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KERATIN AND POLYAMIDE-COATED INORGANIC MATRICES AS SUPPORTS FOR GLUCOAMYLASE IMMOBILIZATION

Łobarzewski J., Paszczyński A., Wolski T.[xx] and Fiedurek J.[x]

Department of Biochemistry and [x]Department of Applied Microbiology, M. Curie-Skłodowska University, 20-031 Lublin, Poland,
[xx]Technological Laboratory, Dept. of Inorganic and Analytical Chemistry, Medical Academy, Lublin, Poland

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SUMMARY. Previously solubilized feather keratin and polyamide were used for coating sand, glass beads and silica gel. These new seven supports were employed for comparative studies on pure glucoamylase / EC 3.2.1.3 / immobilization. The immobilization yield of glucoamylase on keratin and polyamide coated supports was comparable with conventional matrices used earlier. The highest activity per 1 g of support was shown by the enzyme bound to polyamide-coated CPG, and the bests operational stability by the enzyme immobilized on polyamide-coated CPG with keratin subsequently deposited on it.

Since the industrial-scale utilization of immobilized enzymes the search for new, unconventional supports has been a subject of many reports / 1,2,3 /. The application of feather keratin as a support for enzyme immobilization which, similarly to collagen, is theoretically possible encountered obstacles because of its insolubility. The acquisition of a soluble preparation of keratin in diethylsulphoxide / 4 / for first time created an opportunity to produce matrices coated with this protein for enzyme immobilization.

In the past polyamide was usually used for enzyme immobilization in the form of tubes or stripes / 5-18 /.

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But in those forms its surface available for enzyme binding was greatly restricted. For that reason, in the experiments presented we used several supports for enzyme immobilization produced with keratin and polyamide in the pellicular form which highly enlarged matrix accessibility for biotechnological enzyme proteins, thus making matrix application comparable to classical supports such as glass activated with γ -amino-propyltriethoxysilane at much lower costs of production.

MATERIALS AND METHODS

Enzyme source. Glucoamylase / EC 3.2.1.3 / was obtained from the medium after a culture of Aspergillus niger C, and then purified by affinity chromatography. The procedure was described erlier Paszczyński et al. / 19 /. Thus obtained glucoamylase preparation was lyophilized and used for experiments. Glucoamylase activity was determined by a glucose oxidase test after Lloyd and Whelan / 20 /. The unit of glucoamylase activity was defined as the amount of the enzyme which liberates umol of glucose per minute / U / from a 2% solution of soluble starch after Zulkowsky. Measurements were carried out at 45°C using 0.15 M acetate buffer, pH 4.5 / 21 /.

Protein assay. Soluble protein was determined by the method after Schacterle and Pollack / 22 /.

Matrix preparation. Polyamide-6 / textile cuttings / and keratin / feathers / used as material in the experiments constitute troublesome industrial wastes. Polyamide was dissolved in 15% formic acid applying a technique described in patent application / 23-25 /. Keratin was dissolved in dimethylsulphoxide according to patent / 4 /. Both substances were used as polymers with active peptide groups to coat inorganic supports by the evaporation of the solvents in vacuum / Fig. 1 /. The following sorbents were coated: exhaustively washed sand of the granulation of 0.1 - 0.3 mm, silica gel of the granulation of 0.06 - - 0.2 mm / Merck, GFR /, and porous glass / CPG / of the granulation of 0.125 mm / Pierce Co., USA /. The sorbents were mixed with the solutions of polyamide / 6% solution /

or keratin / 6% solution / at the ratio of 100 ml per 100 g of support.

Besides these supports, granular polyamide was also prepared. Polyamide was dissolved in 15% formic acid. The precipitation of granulated polyamide was achieved by the alkalization of the solution followed by the removal of solvent excess in vacuum / 24 /. The obtained product was washed with methanol, dried at 60-70°C, and fractionated on sieves using grain size of 0.1 - 0.2 mm.

All the matrices prepared were further activated with glutaraldehyde as described previously / 26 /.

In order to obtain a longer spacer-arm between the matrix and glucoamylase in one of the variant's we used keratin protein coupled with polyamide coating CPG by means of glutaraldehyde using the technique described above. The preparations were then second time activated with glutaraldehyde. A scheme of the support is shown in Fig. 1.

Glucoamylase immobilization. Polyamide- and keratin--coated matrices after activation with glutaraldehyde were coupled with glucoamylase / EC 3.2.1.3 / by the method described previously / 26 /.

RESULTS AND DISCUSSION

One of the greatest recent achievements in the field of the use of inorganic supports for enzyme immobilization was the coating their surface with a hydrophilic organic film layer. The method worked out in detail consists in coating porous glass with glycophase / 27 /. In this way it has been possible to combine the mechanical resistance and chemical stability of glass with the hydrophilic properties and ease of activation characteristic of polysaccharides / 28 /.

The supports coated with polyamide or keratin protein prepared by us seem to constitute a significant advance in this field. It should be noted that both polyamide used for mineral matrix coating and keratin are troublesome

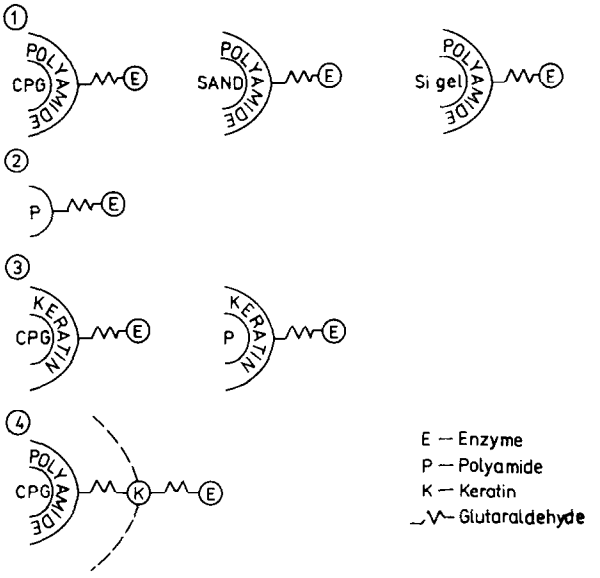


Fig. 1. Scheme of matrices used for glucoamylase immobilization.

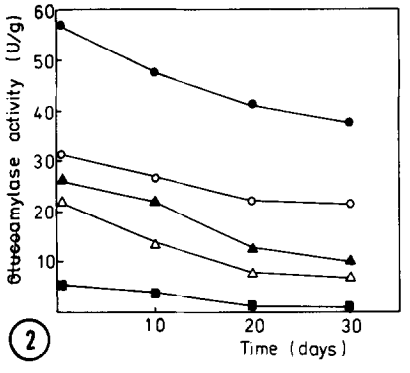
industrial wastes. Thus, in the experiments here presented they have found good practical use.

Up to check the properties of these supports the preparation of glucoamylase / EC 3.2.1.3 / previously purified by our own method was immobilized on seven different matrices / Fig. 1 /.

The obtained results of both immobilization efficiency / Table 1 / and operational stability of glucoamylase / Table 1, Fig. 2 / reveal an activity comparable with the literature data and considerable stability / 1,26 /. The preparations of soluble and insoluble glucoamylase did not show any great differences in their activities during incubation for 1 hour in the temperature range of 20 - 50°C / Fig. 3 /. Even at 50°C the stability of the preparation used by us was quite considerable. On the other hand, glucoamylase from the same source immobilized on glass beads coated with polyethyleneimine was more labile and at 50°C its half-life was less than a week / 29 /.

Table 1. Activities of glucoamylase immobilized on various carriers

Carriers bound to glucoamylase	Amount of enzyme preparation / mg/g /	U/g	Protein	U/mg of protein
CPG coated with polyamide	25	1.33	1.7	0.78
	50	49.20	1.9	26.00
	100	55.20	2.7	20.05
	500	45.30	2.3	18.90
Sand coated with polyamide	25	1.94	0.45	4.30
	50	2.60	0.60	4.30
	100	15.90	2.40	6.62
	500	12.80	2.20	4.80
Silica gel coated with polyamide	25	2.05	0.74	2.77
	50	9.32	4.40	2.11
	100	21.20	4.40	4.80
	500	11.00	3.50	3.14
Keratin immobilized on polyamide coated CPG	100	31.30	8.50	3.60
Polyamide beads	100	25.50	6.70	3.80
Keratin immobilized on polyamide	100	20.00	4.40	4.50
Glass coated with keratin	100	5.30	1.70	3.11



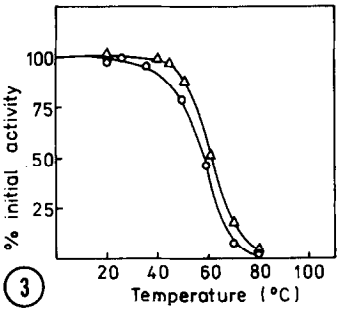


Fig. 2. Operational stabilty of glucoamylase immobilized on following matrices: / — ● — / CPG coates with polyamide; / — ○ — / keratin immobilized on polyamide coated CPG ; / — ▲ — / polyamide beads; / — △ — / keratin immobilized on polyamide; / — ■ — / CPG coated with keratin.

Fig. 3. Comparison of thermal stability of glucoamylase / — △ — / soluble and / — ○ — / immobilized on CPG coated with polyamide.

The inconvenience of the method described by Wasserman / 29 / also consisted in the necessity of enzyme contact during immobilization with a glutaraldehyde solution, which could be a cause of the instability of bound protein and low thermostability of the preparation obtained / 30 /.

The optimalization of the degree of glucoamylase immobilization was performed on three polyamide matrices / Table 1 /. According to the literature, the application of glucoamylase amounts per 1 g of support greater than 100 mg does not increase the degree of enzyme immobilization / Table 1 /. In the presented experiments the proportions of glucoamylase more than 100 mg per 1 g of support did not prove advantageous either / Table 1 /. The highest activity of immobilized glucoamylase was obtained on polyamide covering glass beads / Table 1 /.

Taking into consideration the support's U/g, the values of glucoamylase immobilized on polyamide coating glass / 100 mg 55 U/g / are comparable with the activity of glucoamylase immobilized on keratin coupled with polyamide covering CPG / 31.3 U/g / / Table 1 /. As can be seen in Table 2, the operational stability of the latter glucoamylase preparation, however, is highest in this case. Glucoamylase immobilized on keratin coupled with polyamide covering CPG shows particularly good properties in comparison with pellicular polyamide / Table 1, Fig 2 /. It may result from an increased influence of the distance between glucoamylase and the matrix, which diminishes the negative effect of the matrix or stabilizes its active conformation / Fig. 1 /. Most likely it also facilitates the access of high-molecular substrate, to the active centre of the enzyme.

Table 2. Operational stability of glucoamylase immobilized on various carriers. Percentage of the decrease of enzyme activity after 30 days of incubation at 25°C.

Kind of matrix bound with glucoamylase	Decrease in immobilized glucoamylase activity after 30 days in %
Keratin immobilized on polyamide coating glass	29
Polyamide on glass beads	32
Polyamide beads	61
Keratin on polyamide beads	65
Keratin on glass beads	75

The specific activity of immobilized glucoamylase calculated per 1 mg of bound protein is highest in the case of the preparation immobilized with polyamide covering glass beads / Table 1 /. In other cases the activity ranges from 2.11 to 6.62 / Table 1 /. The amount of protein bound by the application of seven unconventional matrices is similar to the data reported in the literature, while avoiding the necessity of using γ aminopropyltriethoxy-silane so far employed in most experiments for the activation of matrices based on SiO_2.

Virtually all the five analysed preparations of glucoamylase immobilized on keratin or polyamide retained a considerable percentage of activity even after 30 days of experimenting / Table 2, Fig. 2 /. Drops in activity were in the range of about 50%.

It seems that the application of pellicular matrices for supports of polyamide or keratin, which so far has

not been employed for this purpose, may provide new, simple, and cheap supports for enzyme immobilization on a biotechnological scale. We are of the opinion that there are further prospects of modifying the matrices described which may lead to the acquisition of more stable glucoamylase preparations competitive with the results obtained by us previously / 26 /.

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