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CHARACTERISTICS OF THE GLUCOSE ISOMERASE FROM Streptomyces atratus

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The molecular mass (Mr) of highly purified glucose isomerase from Streptomyces atratus has been determined. The native glucose isomerase, which has affinity for xylose, consists of an oligomeric protein containing 1552 amino acid residues and formed of four subunits. The enzyme is stable in the range of temperatures from 40 to 80°C and at pH values of from 6.0 to 11.0.

It is known DS-Na is capable of causing the dissociation of many proteins [1-5]. To achieve complete dissociation, the enzyme was treated with 1% DS-Na containing 0.1% of 2-mercaptoethanol. The dissociation products formed under these conditions were separated by electrophoresis in PAAG containing DS-Na, and also by the method of sedimentation equilibrium in an ultracentrifuge.

Since in the present of detergents the molecular mass of the enzyme decreases by a factor of approximately four, it was assumed that the St. atratus glucose isomerase consists of four subunits. The molecular mass of the St. atratus glucose isomerase with DS-Na, determined by the sedimentation equilibrium method was 43 kDa, and its sedimentation constant was 4.8S.

When the enzyme under investigation was treated with 6 M and 8 M urea, a marked decrease in enzymatic activity was observed. The molecular mass of this enzyme, determined by thin-layer electrophoresis in PAAG in the present of 6 M urea, was likewise 40 kDa.

A comparison of the amino acid composition of the St. atratus glucose isomerase with those of enzymes isolated from other sources showed similarity with respect to the number of residues of hydrophobic and acidic amino acids [7-9]. Table 1 shows the amino acid composition of the St. atratus enzyme. It can be seen that, in this enzyme, residues of acidic amino acids (glutamic and aspartic acids) and of hydrophobic amino acids (alanine and leucine) predominate over those of basic amino acids (arginine and lysine). The assumption exists that the predominance of hydrophobic over hydrophilic amino acids will lead to a

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TABLE 1. Amino Acid Composition of the Glucoside Isomerase from Streptomyces atratus

Amino acid	Number of residues in the protein molecule after hydrolysis: 24	48	72	Number of residues in the molecule,
Lysine	39,8	39,0	40	40
Histidine	103,7	104,2	104	104
Arginine	79,8	80,1	80,2	80
Aspartic acid	188,3	188,0	188,1	188
Threonine	47,7	47,5	46,9	48
Serine	35,5	35,3	34,5	36
Glutamic acid	216,1	216	215,8	216
Proline	63,9	64	63,7	64
Glycine	208,1	208,3	208,3	208
Alanine	196,3	196,1	196,3	196
Valine	52,1	52,2	52	52
Methionine	11,5	11,7	11,9	12
Isoleucine	31,9	32,1	31,5	32
Leucine	128,0	128,2	127,5	128
Tyrosine	27,7	27,5	27,7	28
Phenylalanine	80,3	80,5	80,2	80
Tryptophan				20
Total				1552

TABLE 2. Substrate Specificity of the Glucose Isomerase

Substrate	Degree of formation of ketoses	Substrate	Degree of formation of ketoses
D-Xylose	+++	D-Ribose	+
L-Xylose	—	L-Ribose	—
D-Glucose	++	D-Lactose	—
D-Mannose	—	A-Arabinose	+
D-Galactose	—	L-Arabinose	—

hydrophobic interaction between various sections of the enzyme molecule [6, 11]. Like many glucose isomerases from various microorganisms, an absence of cysteine from the amino acid composition is characteristic for the St. atratus enzyme [1, 12].

It is known that the majority of glucose isomerases from the genus Streptomyces are capable of isomerizing several aldosugars having 5 or 6 carbon atoms [13]. The St. atratus glucose isomerase also exhibits activity in the isomerization of the D-xylose, D-glucose, D-ribose, D-arabinose, and D-rhamnose into their corresponding ketoses (Table 2). Figure 1 shows results on the influence of various concentrations of D-xylose and D-glucose on the rate of the enzymatic reaction. The values of K_M for D-xylose and D-glucose were determined from the Lineweaver–Burk graph and amounted to 0.05 and 0.14 M, respectively. It can be seen from these values that this enzyme exhibits its highest affinity in relation to D-xylose.

The influence of the pH on the stability and activity of the St. atratus glucose isomerase was determined in the pH range from 5.7 to 13.0 using appropriate buffers. The purified enzyme was found to be active from pH 7.0 onward, and its optimum glucose isomerase activity was observed in the pH range from 8.0 to 9.5 (Fig. 2). To determine its pH-stability, the St. atratus glucose isomerase was first incubated at 35°C in buffer solutions with various pH values for 15 min. The residual activities were determined at pH 8.0. As can be seen from Fig. 2a, the enzyme was stable at pH values of from 6.0 to 11.

The optimum temperature of the St. atratus glucose isomerase was determined under the usual conditions at temperatures of from 40 to 100°C. It was found that the temperature optimum for the action of this glucose isomerase was 75-80°C (Fig. 2b). In order to determine thermal stability, a 5% solution of the enzyme in 0.005 M phosphate buffer (pH 8.0) was also incubated at various temperatures (from 40 to 100°C) and its residual activity was determined at 75°C. On the basis of these experiments it can be stated that the glucose isomerase from St. atratus is stable in the range of temperatures from 40 to 80°C, and a further rise in the temperature leads to the denaturation of the enzyme (Fig. 2b).

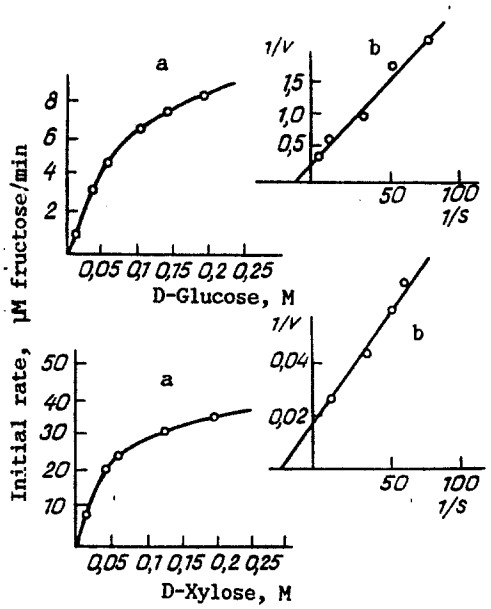

Fig. 1. Dependence of the rate of the enzymatic reaction on the concentration of the substrate: a) graph in the Michaelis–Menten coordinates; b) graph in the Lineweaver–Burk coordinates.

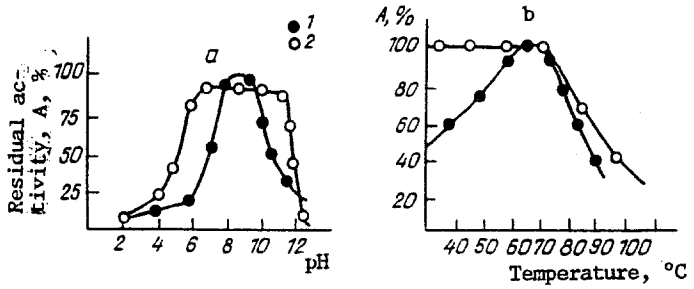

Fig. 2. Effect of the pH (a) and the temperature (b) on the activity and stability of the St. atratus glucose isomerase: a) [E] = 0.05 mg/ml; [S] = 0.1 M; 1) pH optimum; 2) pH-stability; b) pH = 8.0, [E] = 0.05 mg/ml, [S] = 0.1 M; 1) temperature optimum; 2) thermal stability.

Thus, one of the main features of this enzyme is its high thermal stability. This unique feature of glucose isomerases makes them valuable materials for elucidating the mechanisms of thermal stability and the causes of the denaturation of proteins. The temperature optimum of the growth of the producing agents is 30 to 45°C, while the glucose isomerases obtained from them have enzymatic activity mainly in the range from 70 to 90°C, and this at high pH values [14]. This suggests the presence of additional stabilizing factors in the reaction medium. It is known that the presence of the substrate has a great influence on the thermal stability of various enzymes. This is apparently due to the fact that on binding with the enzyme and forming an enzyme-substrate complex, it stabilizes the secondary and tertiary structures of the enzyme, making it more resistant to any unfavorable actions [15].

Many other reasons for thermal stability exist, one of which is the amino composition of an enzyme. As has been noted above, hydrophobic amino acid residues predominate in the molecules of glucose isomerases, and these apparently give rise to numerous hydrophobic interactions within the enzyme molecules. In actual fact, it is known from literature sources that the noncovalent bonds through which the second and tertiary structures of a protein are formed also determine the stability of the enzyme, while disulfide bonds are not capable of exhibiting such properties [16]. The low content of tryptophan and methionine and the absence of cysteine in the St. atratus glucose isomerase apparently form another example of the inability of these amino acids to form covalent bonds [6].

The stability at high temperatures of the glucose isomerases from various microorganisms is possibly connected with the presence of a large number of acidic amino acid residues. Reports exist according to which, probably, the carboxy group, unlike a group possessing basic properties, considerably enhances the thermal stability of proteins [16].

EXPERIMENTAL

The conditions for the growth of the St. atratus culture and the methods of isolating and purifying the glucose isomerase from this culture and the determination of some of its physicochemical properties and enzymatic activity have been described previously [10].

Dissociation in DS-Na. Before being deposited on a PAAG plate, the sample and marker proteins were dissociated in DS-Na solution. For this purpose, dry samples of the glucose isomerase and of the marker proteins were dissolved in 0.1 M electrode buffer (Tris-glycine buffer), pH 7.8, containing a 1% solution of DS-Na and the mixture was incubated at 100°C for 3 min. A 0.1% solution of DS-Na was added to the electrode buffer intended for the performance of electrophoresis.

Dissociation in Urea. The enzymes and marker proteins were incubated in 6 M urea in 0.01 M Tris-HCl buffer (pH 7.8) at 4°C for 30 min. The urea-modified preparations so formed were passed through PAAG containing 0.01 M urea on a plate.

Electrophoresis in 7.5% PAAG on a plate and sedimentation analysis were conducted as described in [10].

Amino Acid Analysis. To determine its amino acid composition, a weighed sample of the homogeneous enzyme was dissolved in 5-8 ml of 6 N hydrochloric acid. Hydrolysis was carried out in a vacuum-sealed tube at 110°C for 24, 48, and 72 h. The hydrolysate was evaporated and, after the addition of a fresh portion of water, it was dried in a vacuum desiccator over alkali. Amino acid analysis was performed on a AAA-881 amino acid analyzer (Prague).

The number of tryptophan residues was determined spectrophotometrically [18].

Substrate Specificity. The ketosugars obtained as the result of the isomerization of aldosugars were identified by the cysteine-carbazole method [19] and by paper chromatography. Before chromatography, mixtures each with the following composition were prepared: 0.1 ml of the enzyme solution and 0.1 ml of a 1% solution of one of various sugars. Each mixture was incubated at 70°C for 5 h and was then deposited on chromatographic paper (Whatman No. 1) and was separated in the butanol–pyridine–water (6:4:3) system. Sugars were revealed by using the naphthoresorcinol reagent.

As the substrates we used D-xylose, L-xylose, D-glucose, D-ribose, D-arabinose, L-arabinose, L-ribose, D-mannose, D-galactose, and D-lactose. To determine K_M values, enzymatic activity was determined by the usual method with a variation in the concentration of substrate from 0.001 to 3 M. The K_M values were determined from Lineweaver–Burk graphs.

Determination of the pH Optimum and of the pH Stability. To determine the pH optimum and stability of the St. atratus glucose isomerase we used the following buffers: 0.2 M acetate buffer in the pH range from 3 to 5; 0.2 M Na, K phosphate buffer, pH from 6 to 8; 0.2 M glycine–NaCl–NaOH buffer, pH from 8.5 to 10.0; and 0.2 M Na_2CO_3 + NaOH buffer, pH 11 to 12.5. The pH-stability of the enzyme was determined from the residual activity under the usual conditions at pH 8.0 after its preliminary incubation in the appropriate buffers at 35°C for 30 min.

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INFLUENCE OF METAL IONS ON THE ACTIVITY AND STABILITY OF THE GLUCOSE ISOMERASE FROM Streptomyces atratus

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The influence of a number of bivalent metals on the activity of Streptomyces atratus glucose isomerase has been studied. Mg^{2+} ions are activators and Co^{2+} ions are stabilizers of the glucose isomerase activity. The effective kinetic parameters for the action of the enzyme have been determined.

It is known that the activity of glucose isomerases depends on the presence in the reaction mixture of several metal cations that are cofactors of the enzyme. In the main, the maximum activity of glucose isomerase is shown in the presence of Co^{2+} and Mg^{2+} ions [1]. However, so far as concerns the glucose isomerase of Lactobacillus brevis 74, the greatest activating action is exerted by the simultaneous presence of cobalt and chromium ions [2]. For the majority of glucose isomerases from various sources the main activators are Mg^{2+} ions, while Co^{2+} ions play the role of stabilizers [3-6]. It is known from the same sources that the maximum activating effect is observed under the simultaneous action of Mg^{2+} and Co^{2+} ions. Values of K_M for Mg^{2+} and Co^{2+} ions have been calculated for glucose isomerases from various microorganisms [1].

The aim of the present paper is to describe results relating to the influence of metal ions on the enzymatic activity of Str. atratus glucose isomerase.

The presence of Mg^{2+} ions and, to a smaller degree, Co^{2+} ions is necessary for the performance of the isomerism of aldosugars into ketosugars with the participation of the Str. atratus glucose isomerase. Elimination of metal ions from the enzyme solution by dialysis against a buffer containing 0.01 M EDTA led to the complete disappearance of enzymatic activity.

On the performance of similar studies with the glucose isomerase of L. brevis 74, Toluelova et al. [2] established that the enzyme was weakly bound by a metal and, consequently, no covalent bonds exist between protein and metal.

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