IMMOBILIZATION OF MOLD BETA-GALACTOSIDASE

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We immobilized beta-galactosidase (EC 3.2.1.23) from the mold *Aspergillus oryzae* by various methods on the derivatives of bead cellulose manufactured by Czechoslovak industry. The activity, specific activity and operational stability of the immobilized enzyme were determined. The best results were obtained with the mold enzyme which had been immobilized by ionic adsorption to DEAHP bead cellulose (Ostsorb DEAHP); its activity was 275 U/g and its operational stability at 40°C was 30.6 days. The pH-optimum of this enzyme is 4·3 and the optimum temperature determined from the linearized Arrhenius plot is 65°C. Beta-galactosidase of *Kluyveromyces fragilis* was also immobilized on the same supports and its properties determined for comparison. The immobilized enzyme from the yeast *Kluyveromyces fragilis* showed a higher activity in all experiments yet its operational stability was 5 to 14 times lower in the individual cases.

Immobilized beta-galactosidase, an enzyme cleaving lactose to glucose and galactose, is one of the enzymes which have received considerable application in industry. Whereas until recently immobilized beta-glactosidase was mainly employed for the removal of lactose from milk, during the past few years this enzyme has received application in the production of sweet sirups from whey.

Beta-galactosidase has been immobilized by covalent bonding to nylon[1], polyvinyl alcohol[2], cellulose and chitin[3] and even to granular bone[4] which all served as very inexpensive supports. In none of these cases. however,was covalent bonding suitable for the immobilization of yeast beta-galactosidase[1]. Several methods of covalent bonding were tested: the classical cyanogen bromide method, the cyanuric chloride bonding[5] and bonding via epichlorohydrine[2]. Cellulose[6] and xanthate[7] were the supports used most frequently. Of the various adsorption methods interest deserves, in addition to the classical adsorption to ion-exchange resins, the prospective affinity adsorption to immobilized reactive dyes[5]. The stability of beta-galactosidase immobilized by various methods has been studied in detail by Greco et al[8].; this author, however, has not reported any relations of general validity between the mode of binding, the properties of the enzyme and support and their stabilities.

EXPERIMENTAL

Kluyveromyces fragilis beta-galactosidase was a product of Novo Industri(Denmark), supplied under the brand name Lactozyme 3000. The optimum temperature for this enzyme is 45°C and its pH-optimum lies between 6·5 and 7·0. *Aspergillus oryzae* beta-galactosidase was a product of Roehm, Darmstadt (F.R.G.) supplied under the brand name beta-galactosidase 7041 C. The optimum temperature for this enzyme is 60°C and its pH-optimum is 4·5.

Both enzymes were used without any purification. Immobilized beta-galactosidase covalently bonded to an organic polymer, a product of Roehm, Darmstadt (F.R.G.), supplied under the brand name Plexazym LA-1, was used as a standard for comparison experiments.

All the supports used were of Czechoslovak origin. Ostsorb supports are derivatives of bead cellulose, manufactured by Spolek pro chemickou a hutní výrobu, Ústí nad Labem. Ostsorb DEAHP is the diethylaminohydroxypropyl derivative, Ostsorb DEAE the diethylaminoethyl derivative, Ostsorb DETA the diethylenetriamino derivative, Ostsorb EDA the ethylenediamino derivative, and Ostsorb Phenyl the phenyl derivative of bead cellulose. Glutaraldehyde was a product of Koch-Light. The remaining chemicals used in this study were products of Lachema, Brno and were of analytical purity grade.

The beta-galactosidase activity was determined by the slightly modified method of Mahoney and Adamchuk[9]. Mold beta-galactosidase was assayed at pH 4·5, yeast beta-galactosidase at pH 6·6 in the presence of 0·1 mM $MnCl_2$ using potassium salts for the preparation of the buffer. (The enzyme is inhibited by sodium ions and activated by manganese(II) ions). The assay was carried out at 37°C in both cases. The activity of the immobilized enzyme was determined directly by the reaction of a weighed-out quantity of the immobilized enzyme with the substrate, with shaking in a water bath under the same conditions as those used with the soluble enzyme. The activity was expressed in units per g of wet support.

The proteins were determined according to Hartree[10]. The operational stability was estimated by allowing a 5% lactose solution to flow through a jacketed 0·9 × 4 cm column of the enzyme thermostated at 40°C for 1 week. The quantity of glucose was determined in the samples withdrawn by the Bio-La-Test. The calculations were made according to O'Neill[11]. The immobilization of the enzymes by glutaraldehyde was performed according to Cabral et al.[12].

RESULTS AND DISCUSSION

We have immobilized mold and yeast beta-galactosidase by adsorption and covalent bonding to a series of Czechoslovak supports and compared the activities of the immobilized enzymes (EU/g), the yields of immobilized activity and the operational stabilities of the immobilized enzymes. The results are summarized in Table I. Even though the activity of the immobilized enzyme was higher for the yeast enzyme in all cases, the operational stability was 5 to 14 times higher, depending on the mode of immobilization, for the mold enzyme. These differences in operational stability are more marked for immobilization by covalent bonding than for immobilization by adsorption. When immobilization by adsorption is used the highest activity is obtained with yeast beta-galactosidase and Ostsorb DEAE. Relatively high are also the maximum activities obtained when yeast beta-galactosidase is covalently bonded to Ostsorb DETA and Ostsorb EDA. All methods of immobilization of yeast beta-galactosidase are very efficient yet the operational stabilities of this

TABLE I

Comparison of immobilization of mold and yeast beta-galactosidase

Enzyme	Support	Mode of immobilization	Activity EU/g	Operational stability (days at 40°C)	% of Bound activity[a]
Lactase 7041 C (mold)	Ostsorb DEAHP	ionic adsorption	275·0	30·6	1·97
	Ostsorb DEAE	ionic adsorption	284·1	14·9	4·30
	Ostsorb DETA	covalent bonding[b]	266·0	34·8	3·07
	Ostsorb EDA	covalent bonding	248·1	35·2	2·21
	Ostsorb phenyl	hydrophobic ads.	5·4	—[c]	0·06
Lactozym 3000 (yeast)	Ostsorb DEAHP	ionic adsorption	437·0	4·9	9·70
	Ostsorb DEAE	ionic adsorption	923·9	3·8	10·50
	Ostsorb DETA	covalent bonding	610·3	3·0	7·00
	Ostsorb EDA	covalent bonding	624·5	2·5	7·16
Plexazym LA-1	synth. polymer	covalent bonding	490·0	44·4	—

[a] % of Bound activity — ratio of bound activity to total input activity in %; [b] glutaraldehyde; [c] not determined.

immobilized enzyme are low (cf. Table I). This fact is not a result of the properties of the supports as evidenced, among others, also by the results obtained with the immobilization of yeast beta-galactosidase, yet it is directly related to the properties of yeast beta-galactosidase. This enzyme generally shows lower values of operational stability, the half life of inactivation being of the order of five days; this is caused by the generally higher sensitivity of the yeast enzyme to steric interference. Slightly lower values of both maximal and specific activity were obtained with mold beta-galactosidase yet its operational stability was relatively high.

The immobilization by adsorption to Ostsorb DEAE and Ostsorb DEAHP leads to slightly higher maximal and specific activities than immobilization by covalent bonding to Ostsorb DETA and Ostsorb EDA. The operational stability of beta-galactosidase immobilized by adsorption is slightly lower than the operational stability of the same enzyme immobilized by covalent bonding. A general rule holds in this case that covalent bonding generally results in higher operational stabilities. Yeast beta-galactosidase represents an exception from this general rule because of its increased sensitivity to steric effects.

It is obvious that immobilized mold beta-galactosidase will receive wider application in industry than immobilized yeast beta-glactosidase. The higher activities obtained with the yeast enzyme do not represent an advantage since they cannot be utilized in full. Because of the high hydrodynamic resistance of the column bed flow rates cannot be applied which would permit the activity of the enzyme to be utilized in full. A decrease of the hydrodynamic resistance by replacing the support with a support of larger particle size would result in a lower utilization of the enzyme because of internal diffusion. The flow rate could be also increased by increasing the column cross section and simultaneously decreasing the column height. Such an arrangement would make the construction of the reactor more complicated should a uniform flow over the entire cross section be achieved. This would necessarily increase the price of the reactor and make the operation of the column more demanding. Hence, as the optimal preparation should be regarded mold beta-galactosidase immobilized by adsorption to Ostsorb DEAHP. The operational stability of this preparation is good and so is also its activity; the preparation is acceptable also from the economical point of view because it can be obtained by a simple procedure at a low cost. We have determined its optimal pH and optimal temperature as complementary characteristics.

The optimal pH was determined over the range 2·5 – 7 and the value obtained was compared with that found for a commercial preparation of mold beta-galactosidase. The result is shown in Fig. 1. As could be expected with adsorption to an anion exchange resin the optimal pH is slightly shifted to the acid region compared to the native enzyme.

The optimal temperature was assayed by measuring the temperature dependence of the activity of the enzyme in the temperature range 30 – 75°C. We evaluated the

data by the linearized Arrhenius plot as shown in Fig. 2. The profile of this dependence shows two inflections. The activation energy at temperatures below 55°C is 22·59 kJ/mol; this is a value usually observed with enzymatic reactions and corresponding to the temperature profile of the kinetic constant. Hence, the reaction is kinetically controlled in this range. The activation energy at temperatures between 55 and 65°C is low (– 1·19 kJ/mol). We assume that the reaction is controlled by diffusion in this region yet a simultaneous effect of the denaturation of the enzyme can also be observed. Finally, denaturation becomes prevalent at temperatures above 65°C.

Our results of immobilization experiments with yeast and mold beta-galactosidase were compared with the results obtained with a commercial preparation of this immobilized enzyme (Plexazym LA-1). As obvious from Table I, the maximal activity of the commercial preparation of beta-galactosidase is higher than that of the mold enzyme immobilized on Czechoslovak supports. The parameters of the commercial enzyme are comparable to those of yeast beta-galactosidase immobilized on Czechoslovak supports yet the stability of these preparations is considerably lower. The activity of mold beta-galactosidase immobilized on Czechoslovak supports is lower than the activity of the commercial preparation of immobilized beta-galactosidase, yet the operational stabilities are comparable, even though the value for the enzyme immobilized on supports of home origin is also lower. This difference can be accounted for, especially as regards the activity values, by the fact that the starting

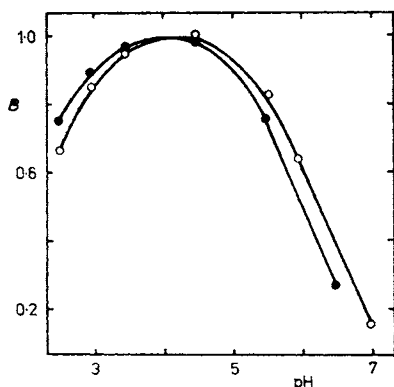

FIG. 1

pH-Profile of activity of soluble (○) and immobillized (●) on Ostsorb DEAHP mold beta-galactosidase at 37°C; *B* is relative activity

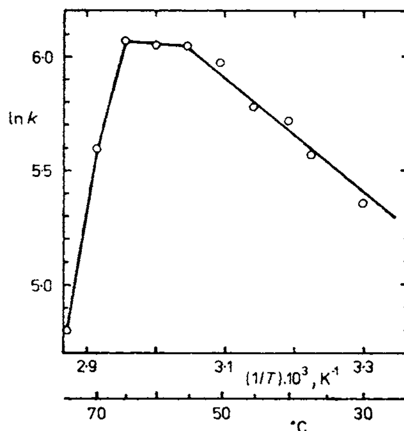

FIG. 2

Arrhenius plot for calculation of activation energy (beta-galactosidase 7041 C immobilized by adsorption to Ostsorb DEAHP)

material for manufacturing the commercial preparation is the native enzyme of a higher purity; hence no contaminating proteins are simultaneously immobilized. The efficiency of the immobilization is thus increased. We are lacking so far a more detailed explanation of the differences in operational stability. It would appear that there exists a real possibility of an additional increase in operational stability by optimalization of the conditions of immobilization.

In the light of our measurements we regard as the best preparation mold beta-galactosidase immobilized by adsorption to Ostsorb DEAHP since the immobilization of the enzyme is simple in this case, the unadsorbed enzyme can be repeatedly used and the regeneration of the support is easy. All these features are favorable from the viewpoint of industrial application of the enzyme.

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