9. A. P. Sinitsyn, V. Nadzhemi, and A. A. Klesov, Prikl. Biokhim. Mikrobiol., 17, No. 3, 315 (1981).
10. M. M. Vorob'ev, E. A. Paskonova, S. V. Vitt, and V. M. Belikov, Biotekhnologiya, 4, 40 (1986).
11. G. A. Piyakina and T. S. Yunosov, Khim. Prir. Soedin., 359 (1986).
12. R. F. Itzhaki and D. M. Gill, Anal. Biochem., 9, 401 (1964).
13. A. N. Gurov, M. A. Muchin, N. Larichev, and N. V. Lozinskaya, Colloids and Surfaces, No. 6, 35 (1983).
14. H. R. Maurer, Disk-Elektrophorese, Theorie und Praxis, Walter de Gruyter, Berlin (1968).

ISOLATION, PURIFICATION, AND SOME PHYSICOCHEMICAL PROPERTIES OF GLUCOSE ISOMERASE FROM Streptomyces atratus

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A highly purified glucose isomerase with a specific activity 23.5 times greater than that of a homogenate of the mycelium has been obtained from Streptomyces atratus by methods of homogenization from the freeze-dried biomass, heat treatment (70°C, 10 min), ion-exchange chromatography, and gel filtration. The yield of enzyme on the initial biomass was 51.8%. The molecular mass of the enzyme has been determined by various methods as 160 kDa but in the presence of sodium dodecyl sulfate in thin-layer PAG it amounts to 40 kDa.

Glucose isomerase [xylose isomerase; D-xylose ketol-isomerase (EC 5.3.1.5)] – an enzyme catalyzing the isomerization of certain aldosugars and ketosugars, is attacting the attention of many researchers by the possibility of its use for the industrial production of glucose-fructose syrups and of crystalline fructose [1-3]. Among the numerous microorganisms of various taxonomic groups described in the literature as producers of glucose isomerases with a high activity and productivity, the streptomycetes stand out [4]. Glucose isomerases have been obtained in a highly purified state from various species of streptomycetes by very simple methods of purification [5-7]. The aim of the present work was to obtain a homogeneous glucose isomerase from a culture of Str. atratus, Uz GIT-1, and to study its physicochemical properties.

The glucose isomerase from Str. atratus is an intracellular enzyme localized in the periplasmic space and in the cell wall of the producing organism [8] and various approaches have therefore been used for its isolation (both with and without the disruption of the cells). The most effective method of isolating the enzyme proved to be homogenization from the freeze-dried biomass. We used this method in subsequent work. A homogenate of the mycelium was centrifuged at 8000g for 30 min, and then the precipitate was removed. The cell-free buffer extract obtained in this way did not contain a large amount of ballast proteins. Since glucose isomerase possesses thermal stability, the following stage of purification was heat treatment at 70°C for 10 min in the presence of Mg^{2+} and Co^{2+} ions. The product was freed from denatured proteins by centrifugation at 6000g for 10 min. After this, the enzyme solution was deposited on a column containing DEAE-cellulose that had previously been equilibrated with 0.05 M phosphate buffer, pH 7.8. 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/h (Fig. 1). This concentration of sodium chloride was established after the preliminary performance of stepwise and linear gradient elution. The fractions with enzymatic activity were collected and were concentrated in a dialysis bag against polyethyleneglycol at 4°C for 5 h. The concen-

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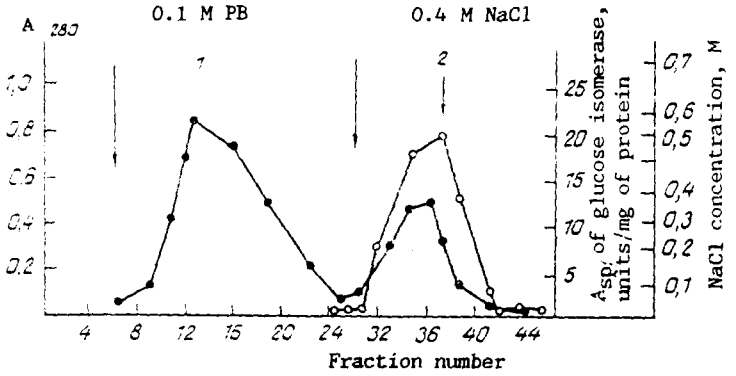

Fig. 1. Ion-exchange chromatography on DEAE-cellulose (column 4 × 20 cm; rate 60 ml/h; elution with 0.1 M phosphate buffer with a NaCl gradient from 0 to 0.7 M); 1) protein — extinction at 280 nm; 2) fractions with enzymatic activity.

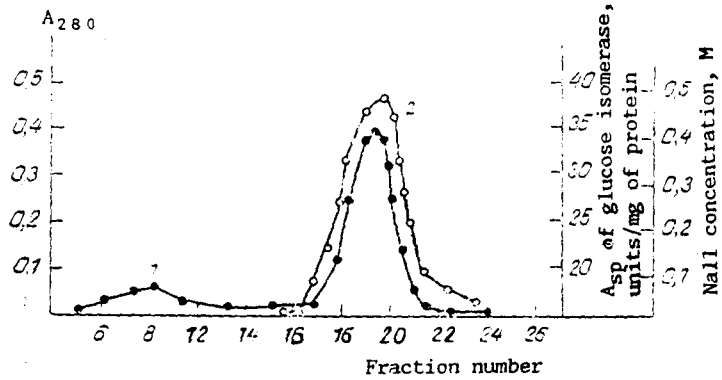

Fig. 2. Rechromatography of the glucose isomerase on a column of DEAE-Sephadex A-50 (column 2 × 25 cm; rate 30 ml/h; elution was carried out by the same method as from DEAE-cellulose).

trate obtained was desalted by passage through a column of Sephadex G-25. The desalted protein fractions were combined and subjected again to ion-exchange chromatography on DEAE-Sephadex A-50. This was washed and the glucose isomerase was eluted by the same method as from the DEAE-cellulose (Fig. 2). The last stage of purification was gel chromatography on a column of Sephadex G-100 (Fig. 3).

Thus, glucose isomerase has been obtained from Streptomyces atratus and a 23.5-fold purification of the enzyme has been achieved, the activity yield amounting to 51.8% (Table 1).

The homogeneity of the purified enzyme was checked by the method of analytical ultracentrifugation and by thin-layer electrophoresis in PAG.

The molecular mass of the enzyme was determined by the use of the methods of sedimentation, gel chromatography, and PAG electrophoresis.

It was established by gel filtration that the glucose isomerase from Str. atratus had a molecular mass of 160 kDa, while electrophoresis in a thin layer PAG containing sodium dodecyl sulfate (DDS—Na) gave a figure of 40 kDa. Such results give grounds for assuming that this enzyme has a subunit structure and apparently consists of four subnits, but this hypothesis requires additional confirmation.

The isoelectric point of the homogeneous enzyme obtained, determined by the method of isoelectric focusing on Ampholines, was 5.2.

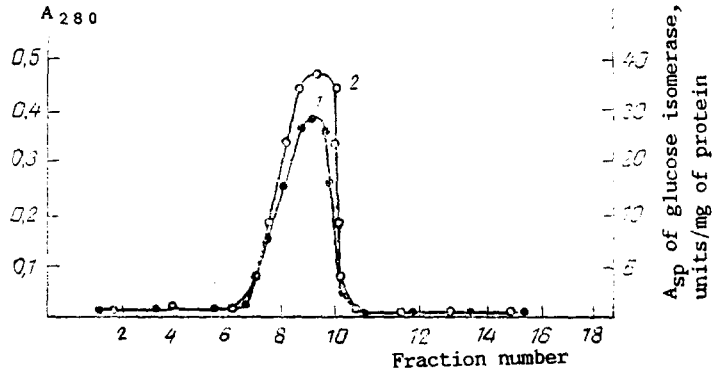

Fig. 3. Gel chromatography of the enzyme fractions obtained after rechromatography on DEAE-Sephadex A-50 (column 2 × 85 cm; rate 36 ml/h).

TABLE 1. Activities of Glucose Isomerase and Protein Concentrations during the Purification of the Enzyme from Streptomyces atratus

Stage of purification	Amount of protein, mg	Activity		Total	Specific
		total	specific		
Homogenate from the freeze-dried biomass	190	336,6	1,7	1	100
Heat treatment at 70°C for 10 min	20,8	260,8	12,5	7	77,5
Ion-exchange chromatography on DEAE-cellulose	9,22	216,8	23,5	13,8	64,5
Ion-exchange chromatography on DEAE-Sephadex A-50	5	192,2	38,4	21,7	57,1
Gel filtration on Sephadex G-100	4,2	174,3	41,5	23,5	51, 8

EXPERIMENTAL

The activity of the glucose isomerase was determined in a reaction mixture containing 1 ml of a 1 M solution of glucose as substrate, 2.5 ml of 0.2 M K, Na phosphate buffer with pH 7.8, 0.5 ml of a 0.1 M solution of $MgSO_4 \cdot 7H_2O$, and 0.5-1 ml of a solution of the enzyme (the volume of the reaction mixture was made up to 10 ml with distilled water). After incubation at 70°C for an hour, the amount of fructose was determined by the cysteine/carbazole method [9]. The amount of fructose in the reaction mixture was judged from the correlation formed. Measurement from the absorption of the colored solution at a wavelength of 560 nm was carried out on a SF-26 spectrophotometer. As the unit of activity of the glucose isomerase we took the amount of enzyme in the presence of which 1 mmole of fructose was formed in the reaction mixture at 60°C.*

Protein was determined by Bradford's method [10] and also in the SF-26 spectrophotometer at wavelengths of 260 and 280 nm. Bovine serum albumin (Reneal) was used as standard.

The molecular mass of the enzyme was determined by gel filtration in the FPLC system (Pharmacia). The standards used were lactalbumin (14 kDa), trypsin inhibitor (20 kDa), ovalbumin (45 kDa), albumin (67 kDa), phosphorylase B (94 kDa), alcohol dehydrogenase (140 kDa), and fructofuranosidase (210 kDa).

Electrophoresis was conducted in a gradient (10-15%) of polyacrylamide gel in the presence of 0.1% of DDS–Na in a thin layer. The gels were stained with 0.05% Coomassie Brilliant Blue in 10% acetic acid containing 45% of ethanol. The molecular masses of the proteins detected were calculated from a calibration graph of the dependence of the relative electrophoretic mobility on the molecular mass of standard proteins that had been plotted.

Isoelectric focusing was carried out by Vesterberg and Svensson's method [12]. A column with a capacity of 110 ml (LKB) was used for fractionation.

*No time is specified – Translator.

To create a density gradient we used sucrose, and, to a create a pH gradient, ampholite supports with a pH range of 3-5 (LKB). A density gradient of sucrose was created by mixing a light solution containing water and Ampholines and a heavy solution containing water, sucrose, and Ampholines. The sample that was to be subjected to isoelectric focusing was added to the light component.

On the use of an analytical column, the heavy electrode solution contained distilled water (14 ml) and sucrose (12 mg). The anode was located at the bottom of the column. Electrofocusing was carried out for 48 h with a voltage at the beginning of the experiment of 500 V and at the end of the experiment one of 1200 V and current strengths of 5 and 2 mA, respectively.

The sedimentation analysis of the enzyme was carried out on a MOM-3170 analytical ultracentrifuge. When a solution of the protein with a concentration of 7 mg/ml in 0.05 M sodium phosphate buffer, pH 7.8, was sedimented at 56,000 rpm with an exposure interval of 10 min one symmetrical schlieren peak was observed.

LITERATURE CITED

1. T. C. Gams, Stärke, 28, 344 (1976).
2. E. G. Polishchuk and L. A. Nakhapetyan, Biotekhnologiya, No. 5, 79 (1986).
3. P. I. Wang and B. F. Jonson, Biotechnology Lett., 2, 273 (1980).
4. I. V. Ulezlo, A. V. Ananichev, and A. M. Bezborodov, Usp. Biol. Khim., 27, 136 (1986).
5. R. A. Johnson, R. L. Antrim, and N. E. Lloyd, US Patent 4,610,965 (1984).
6. B. Kralova, K. Demnerova, and L. Skrivan, Biotechnology Lett., 7, 893 (1985).
7. H. S. Pawar, K. Kamnan, and M. S. Srivasan, Biochem. Biophys. Res. Commun., 155, 411 (1988).
8. I. G. Sultanova, Z. R. Akhmedova, and K. D. Davranov, Uzb. Biol. Zh., No. 1, 11 (1989).
9. Z. Dische and E. Borenfreund, J. Biol. Chem., 192, 583 (1951).
10. M. Bradford, Anal. Biochem., 72, 248 (1976).
11. O. Vesterberg and A. Svensson, Acta Chem. Scand., 20, 820 (1966).

COMBINED IMMOBILIZATION OF LYTIC AND PROTEOLYTIC ENZYMES

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Lytic enzymes immobilized together with proteinases (lytic enzymes complex of Act. recifensis var. liticus 2435 and lysozyme) with retention of 80-150% of the lytic activity have been obtained. The properties of the preparations obtained have been studied.

The broad possibilities of the use of lytic enzymes in medicine are based on their direct action on microorganisms, and also on their immunostimulating, antiphlogistic, antiallergic, analgesic, and thrombocytopoesis-stimulating actions [1, 2]. However, the instability of the enzyme preparations and their high cost have led to a search for methods of immobilizing them [3-7]. In view of the fact that lytic enzymes could supplement the spectrum of the necrolytic action of proteinases immobilized on dressing material, we have carried out their co-immobilization.

Earlier, in the immobilization of lysozyme on a polyacrylamide support and on derivatives of Silokhrom, the percentage retention of lytic activity determined from the lysis of

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