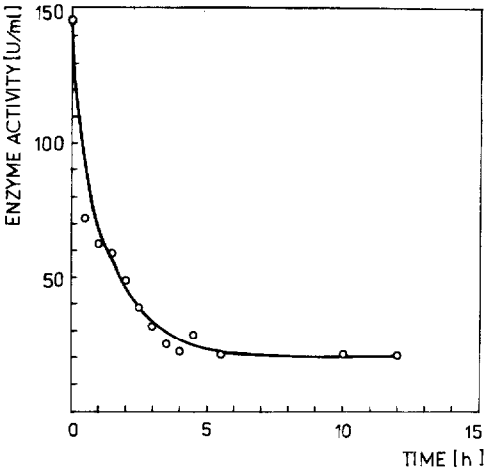

Fig. 3. Time course of the sorption of cyclodextrin glucosyltransferase on modified starch. A 0.5-g amount of modified starch was suspended in 50 ml of enzyme solution at 10 °C and the suspension was stirred continuously. The enzyme activity in solution was measured at the time intervals indicated.

coefficient was 96 ± 6 at 5 °C. The distribution coefficient used is defined as the ratio of the concentration of the enzyme in the sorbent to that in the solution.

With regard to the temperature dependence of the sorption, a buffer can be used for elution at temperatures above 30 °C. As we assumed that the decrease in sorption at elevated temperatures was due to the dissociation of the enzyme–substrate complex into the enzyme and the product, we used this fact and obtained the enzyme from the sorbate merely by heating at 50 °C for 2 h in the presence of a small volume of water. During this period, complete liquefaction of the sorbent took place and a concentrated enzyme solution of activity 893.8 U/mg of protein was obtained, which represented a 15.25-fold increase in specific activity in comparison with that of the cultivation broth.

We repeated the enzyme isolation from the cultivation broth by the described method ten times using various batches of cultivation broth. The average yield was 70 ± 6%.

The method described for the isolation of cyclodextrin glucosyltransferase is simple, highly specific and provides relatively good yields of the enzymic activity. It is suitable for laboratory, macropreparative and industrial applications. The procedure is the subject of a Czechoslovakian patent application [8].

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