Chemistry of Natural Compounds, Vol. 30, No. 5, 1994

IMMOBILIZATION OF *Streptomyces atratus* GLUCOSE ISOMERASE ON VARIOUS SUPPORTS

B. A. Tashpulatova, Zh. Tashpulatov, I. G. Sultanova, and N. Yu. Zukhrutdinova

UDC 577.15.154.633.3.81

The immobilization of Streptomycs atratus *glucose isomerase has been achieved and the periods of half-life of the immobilized glucose isomerase have been determined on various supports, among which the most acceptable are cotton lint and polyurethane. The stability of the immobilized preparations has been demonstrated.*

In recent years, ever greater attention has been devoted to the use of immobilized glucose isomerase for the production of glucose-fructose syrup [1]. At the present time commercial preparations of glucose isomerase immobilized mainly on gelatin (Maxazyme) [2], DEAE-cellulose (Sweetase) [3], and chitosan (Godoagt) [4], and others, are being produced.

With the aim of using more accessible and cheaper support materials for the immobilization of enzymes and their use in the large-scale manufacture of glucose-fructose syrup, we have selected supports that are waste products from local industries — acetate fiber, the fibrous substances obtained after the unwinding of cocoons, cotton lint, and polyurethane.

Cells of *Streptomyces atratus* — a producing agent of glucose isomerase — were immobilized on the supports by physical absorption. Preliminary modification of the supports permitted a substantial increase in the strength of binding of the absorption-immobilized enzyme. The modifying agents used were sodium citrate and an azo reagent.

The *Str. atratus* glucose isomerase has a low stability in the acid pH region and, therefore, in its absorption on various supports a loss of catalytic activity may occur, since, because of the presence of sodium citrate, the surface of the supports acquires an acid nature. To prevent inactivation of the immobilized enzyme preparation obtained in this way, before the performance of the isomerization reaction it was kept for 6 h in a buffer solution with pH 7.8. Moreover, the majority of supports are capable of selectively and strongly sorbing metal ions [5] and, therefore, on the absorption immobilization of metal-dependent enzymes, especially glucose isomerase, a partial or complete loss of catalytic activity may take place due to the departure of a metal ion from the active center of the enzyme and its binding to the surface of the support. In order to eliminate this undesirable phenomenon, before the immobilization procedure the support is treated with a 0.5 M solution of $MgSO_4 \cdot 7H_2O$ and a $5 \cdot 10^{-3}$ M solution of $CoCl_2$, thereby saturating the centers of metal-ion sorption on the support.

Preliminary treatment of the supports with metal ions also ensures the strength of binding of the absorption-immobilized enzymes. The efficacy of sorption is increased in this case, apparently, through the formation of a complex of the protein with Mg and Co, which, as is assumed, are strongly bound with the surface of the support. In other words, the Mg^{2+} and Co^{2+} ions play the part of a bridge linking the enzyme molecule with the support. When sodium citrate and an azo reagent are used as modifying agents, a layer with a large number of functional groups (hydroxy, carboxy, etc.) is formed on the surface of the support. Therefore, by treatment with a solution of sodium citrate and the azo reagent it is possible to raise the efficiency of sorption and to improve the catalytic characteristics of the immobilized enzyme.

We have studied the properties of preparations obtained by the immobilization of glucose isomerase on various supports. As can be seen from Table 1, cotton lint and polyurethane proved to be effective supports. The smallest amount of protein was bound with the cocoon fibers and the acetate fiber. This obviously shows a dependence of the magnitude of the surface area of the matrix on binding with the enzyme molecule [sic], all the more since it is known from the literature that the sorption capacity of a support is proportional to its specific surface [5]. The results of experiments on the thermal stability

Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (82712) 41 71 29.
Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 670-672, September-October, 1994. Original article submitted January 5, 1994.

0009-3130/94/3005-0619$12.50

TABLE 1. Characteristics of Preparations of Glucose Isomerase Immobilized on Various Supports

Support	Amount of bound protein, %	Activity, units/g of support	Half-life, days
Acetate fiber	33.5	70.5	3
Cocoon fiber	25.6	50	2
Cotton lint	75	243.5	21
Polyurethane	60	250	20

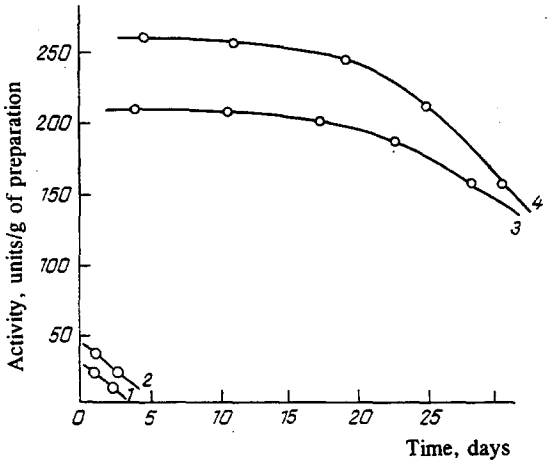

Fig. 1. Parameters of the activity of glucose isomerase immobilized on various supports: 1) on cocoon fibers; 2) on acetate fiber; 3) on polyurethane; 4) on cotton lint.

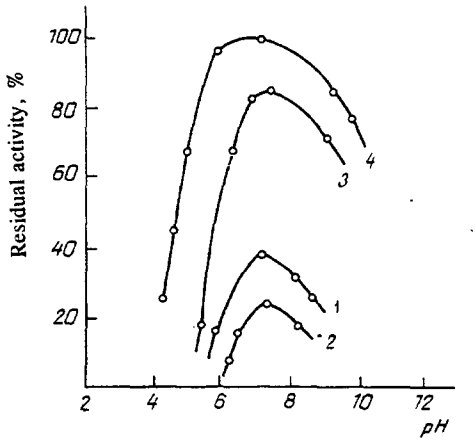

Fig. 2. Activity of glucose isomerase immobilized on various supports as a function of the pH. For the arbitrary symbols, see Fig. 1.

of the immobilized glucose isomerase preparations showed that the activity of the enzyme changed little over 10 days in a column reactor with a preparation immobilized on cotton lint or polyurethane. For the other preparations, thermal inactivation began fairly rapidly.

We investigated the stability of the immobilized preparations as a function of the pH. The highest stability of the glucose isomerase with a change in pH from 5.0 to 10.0 was shown when it was immobilized on cotton lint and polyurethane.

In a study of the immobilized preparations under the optimum conditions, differences were found in the times of inactivation. When preparations immobilized on cocoon fibers and acetate fiber were stored, complete inactivation of the enzyme was observed after only two weeks, while glucose isomerase on cotton lint and polyurethane did not lose activity in the course of 1.5 years.

Thus, it has been established that in the absorption immobilization of glucose isomerase on various supports the most acceptable are cotton lint and polyurethane.

EXPERIMENTAL

The growth of a culture of *Str. atratus* and the production of its biomass have been described in [6].

As supports for the immobilization of cells of *Str. atratus* — the producing agent of glucose isomerase — we used acetate fiber, the fiber formed in the unwinding of cocoons, cotton lint, and polyurethane production wastes.

The supports were modified by stirring them (5 g) in a 16% solution of sodium citrate and a 2.5% solution of azo reagent with the addition of 0.5 M $MgSO_4 \cdot 7H_2O$.

The amount of fructose formed as a result of the reaction in the solution at the outlet from a laboratory column reactor was determined by the cysteine-carbazole method [7].

As the unit of glucose isomerase activity we took the amount of enzyme leading to the formation of 1 μmole of *D*-fructose in 1 min at 70°C and a pH of the substrate of 7.8. The substrate used was a 20% solution of crystalline glucose containing $5 \cdot 10^{-3}$ M $MgSO_4 \cdot 7H_2O$.

The amounts of protein in the enzyme solution and on the support were determined by Lowry's method [8].

The stability of an immobilized preparation was determined as the time of half-inactivation of the glucose isomerase at 70°C and pH 7.8 in 0.005 M Na,K phosphate buffer containing $5 \cdot 10^{-3}$ M $MgSO_4 \cdot 7H_2O$.

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