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Studies on Glucose Isomerase from a *Streptomyces* Species

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Production and properties of glucose isomerase from a Co^{2+}-sensitive *Streptomyces* species were studied. After 4 days of shaking cultivation at 30°C and 200 rpm, a maximum of 1.1 enzyme units per ml of broth was obtained. Cell-free glucose isomerase, obtained from mycelia heat-treated in the presence of 0.5 mM Co^{2+}, showed a 3.5-fold increase in specific activity over enzyme obtained from untreated mycelia. The optimum pH and temperature for the glucose isomerase were 7 to 8 and 80°C, respectively. The Michaelis constant for fructose was 0.40 M. Mg^{2+} was found to enhance the glucose isomerase activity, whereas the effect of Co^{2+} on enzyme activity depended on the manner in which the enzyme was prepared. This glucose isomerase was quite heat stable, with a half-life of 120 h at 70°C.

Glucose isomerase is a commercially important enzyme used in the United States to manufacture billions of pounds of high-fructose corn syrups each year at low cost (8). These syrups are used in consumer products such as carbonated beverages and baked goods (2).

This enzyme has been the subject of interested study ever since Marshall and Kooi (6) reported its potential as a catalyst in the formation of D-fructose from D-glucose. After the discovery in 1961 of a glucose isomerase from *Aerobactor cloacae* (12), other microbial sources for the enzyme, including *Lactobacillus brevis* (15), *Bacillus coagulans* (3), and several *Streptomyces* species (4, 9-11, 13, 14), have been described. It was reported for *Streptomyces* species that addition of Co^{2+} into the culture medium was required to stimulate the formation of glucose isomerase (4, 9, 11, 14). In this paper we report a *Streptomyces* species that can be cultivated in the absence of Co^{2+} with high production of glucose isomerase and whose growth is inhibited in the presence of Co^{2+}. In addition, some properties of the glucose isomerase from this organism are described in this presentation.

MATERIALS AND METHODS

Culture. An unidentified *Streptomyces* species was isolated and maintained on nutrient agar slants (Difco) at 4°C. Stock cultures were transferred twice on nutrient agar in an interval of 4 to 5 days before use. Seed culture was inoculated from a slant into a 250-ml Erlenmeyer flask containing 50 ml of culture medium of 1% tryptone (Difco), 0.7% yeast extract (Difco), 1% xylose, and 0.1% $MgSO_4 \cdot 7H_2O$ (pH 7.0 to 7.2) and grown at 30°C for 24 to 30 h. Main cultures were started from 2-ml portions of seed culture inoculated into 500-ml Erlenmeyer flasks, each of which contained 100 ml of culture medium. The cultures were then incubated at 30°C in a controlled-environment incubator shaker (New Brunswick Scientific Co., Inc., New Brunswick, N.J.) at a speed of 200 rpm for ca. 4 days.

Enzyme preparations. Mycelium pellets cultivated during incubation were collected by centrifugation, washed several times with deionized water, and homogenized in a Waring blender. Cell-free enzyme was obtained by disrupting the resulting cell suspension. This was done either by sonic oscillation on ice for 30 min with a Branson sonifier at 8 kc (Branson Instruments, Inc., model S-125, Stamford, Conn.) or at 20,000 lb/in^2 pressure at room temperature with a French pressure cell press (American Instrument Co., Silver Spring, Md.). This step was followed by centrifugation at 12,000 × *g* and 4°C for 15 min to remove cell debris.

Enzyme assay. Glucose isomerase catalyzes the formation of fructose from glucose and vice versa. Results of preliminary studies in our laboratory showed the reaction to be highly reversible. We found that the forward reaction proceeds at 1.1 times the backward rate for the enzyme studied by us. Thus, we used the backward reaction (fructose to glucose) as a measure of isomerase activity, this choice being dictated by the availability of a glucose analyzer (Beckman Instruments, Fullerton, Calif.), which greatly simplified the enzyme assay procedure. We believe that assaying the formation of glucose from fructose is a valid measure of glucose isomerase activity, even though the reaction of practical interest is the conversion of fructose from glucose.

Glucose isomerase activity was determined by an initial rate assay at 70°C. A 1.0-ml portion of appropriately diluted glucose isomerase was mixed with 1 ml of assay solution containing 200 mM fructose, 10 mM $MgSO_4 \cdot 7H_2O$, 1 mM $CoCl_2 \cdot 6H_2O$, and 100 mM potassium phosphate buffer (pH 7.0) and incubated

at 70°C for a period of 30 min to 1 h. The glucose formed was measured in the glucose analyzer (1). Assay conditions were selected to ensure that the enzyme activity was a linear function of both time and enzyme concentration. One unit of isomerase activity is the quantity of enzyme that catalyzes the formation of 1 μmol of glucose per min under the conditions described above.

Protein determination. Protein was determined by the method of Lowry et al. (5) using bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) as a standard.

RESULTS AND DISCUSSION

Production of glucose isomerase from *Streptomyces* species has been documented by several investigators (4, 9–11, 13, 14). One condition found necessary to activate the formation of glucose isomerase was the addition of 0.024% Co^{2+} to the culture medium (4, 9, 11, 14). The *Streptomyces* species studied by us, however, exhibited a somewhat different behavior; 0.024% Co^{2+} caused strong inhibition, especially in the early stages of propagation of mycelium and the production of glucose isomerase, whereas the absence of Co^{2+} gave the best results (Fig. 1). A maximum enzyme activity of 1.1 U/ml of broth was obtained after 96 h of cultivation in media containing no Co^{2+}.

Heat treatment of homogenized mycelia in the presence of 0.5 mM Co^{2+} at 70°C for 30 min was found to be a simple and effective purification technique for glucose isomerase. Activities measured for cell-free enzyme obtained from untreated cells and from cells treated in the presence and absence of Co^{2+} are presented in Table 1. Cell-free enzyme obtained from cells heat-treated in the presence of Co^{2+} showed a 3.5-fold increase in specific activity over the untreated preparations. The increase in specific activity indicated that substantial quantities of non-isomerase proteins were denatured during heat treatment. The results also showed that Co^{2+} exerted a protective effect on glucose isomerase activity, apparently by enhancing the heat stability of the enzyme, an effect also noted by Tsumura and Sato (13).

The effect of the sequence of sonification and heat treatment on enzyme preparations was studied. As shown in Table 2, heat treatment of the disrupted-cell suspension resulted in very little increase in enzyme specific activity. On the other hand, a marked increase in enzyme activity was noted when the cell suspension was first heat-treated and then sonified. This clearly indicated that the cells must be heat-treated before disruption to obtain a pro-

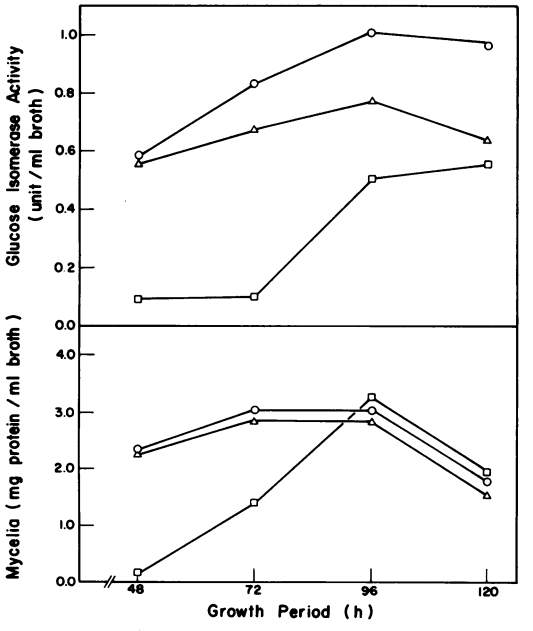

FIG. 1. *Effect of Co^{2+} on the time course of glucose isomerase production and mycelium propagation. No Co^{2+} (○); 0.012% Co^{2+} (△); 0.024% Co^{2+} (□). Activity was determined using a homogenized mycelium suspension.*

TABLE 1. *Effect of heat treatment[a] and Co^{2+} on enzyme preparations*

Treatments on mycelium suspension	Cell-free enzyme		
	Activity (U/ml)	Protein (mg/ml)	Sp act (U/mg of protein)
None	0.40	0.90	0.45
Heat treatment in absence of Co^{2+}	0.18	0.45	0.40
Heat treatment in presence of 0.5 mM Co^{2+}	0.74	0.50	1.45

[a] Heated at 70°C for 30 min.

TABLE 2. *Effect of the sequence of sonification and heat treatment[a] on enzyme preparation*

Prepn	Sp act (U/mg of protein)	
	Sonification, then heat treatment	Heat treatment, then sonification
Disrupted-cell suspension before centrifugation	0.21	0.31
Centrifuged fractions		
Supernatant	0.29	1.45
Precipitate	Negligible	0.003

[a] Heated at 70°C in presence of 0.5 mM Co^{2+} for 30 min.

nounced purification effect. In addition, the data also showed that the precipitates obtained from centrifugation of the heat-treated, disrupted cells contained relatively little enzyme activity.

In laboratory work, disruption of homogenized mycelia was generally accomplished through sonic oscillation. Since this method did not seem practical for large-scale industrial application, an alternate method involving disruption of the cells with a French pressure cell press was examined. As shown in Table 3, both sonic oscillation and pressure treatment resulted in quantitative release of isomerase activity from mycelium suspension. The apparent yields of glucose isomerase released from cells by both treatments were greater than 100%. This may be attributed to the diffusion resistance of the cell membrane of whole cells to substrate and products. It was also interesting to note that a higher yield of enzyme activity was obtained with pressure treatment. These results suggest that the application of a French pressure cell press for large-scale disruption of *Streptomyces* mycelia to obtain a cell-free glucose isomerase preparation is indeed practical.

Figure 2 shows the effect of temperature on glucose isomerase activity. The optimum temperature was found to be 80°C, which is in agreement with reports of other investigators (4, 7, 9, 11, 14). As shown in Fig. 2, the enzyme activity at 80°C is two times that at 70°C. However, degradation of keto-sugars, as evidenced by pronounced discoloration of an aqueous sugar solution, occurs at the higher temperature. Therefore, the temperature of 70°C was used to examine other properties of the enzyme.

When glucose isomerase activity was examined in the pH range of 5.5 to 8.0 using potassium phosphate buffer, a sharp increase in enzyme activity occurred between pH 6.5 and 7.0, as shown in Fig. 3. The optimum pH appears to be between pH 7 and 8, a result similar to that reported by Strandberg and Smiley (9), Taka-

TABLE 3. *Release of glucose isomerase from Streptomyces cells by sonification and pressure treatment*

Enzyme source	Yield (%)[a]	U/10 ml
Whole-cell suspension	100.0	6.3
Sonified		
Supernatant	109.0	6.9
Precipitate	2.7	0.17
Total	111.7	7.2
Pressure treated		
Supernatant	112.0	7.07
Precipitate	15.7	0.99
Total	127.7	8.06

[a] Activity obtained from whole-cell suspension was considered to be 100%.

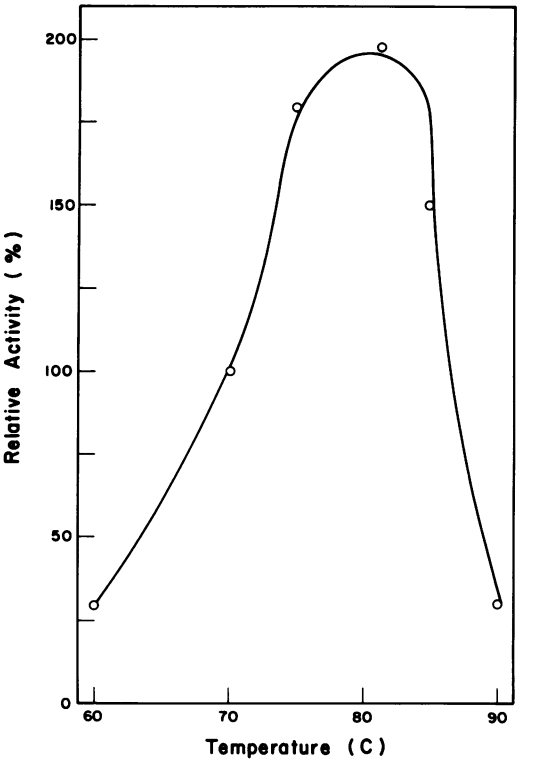

FIG. 2. *Effect of temperature on soluble glucose isomerase activity. At 70°C, 1.45 U per mg of protein corresponds to 100% relative activity.*

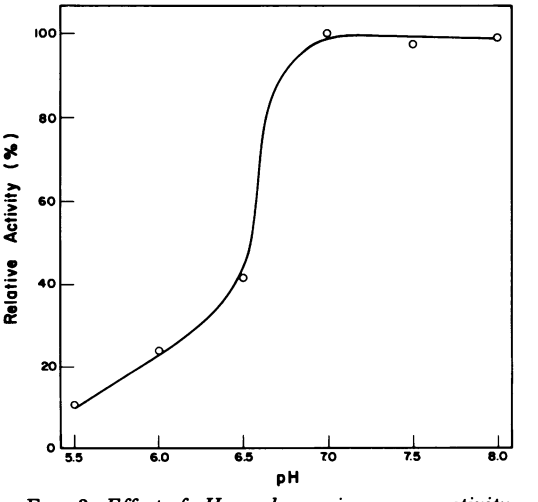

FIG. 3. *Effect of pH on glucose isomerase activity. Activity was determined using a homogenized mycelium suspension. One hundred percent relative activity corresponds to 0.6 U per mg of protein.*

saki et al. (11), and Sanchez and Smiley (7) for their *Streptomyces* species but lower than the pH 9.5 obtained by Tsumura and Sato (13) for *Streptomyces phaeochromogenes*.

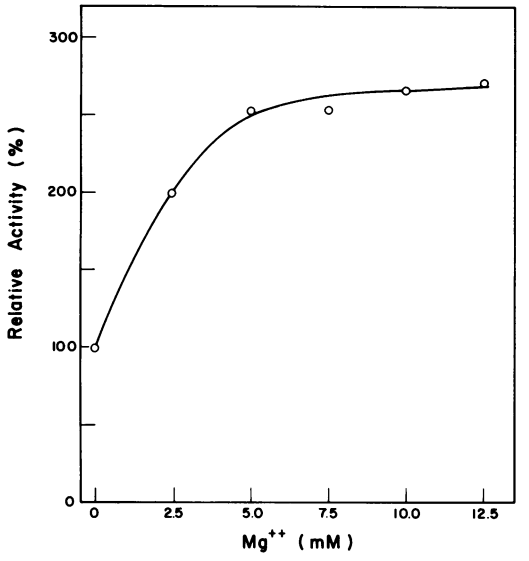

FIG. 4. *Effect of Mg^{2+} on soluble glucose isomerase activity at 70°C. Activity in the absence of Mg^{2+} was assigned a relative activity of 100% and corresponds to 1.45 U per mg of protein.*

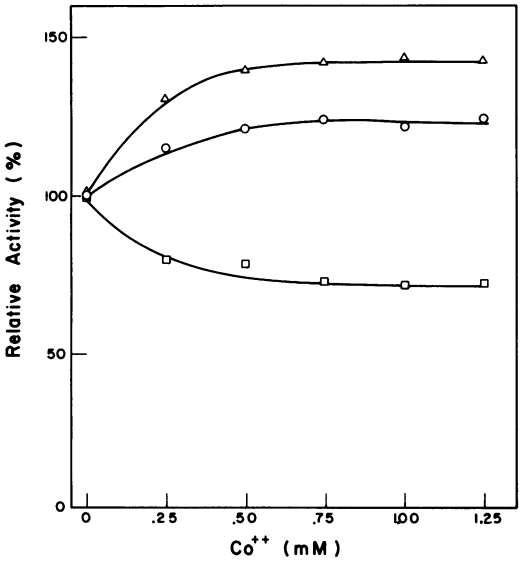

FIG. 5. *Effect of Co^{2+} on activity of several soluble glucose isomerase preparations. One hundred percent relative activity corresponds to 0.32 U per mg of protein for non-heat-treated enzyme (○), 0.32 U per mg of protein for enzyme heat-treated in the absence of Co^{2+} (△), and 2 U per mg for enzyme heat-treated in presence of Co^{2+} (□).*

Small quantities of Mg^{2+} and Co^{2+} present in the enzyme reaction mixture strongly affected the glucose isomerase activity, as shown in Fig. 4 and 5. A maximum of 1.5-fold enhancement of enzyme activity by Mg^{2+} was obtained at the 5 mM concentration level. Above this, no further marked change in enzyme activity was observed (Fig. 4).

The effect of Co^{2+} on cell-free glucose isomerase activity varied, depending on the manner in which the homogenized cells were treated

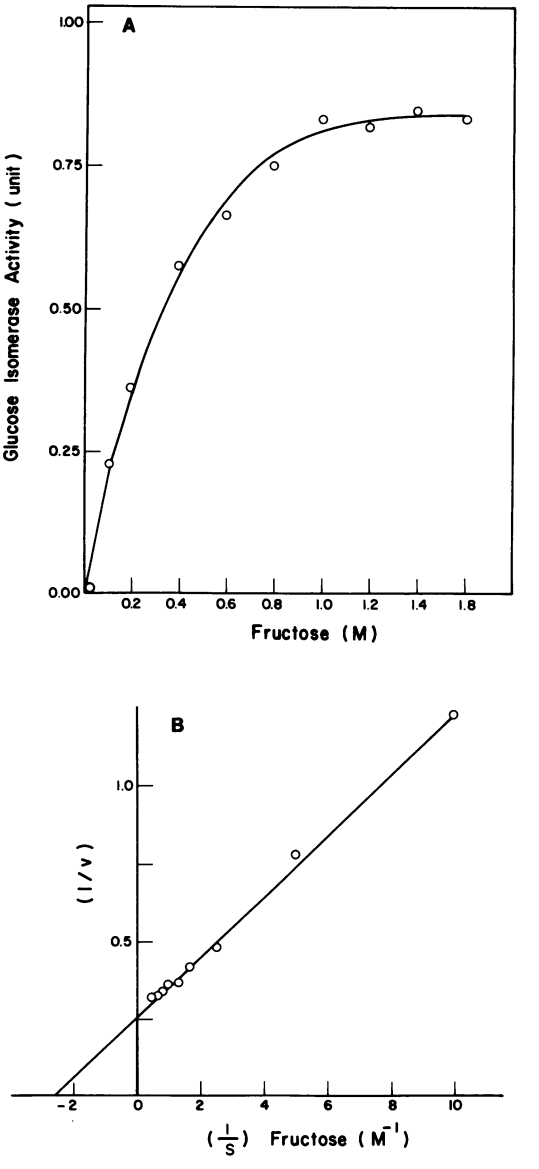

FIG. 6. *(A) Effect of fructose on soluble glucose isomerase activity. (B) Effect of fructose on soluble glucose isomerase activity (Lineweaver-Burk plot).*

before disruption. Co^{2+} had an inhibitory effect on activity for cell-free enzyme obtained from cells heat-treated in the presence of Co^{2+}. On the other hand, cell-free enzyme from cells heat-treated in the absence of Co^{2+} showed an increase in activity of over 40% in the presence of ca. 0.5 mM Co^{2+}. The enzyme from untreated cells also showed a maximum of 20% increase in activity under the same conditions. In all cases, no further effect on enzyme activity was evident above the 0.5 mM Co^{2+} level.

The effect of fructose concentration on glucose isomerase activity was studied in the presence of 0.6 mM Co^{2+}, 5 mM Mg^{2+}, 50 mM potassium phosphate buffer (pH 7.0), and various amounts of fructose in the incubation mixture. The results are shown in Fig. 6A. A replot of this data in the form of a Lineweaver-Burk plot is presented in Fig. 6B. The Michaelis constant for fructose extrapolated from this graph is 0.40 M.

The thermal stability of glucose isomerase was examined. Results of this study are shown in Fig. 7. A mixture of cell-free enzyme (1.2 mg of protein per ml), 5 mM $MgSO_4 \cdot 6H_2O$, 0.8 mM $CoCl_2 \cdot 6H_2O$, and 50 mM potassium phosphate buffer (pH 7.0) was incubated at 70°C. Periodically, this mixture was sampled and assayed for residual enzyme activity. As shown in Fig. 7, the enzyme showed a biphasic stability curve. The first phase occurs between 0 and 10 h, when a 20% drop in activity occurred, and the second phase occurred after 10 h; during this phase, the enzyme was relatively stable, with a half-life of approximately 120 h.

In view of the properties of glucose isomerase, the *Streptomyces* species studied by us seems quite similar to those reported by other investigators (9, 11, 14) with respect to metal requirements, temperature, and pH optima for enzyme activity. However, unlike others, the *Streptomyces* species studied by us can grow in the absence of Co^{2+} with a high yield of glucose isomerase and is actually inhibited by Co^{2+}. In addition, the glucose isomerase can be partially purified, with a 3.5-fold increase in specific activity, by simple heat treatment. These characteristics, along with the high thermal stability of the enzyme, make this organism attractive as a possible microbial source for glucose isomerase production in industry.

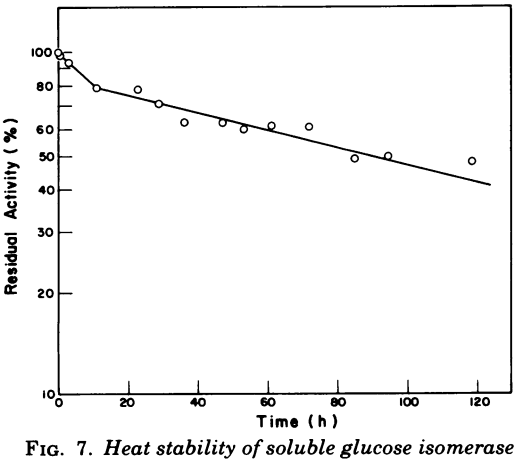

FIG. 7. *Heat stability of soluble glucose isomerase at 70°C.*

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