Chemistry of Natural Compounds, Vol. 32, No. 6, 1996

ISOLATION AND SOME PROPERTIES OF GLUCOSE ISOMERASE FROM A HYBRID STRAIN OF *Streptomyces atratus*

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UDC 576.872.1

A highly effective easily reproducible method of obtaining high-purity preparations of glucose isomerase from initial and hybrid strains of Streptomyces atratus *has been developed. Two enzymes have been characterized, with molecular masses of 160 and 120 kDa, isoelectric points of 5.2 and 5.0, pH optima of 8.0 and 9.0, and temperature optima of 80 and 90°C, respectively. The activity of glucose isomerase preparations obtained by purification from a hybrid strain of* Streptomyces atratus *was 60-70% greater than that of analogous preparations from the initial parental strain.*

The aim of the present work was a comparative study of the enzymatic activity and other properties of preparations of glucose isomerase (GII) from initial and hybrid strains of *Streptomyces atratus*.

The method of cell engineering has been used to obtain new active strains of microorganisms producing glucose isomerase. Hybrids forming a more active glucose isomerase have been obtained by fusing the protoplasts of two strains of the genus *Streptomyces* (*S. atratus* and *S. graminofaciens*).

An investigation of the genetic stability of the hybrid strains with respect to morphological characteristics and biochemical activity has shown that these characteristics are stably reproduced over tens of generations.

To obtain preparations of the enzyme glucose isomerase, we selected two strains of *S. atratus* – initial (parental) and hybrid. In a study of the influence of carbohydrates on the biosynthesis of glucose isomerase in the selected producing strains, the highest GII activity appeared in media containing xylose or xylitol in a concentration of 1%. When other carbohydrates — glucose, sucrose, starch — were added to the medium, good growth of biomass was observed but the GII activity was zero.

The results obtained show that, for the initial and the hybrid strains of *S. atratus*, xylose and xylitol were specific inductors of the synthesis of glucose isomerase, while other carbohydrates repressed the biosynthesis of the enzyme. The highest GII activity of the strains was observed when they were cultivated in media with organic sources of nitrogen in the form of yeast autolysate and fodder yeast (0.5-1%), this apparently creating the most favorable complex of biologically active substances that is necessary for the synthesis of glucose isomerase by streptomycetes.

In the course of a study of the influence of metal ions on the biosynthesis of glucose isomerase it was established that Mg^{2+} and Co^{2+} ions in a concentration of 0.01% lead to an increase in GII activity, while other ions have no appreciable influence on the growth and activity of *S. atratus* strains.

A comparative investigation of the biosynthesis of GII during the growth of the initial and hybrid strains of *S. atratus* by a published method [3] showed a similarity in the general features of these processes in the strains concerned. The maximum accumulation of biomass and GII activity was observed in the exponential growth phase, which corresponds to cultivation for 72 h.

Although the principles of isolation and purification are similar for the majority of enzymes, in the case of glucose isomerase there are special features. The GII of *S. atratus* is an intracellular enzyme localized in the periplasmic space and in the cell wall of the producing agent, and, therefore to destroy the cell wall and to isolate the enzyme we used extraction from the freeze-dried biomass. After extraction at 40°C for two hours the residue was separated off by centrifugation at 8000 rpm

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Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 903-907, November-December, 1996. Original article submitted March 11, 1996.

0009-3130/96/3206-0886$15.00

TABLE 1. Purification of the *S. atratus* Glucose Isomerase

Stage	Protein, mg		GII activity				Activity yield, %		Degree of purification, times	
			total, units		specific, units/mg of protein					
	A*	B	A	B	A	B	A	B	A	B
Mycelium homogenate	180.6	310.2	320.6	980.6	1.8	3.1	100	100	1	1
Heat treatment at 70°C for 15 min	15.7	30.2	260.5	670.4	16.5	22.2	81.2	68.4	9.1	7.5
Chromatography on DEAE-cellulose	8.25	15.4	215.7	540.3	26.2	35.2	67.3	55.1	14.5	11.3
Chromatography on DEAE-Sephadex A-50	5.4	8.5	197.2	425.8	35.6	50.1	61.6	13.4	19.8	16.2
Gel filtration on Sephadex G-100	4.3	5.4	179.3	375.7	41.7	69.6	55.9	38.2	23.2	22.4

A) Initial strain; B) hybrid.

TABLE 2. Some Properties of the *S. atratus* Glucose Isomerase

Properties	Indices	
	initial strain	hybrid strain
Molecular mass, kDa	160	120
Electrophoretic mobility, R_f	0.64	0.78
Isoelectric point	5.2	5.0
pH optimum	8.0	9.0
Temperature optimum	80°	90°

for 15 min. The supernatant liquid, which contained 1.8 and 3.1 GII activity units per 1 ml of protein for the initial and hybrid strains of *S. atratus*, respectively, served as the source of GII.

Table 1 gives the main facts relating to the various stages in the isolation and purification of the enzyme, including the yields in terms of enzyme activity. In the first stage of the purification of the GIIs of both strains, which are heat-stable enzymes, we used the heat treatment of the homogenate at 70°C for 15 min, which enabled us to achieve a 7- to 9-fold purification of the enzymes.

After dialysis, the enzyme solution was deposited on a column of DEAE-cellulose. For the initial and the hybrid strains, the specific GII activities after desorption from the column had risen to 26.2 and 35.1 units/mg of protein, respectively. The electrophoretic analysis of each of the desorbed enzyme preparations revealed three protein bands, one of which was due to GII-ase and the other two to impurity proteins.

To eliminate the impurity proteins we carried out ion-exchange chromatography on DEAE-Sephadex A-50. Desorption was achieved by the same method as for DEAE-cellulose. The last stage of purification was the gel chromatography of the enzyme preparation on a column of Sephadex G-100. The protein was collected and dialyzed, and was then freeze-dried.

The highly purified preparation of glucose isomerase from the hybrid had a specific activity of 69.6 units/mg, which was almost 70% higher than that from the initial strain.

Some physicochemical properties of the purified enzymes are given in Table 2. Comparison showed a difference in the purified glucose isomerases from the initial (parental) and the hybrid strains of *S. atratus* with respect to molecular mass and electrophoretic mobility and the pH and temperature optima. The results obtained indicate the necessity for studying the properties of the glucose isomerases of various producing agents, since they vary appreciably not only in different species but also in experimentally obtained strains of streptomycetes belonging to a single species.

Thus, a method has been developed for obtaining highly purified active enzyme preparations of glucose isomerase from a hybrid strain of *S. atratus* that are promising in the production of glucose–fructose syrups and of pure fructose for the needs of the medical industry.

EXPERIMENTAL

Initial and hybrid strains of *S. atratus* grown as described previously [4] were investigated.

When the maximum amount of biomass had accumulated, the cells were separated off by centrifugation at 3000 *g*, and they were washed three times with distilled water to eliminate traces of the medium and were freeze-dried. The dry biomass was ground in a mortar with the addition of 20 ml of 0.005 M phosphate buffer, pH 7.8, per 1 g of biomass. The enzyme was extracted from the cells by stirring with a magnetic stirrer at 40°C for 15 min. The residue was separated off by centrifugation at 8000 *g* for 15 min. The supernatant was used as the source of glucose isomerase.

Glucose isomerase activity was determined by the cysteine–carbazole method [3].

To determine isomerization activity, the cell extract, at pH 8.0, was incubated at 70°C for 60 min, since during this period the amount of product (fructose) rose linearly with the time. As the unit of GII activity we took the amount of enzyme that ensured the formation of 1 μmole of *D*-fructose in 1 min at a glucose concentration of 1 M.

In the first stage of purification, the enzyme solutions were subjected to heat treatment in a water bath at 70°C for 15 min. Then the residue was separated off by centrifugation (8000 *g*), and the supernatant liquid was dialyzed in the cold against 0.05 M phosphate buffer, pH 7.8, for 10-12 h. After dialysis, the enzyme solutions from the initial and the hybrid strains, containing 15.7 and 30.2 mg of protein and GII activities of 16.5 and 22.2 units/mg of protein, respectively, were deposited on a column of DEAE-cellulose (4 × 20 cm) previously equilibrated with the above-mentioned buffer.

The glucose isomerase was eluted at a concentration of 0.4 M NaCl in 0.1 M phosphate buffer at the rate of 60 ml/min, with the collection of 3-4-ml fractions. The active fractions were combined and were concentrated in a dialysis bag against

poly(ethylene glycol) at 4°C for 5 h. The concentrate obtained was desalted by passage through a column of Sephadex G-25. The desalted protein fractions were combined and subjected to ion-exchange chromatography on DEAE-Sephadex A-50 (2 × 25 cm column). Washing and elution (at a rate of 30 ml/h) were carried out by the same method as for the DEAE-cellulose. The last stage of purification was gel filtration on a column of Sephadex G-100 (2 × 85 cm, rate 36 ml/h).

Protein in the eluate was determined spectrophotometrically from the absorption at 280 nm. At various stages of purification, protein was determined by Bradford's method [5]. The purity of the preparations was checked by disk electrophoresis in polyacrylamide gel (PAAG) on a LKB instrument (Sweden).

The molecular masses of the enzymes were determined by gel chromatography on a column (1.5 × 120 cm) of Sephadex G-100 equilibrated with 0.005 M phosphate buffer, pH 7.8, and by electrophoresis in PAAG in the presence of SDS, using standard proteins with known molecular masses.

Isoelectric focusing was carried out by the method of Vesterberg and Svensson [6] on a LKB instrument (Sweden) in a column with a volume of 110 cm^3 in a zone of Ampholines with pH values from 4.0 to 8.0. Sucrose (0-50%) was used to form a gradient.

The stability of the glucose isomerase at various pH values and temperatures was determined as described previously [7].

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