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Purification, Immobilization, and Some Properties of Glucose Isomerase from *Streptomyces flavogriseus*†

W. P. CHEN‡ AND A. W. ANDERSON*

Department of Microbiology, Oregon State University, Corvallis, Oregon 97331

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Glucose isomerase (EC 5.3.1.5) produced from *Streptomyces flavogriseus* was purified by fractionation with $(NH_4)_2SO_4$ and chromatography on diethylaminoethyl (DEAE)-cellulose and DEAE-Sephadex A-50 columns. The purified enzyme was homogeneous as shown by ultracentrifugation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Benzyl DEAE-cellulose, triethylaminoethyl-cellulose, and DEAE-cellulose were effective in the immobilization of partially purified glucose isomerase. Several differences in properties were found between purified soluble enzyme, immobilized enzyme (DEAE-cellulose-glucose isomerase), and heat-treated whole cells. Glucose and xylose served as substrate for the enzyme. Whole cells had the highest K_m values for glucose and xylose; the soluble enzyme had the lowest values. The optimum temperature for activity of the soluble and immobilized enzymes was 70°C; that for whole cells was 75°C. The pH optimum for the three enzyme preparations was 7.5. Magnesium ion or Co^{2+} was required for enzyme activity; an addition effect resulted from the presence of both Mg^{2+} and Co^{2+}. The enzyme activity was inhibited by Hg^{2+}, Ag^{+}, or Cu^{2+}. The conversion ratio of the enzyme for isomerization was about 50%. The soluble and immobilized enzymes showed a greater heat stability than whole cells. The soluble enzyme was stable over a slightly wider pH (5.0 to 9.0) range than the immobilized enzyme and whole cells (pH 5.5 to 9.0). The molecular weight of the enzyme determined by the sedimentation equilibrium method was 171,000. A tetrameric structure for the enzyme was also indicated. After operating at 70°C for 5 days, the remaining enzyme activity of the immobilized enzyme and whole cells, which were used for the continuous isomerization of glucose in a plug-flow type of column in the presence of Mg^{2+} and Co^{2+}, was 75 and 55%, respectively. Elimination of Co^{2+} decreased operational stability.

Glucose (or xylose) isomerase (EC 5.3.1.5) catalyzes the reversible isomerization of glucose to fructose (27). Almost every known glucose isomerase is an intracellular enzyme which also isomerizes xylose. The enzyme is used for the commercial production of high-fructose corn syrup (12).

Although numerous glucose isomerases have been produced from different microorganisms (2), only few of them have been purified to a homogeneous state (1, 8, 17, 24, 26, 31). On the other hand, enzymatic and physiochemical properties of glucose isomerases have been extensively studied (5, 6, 8, 17, 20, 21, 24, 27, 31). Glucose isomerase is usually prepared in the form of heat-treated whole cells or immobilized enzyme for the isomerization of glucose; these preparations are relatively stable and capable of being reused under a continuous operation (14, 18, 19, 22, 25; T. Sipos, German patent 2,061,371, January, 1971). A summary of production, purification, immobilization, and properties of glucose isomerases has been prepared (W. P. Chen, Ph. D. thesis, Oregon State University, Corvallis, 1979).

Chen et al. (3) reported the isolation and characterization of *Streptomyces flavogriseus*, which produced a high level of glucose isomerase when grown on straw hemicellulose or H_2SO_4 hydrolysate of straw. In this study, we report the purification, immobilization, and some properties of glucose isomerase produced from *S. flavogriseus*.

MATERIALS AND METHODS

Materials. Aminoethyl cellulose, benzyl diethylaminoethyl (DEAE)-cellulose, carboxymethyl-cellulose, DEAE-cellulose, ecteola cellulose, polyethyleneimine-cellulose, and triethylaminoethyl (TEAE)-cellulose were all purchased from Sigma Chemical Co.,

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‡ Present address: Taiwan Sugar Research Institute, Tainan, Taiwan.

St. Louis, Mo. Carboxymethyl-Sephadex, DEAE-Sephadex, and quaternary aminoethyl-Sephadex were obtained from Pharmacia Fine Chemicals Co., Uppsala, Sweden. Bovine serum albumin, carbonic anhydrase, lysozyme, ovalbumin, phosphorylase, soybean trypsin inhibitor, and sodium dodecyl sulfate (SDS) were purchased from Bio-Rad Laboratories, Richmond, Calif. Corn steep liquor was provided by Clinton Corn Processing Co., Clinton, Iowa. Acrylamide, *N,N'*-methylene bisacrylamide, *N,N,N',N'*-tetramethylenediamine, and ammonium persulfate were from Canalco Inc., Rockville, Md. Coomassie brilliant blue G-250 was from Eastman Kodak Co., Rochester, N.Y.

Microorganism, medium, and growth. A strain of *S. flavogriseus* isolated from soil by Chen et al. (3) was used. The organism was grown in 2-liter flasks maintained at 30°C on a rotary shaker in a medium containing 1% straw hemicellulose, 2.5% corn steep liquor, and 0.1% $MgSO_4 \cdot 7H_2O$. The pH of the medium was adjusted to 7.0 with 10 N NaOH. The preparation of straw hemicellulose was according to the method of Chen et al. (3).

Preparation of enzyme extract. The organism was grown in the medium at 30°C for 36 h, the culture was centrifuged out, and the cell mass (mycelium) was washed twice with distilled water. The cell mass was suspended in 0.05 M sodium phosphate buffer (pH 7.0) containing 0.1% cetyltrimethylammonium bromide (4). The enzyme was extracted for 2 h at 37°C with shaking. The suspension was centrifuged at 12,000 × *g* for 10 min. The supernatant was used as the enzyme extract.

Assay of enzyme activity. The enzyme reaction mixture contained 0.5 ml of 0.2 M sodium phosphate buffer (pH 7.0), 0.2 ml of 1 M glucose, 0.1 ml of 0.1 M $MgSO_4 \cdot 7H_2O$, 0.1 ml of 0.01 M $CoCl_2 \cdot 6H_2O$, and 0.2 ml of enzyme extract. The final volume of the enzyme reaction mixture was made up to 2 ml with distilled water. The mixture was incubated at 70°C for 1 h, and the reaction was stopped by adding 2 ml of 0.5 M perchloric acid. The fructose produced was determined by the modified method of Dische and Borenfreund (9). One unit of glucose isomerase activity was defined as the amount of the enzyme that produced 1 μmol of D-fructose per min under the assay conditions described.

Determination of protein. Protein was determined by the method of Lowry et al. (16). Bovine serum albumin was used as the standard. The protein concentration of each fraction during column chromatography was estimated by measuring the absorbance at 280 nm.

Determination of glucose. Glucose was determined by the use of PGO reagent (Sigma Technical Bulletin no. 510, 1978, Sigma Chemical Co.). PGO reagent is a mixture of peroxidase and glucose oxidase; it contains *O*-dianisidine as a chromogen. To 0.5 ml of glucose solution, 5 ml of PGO reagent was added. The mixture was shaken gently and allowed to stand at room temperature for 45 min. At the end of the incubation period, the absorbance was read at 560 nm on a spectrophotometer (model PMQ II, Carl Zeiss Co.). Pure glucose was used as the standard.

Purification of glucose isomerase. Solid ammonium sulfate was added to the enzyme extract to give a 70% saturation, and the precipitate was discarded. More ammonium sulfate was added to the supernatant to give a 90% saturation. The precipitate was collected, dissolved in 0.05 M sodium phosphate buffer (pH 7.0), and dialyzed overnight against the same buffer. The dialysate was centrifuged at 12,000 × *g* for 10 min. Unless otherwise indicated, all steps of the purification were carried out at 4°C. After dialysis and centrifugation, the supernatant was applied to a column of DEAE-cellulose (2.5 by 32 cm) which was previously equilibrated with 0.05 M sodium phosphate buffer (pH 7.0). The enzyme was eluted with the same buffer containing a linear gradient of NaCl at a flow rate of 50 ml/h. Active fractions (no. 52 to 70) were collected, dialyzed overnight with the same buffer, and centrifuged at 12,000 × *g* for 10 min. The supernatant was applied to a column of DEAE-Sephadex A-50 (2.2 by 30 cm) which was previously equilibrated with 0.05 M sodium phosphate buffer (pH 7.0). The enzyme was eluted with the same buffer containing a linear gradient of NaCl at a flow rate of 30 ml/h. Active fractions (no. 92 to 138) were collected, and the enzyme was precipitated by adding solid ammonium sulfate to 90% saturation. The precipitate was then dissolved in a minimum volume of 0.05 M sodium phosphate buffer (pH 7.0), dialyzed overnight against the same buffer, and centrifuged at 12,000 × *g* for 10 min. The supernatant was stored at −20°C until further use.

SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis was based on the method of Weber et al. (28) with minor modifications suggested by Malencik (personal communication). Polyacrylamide gels (7.5 and 10%) were used for the study of homogeneity of glucose isomerase and the determination of the molecular weight of the enzyme subunit, respectively. The buffer system was 0.01 tris(hydroxymethyl)aminomethane-phosphate buffer (pH 6.8). Electrophoresis was carried out with a Buchler polyanalyst electrophoresis chamber. The power supply (model 400, Bio-Rad Laboratories) was operated at 4 mA per tube. The running time was about 2 h. After electrophoresis, gels were removed from the tubes and fixed in 12.5% trichloroacetic acid for 10 min, then stained for 2 h with Coomassie blue G-250. Destaining was conducted in 7.5% acetic acid using quick gel destainer (Canalco Inc.). The mobilities were plotted against the logarithmic values of molecular weights of standard proteins.

Ultracentrifugation. A sedimentation equilibrium experiment was carried out to determine the molecular weight and homogeneity of the enzyme in a Beckman model E ultracentrifuge (Beckman Inc.) equipped with an absorption optical system. The rotor (AN-D) was cooled to 4°C and run at 6,803 rpm for 3 days. The enzyme in 0.05 M sodium phosphate buffer (pH 7.0) was at a concentration of 0.62 mg/ml. A series of scan patterns at 280 nm were obtained.

Immobilization of glucose isomerase to ion exchangers. A suspension of 500 mg (dry weight) of various ion exchangers in 0.05 M sodium phosphate buffer (pH 7.0) was stirred gently at 4°C. Glucose isomerase solution (5 ml), which was obtained after precipitation with ammonium sulfate (70 to 90% saturation), was added and stirred for 30 min at the same temperature. The enzyme-support complex obtained

was washed and suspended in the same buffer.

Continuous isomerization of glucose. Packed columns of DEAE-cellulose-glucose isomerase or heat-treated whole cells (60°C, 10 min) were continuously operated at 70°C for 5 days. Glucose was dissolved in 0.02 M sodium phosphate buffer (pH 7.0) to give a concentration of 1 M, and the resulting substrate solution was then fortified with mineral salts (see Fig. 8). The substrate solution (70°C) was pumped through the column at a flow rate of 12 ml/h. Hot water (70°C) was pumped through a water jacket which surrounded the column. The effluent was collected as 12-ml fractions in tubes.

RESULTS

Purification of glucose isomerase. Fractionation with ammonium sulfate gave a high degree of purification of the enzyme. The majority of the activity was found in the 70 to 90% fraction. This fraction contained about 50% of the original activity and a purification of around 4.9-fold. After dialysis, the 70 to 90% fraction was chromatographed on a DEAE-cellulose column (Fig. 1). The enzyme activity was eluted using a low concentration of NaCl (0.13 to 0.16 M) and was found in fractions 54 to 70. The enzyme solution from the preceding step was applied to a column of DEAE-Sephadex A-50. The enzyme was eluted at 0.22 to 0.35 M NaCl and was found in fractions 92 to 138 (Fig. 2). The results of the overall purification procedure are summarized in Table 1. The enzyme was purified about 12.6-fold over the extract with an overall yield of 11%.

Homogeneity of purified glucose isomerase. Homogeneity of the purified enzyme was determined by ultracentrifugation and SDS-polyacrylamide gel electrophoresis. The purified enzyme proved to be homogeneous as evidenced by a fairly straight line of a sedimentation equilibrium plot (Fig. 3) and a single band of protein on an SDS-polyacrylamide gel at pH 6.8 (Fig. 4).

Immobilization of glucose isomerase. Various anionic and cationic exchangers were used to immobilize partially purified glucose isomerase. It was found that anionic exchangers were effective in adsorbing glucose isomerase, whereas cationic exchangers were not (data not shown). Benzyl DEAE-cellulose and TEAE-cellulose retained more enzyme activity and protein than other ion exchangers (data not shown). However, these two supports are much more expensive than DEAE-cellulose. To determine the maximum protein and enzyme activity that DEAE-cellulose could adsorb, different concentrations of protein were added to DEAE-cellulose suspensions. The maximum values for protein and enzyme activity that could be bound occurred when 191 mg of protein per g of support was added: 105 mg of protein per g of support and 1,052 U/g of support, respectively. The specific activity of immobilized enzymes prepared on benzyl DEAE-cellulose, TEAE-cellulose, and DEAE-cellulose was higher than that of the soluble enzyme (data not shown). Therefore, the immobilized enzyme prepared by adsorption on DEAE-cellulose was used for further experiment.

Properties of glucose isomerase. A com-

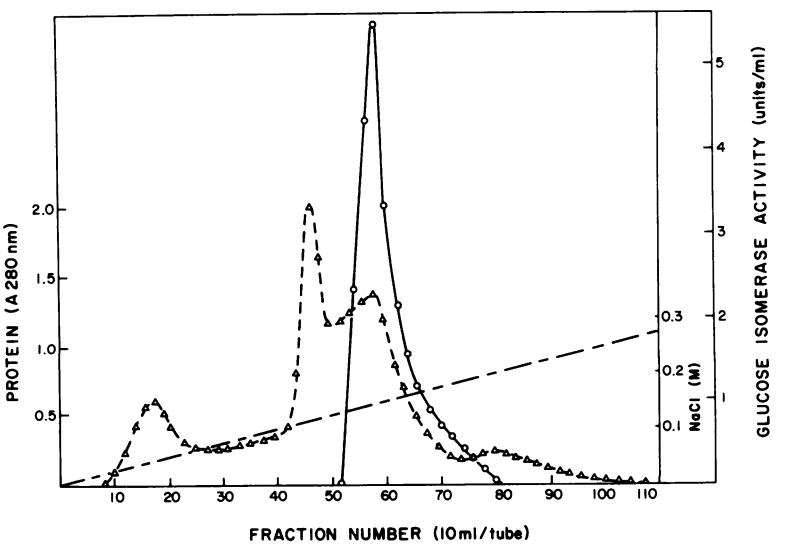

FIG. 1. *Chromatography of glucose isomerase on DEAE-cellulose column. Symbols: glucose isomerase activity* (○); *protein* (△); *NaCl* (-----).

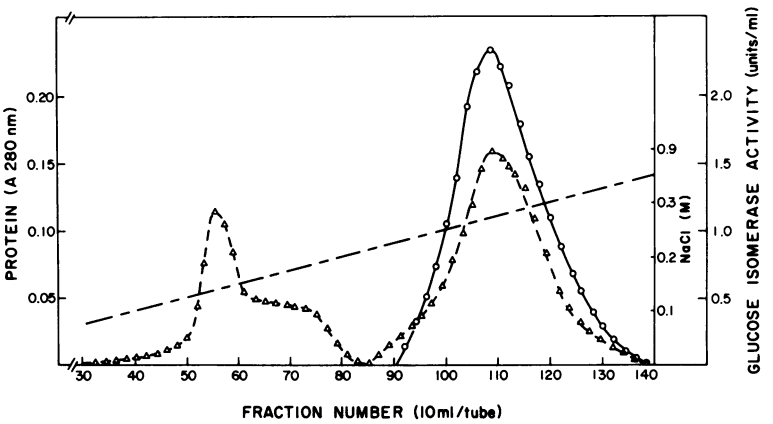

FIG. 2. *Chromatography of glucose isomerase on DEAE-Sephadex A-50 column. Symbols: glucose isomerase activity* (○); *protein* (△); *NaCl* (-----).

TABLE 1. *Purification of glucose isomerase of S. flavogriseus*

Fraction	Protein (mg)	Enzyme activity[a] (U)	Sp act (U/mg of protein)	Yield (%)
Extract	2,860	4,756	1.6	100
$(NH_4)_2SO_4$ fractionation (70–90%)	287	2,242	7.8	49
Dialysis	229	1,876	8.2	41
DEAE-cellulose	131	1,464	11.2	32
DEAE-Sephadex A-50	25	503	20.2	11

[a] One unit of enzyme activity was defined as the amount of enzyme that produced 1 μmol of D-fructose per min under the assay conditions described.

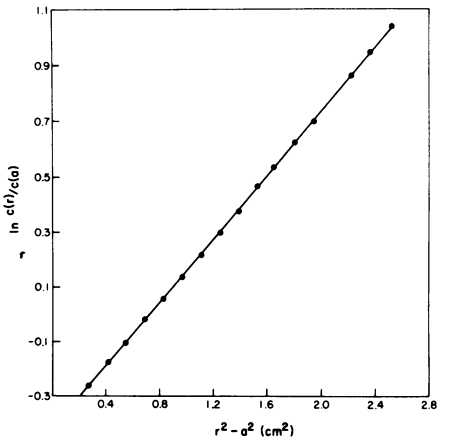

FIG. 3. *Sedimentation equilibrium plot of glucose isomerase as observed by adsorption optical method. c(r) and c(a) are the concentrations of the protein at distances r and a (meniscus) from the axis of rotation (expressed as absorbance at 280 nm).*

parison of properties between purified soluble enzyme, heat-treated (60°C, 10 min) whole cells, and immobilized enzyme (DEAE-cellulose–glucose isomerase complex) was made in this study to provide information for their practical applications. All three enzyme preparations isomerized xylose and glucose to their respective ketoses, but they did not isomerize other pentoses, hexoses, sugar alcohols, α-methyl glucoside, and glucose 6-phosphate (data not shown). Lineweaver-Burk plots of glucose isomerase action were used to calculate K_m for xylose and glucose. The K_m values of soluble enzyme for xylose and glucose were 0.078 and 0.249 M, respectively. The apparent K_m values of immobilized enzyme for xylose and glucose were 0.104 and 0.297 M, respectively. The apparent K_m values of whole cells for xylose and glucose were 0.120 and 0.376 M, respectively.

The optimum temperature for activity of both the soluble and immobilized enzymes was 70°C when the incubation time was 1 h (Fig. 5); the activity of whole cells functioned optimally at 75°C.

Figure 6 shows the effect of pH on the enzyme activity. The pH optimum for activities of three enzyme preparations was 7.5 when the enzyme was incubated for 1 h at 70°C.

Glucose isomerase of *S. flavogriseus* required Mg^{2+} or Co^{2+} for its activity (Table 2). Manganese ion, Ni^{2+}, Ca^{2+}, or Zn^{2+} showed a slight effect, but others were ineffective. It was also found that an addition effect resulted from the presence of both Mg^{2+} and Co^{2+}.

Various chemicals were tested as inhibitors of glucose isomerase from *S. flavogriseus*. Only heavy metals such as Hg^{2+}, Ag^{+}, and Cu^{2+} in-

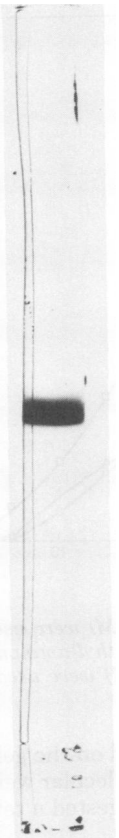

FIG. 4. *SDS-polyacrylamide gel electrophoresis performed on the purified glucose isomerase.*

hibited enzyme activity (data not shown). Sorbitol, mannitol, and tris(hydroxymethyl)aminomethane did not inhibit the enzyme activity, although these chemicals were reported to be inhibitors by several other investigators (20, 26, 27). Chemicals, including *p*-chloromercuribenzoate, maleic acid, and sorbic acid, that can react with sulfhydryl groups of enzymes did not inhibit enzyme activity. As mentioned earlier, the enzyme required Mg^{2+} or Co^{2+} for its activity. However, a cation-chelating reagent, ethylenediaminetetraacetic acid, failed to inhibit enzyme activity, either because its concentration (10^{-3} M) was too low or because the stability of the cation-enzyme complex was greater than that of the cation-EDTA complex (30).

The isomerization of glucose to fructose was reversible (data not shown). At 70°C, the conversion ratio of three enzyme preparations for isomerization was the same, about 50%.

The purified soluble enzyme from *S. flavogriseus* was quite stable, since it retained full activity after incubation at 70°C for 10 min (data not shown). It was also found that almost no difference existed in thermal stability between the soluble and immobilized enzymes; however, the stability of whole cells was less (data not shown).

The soluble enzyme was stable under a wider range of pH (5.0 to 9.0) at room temperature for 24 h than were the immobilized enzyme and the whole cells (pH 5.5 to 9.0).

The sedimentation equilibrium method was used for the determination of molecular weight

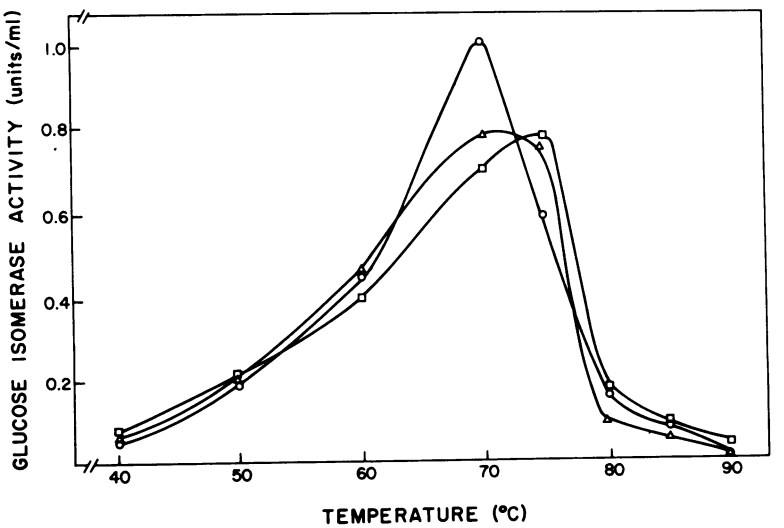

FIG. 5. *Effect of temperature on glucose isomerase activity. Symbols: soluble enzyme* (○); *immobilized enzyme* (△); *whole cells* (□).

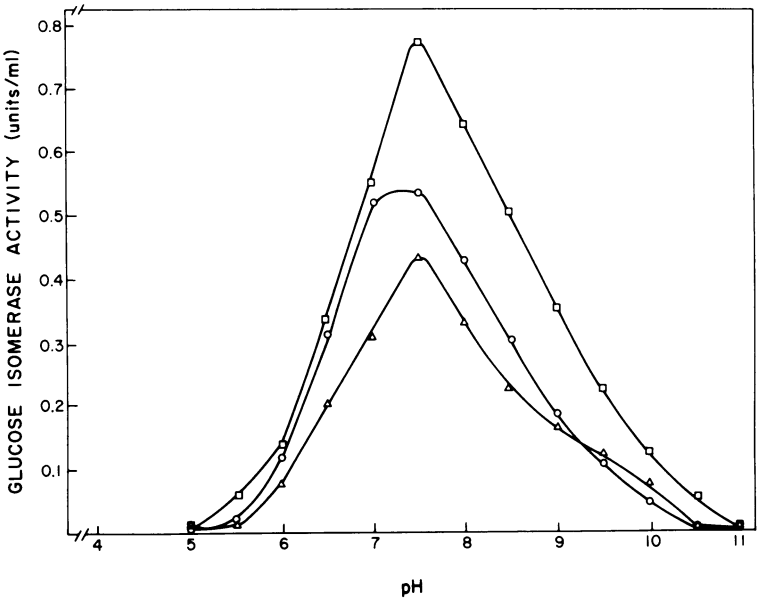

FIG. 6. *Effect of pH on glucose isomerase activity. Acetate buffers (0.05 M) were used for pH 4.0 to 5.0, sodium phosphate buffers (0.05 M) were used for pH 6.0 to 7.5, tris(hydroxymethyl)aminomethane buffers (0.05 M) were used for pH 8.0 to 9.0, and carbonate-bicarbonate buffers (0.05 M) were used for pH 9.5 to 11.0. Symbols: soluble enzyme* (○); *immobilized enzyme* (△); *whole cells* (□).

TABLE 2. *Effect of mineral salts on glucose isomerase activity*

Mineral salt[a]	Relative activity (%)		
	Soluble enzyme	Immobilized enzyme	Whole cells
None	0	0	0
$MgSO_4 \cdot 7H_2O$	100	100	100
$CoCl_2 \cdot 6H_2O$	71	82	76
$MnSO_4 \cdot 4H_2O$	21	28	19
$NiSO_4 \cdot 6H_2O$	17	28	38
$CaCl_2 \cdot 2H_2O$	15	6	19
$ZnSO_4 \cdot 7H_2O$	8	19	14
$BaCl_2 \cdot 2H_2O$	0	0	0
$CuSO_4 \cdot 5H_2O$	0	0	0
$FeSO_4 \cdot 7H_2O$	0	0	0
$Fe_2(SO_4)_3$	0	0	0
$HgCl_2$	0	0	0
$MgSO_4 \cdot 7H_2O$ + $CoCl_2 \cdot 6H_2O$	283	300	252

[a] All salts were present at 5×10^{-3} M. $MgSO_4 \cdot 7H_2O$ + $CoCl_2 \cdot 6H_2O$ were present at 5×10^{-3} M each.

of glucose isomerase. The partial specific volume (0.69 ml/g) of glucose isomerase from *Streptomyces albus* (26) was used in this study for the calculation of molecular weight. The molecular weight of glucose isomerase determined by this method was 171,000. SDS-polyacrylamide gel electrophoresis, the most common way of estimating the molecular weight of protein subunits (10, 11), was also utilized. The purified glucose isomerase migrated on the gel at a position corresponding to a molecular weight of 43,000 (Fig. 7). The results suggested a tetrameric structure for the enzyme.

Continuous isomerization of glucose. Figure 8 shows the stability of the immobilized enzyme and whole cells when used for the continuous isomerization of glucose in a plug-flow column. The immobilized enzyme was more stable than whole cells. The addition of both Mg^{2+} and Co^{2+} enhanced the stability of the immobilized enzyme and whole cells. After operating at 70°C for 5 days, the remaining enzyme activities of the immobilized enzyme and whole cells in the presence of Mg^{2+} and Co^{2+} were 75 and 55%, respectively. However, the remaining activities in the presence of Mg^{2+} alone decreased to 65 and 47%, respectively.

DISCUSSION

Numerous methods are available for immobilization of glucose isomerase (2). Among them, attachment of the enzyme to ion-exchanger materials is considered to be a simple, inexpensive, and promising method (29). In this study, benzyl DEAE-cellulose, TEAE-cellulose, and DEAE-cellulose were effective in adsorbing the enzyme. DEAE-cellulose has been used for immobilization of glucose isomerase by several investigators (14, 15, 18; T. Sipos, German patent 2,061,371, January, 1971), and this process is now used

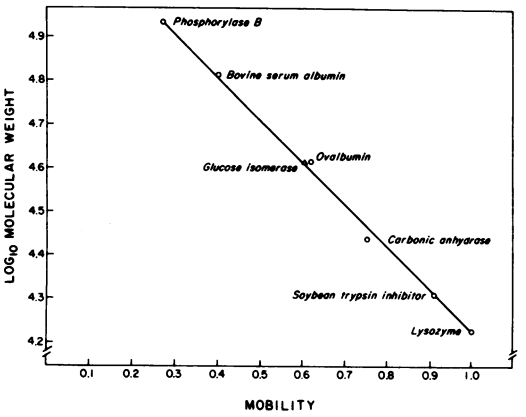

FIG. 7. *Comparison of molecular weight of standard proteins (lysozyme, 14,300; soybean trypsin inhibitor, 21,000; carbonic anhydrase, 30,000; ovalbumin, 43,000; bovine serum albumin, 68,000; phosphorylase B, 94,000) and glucose isomerase by SDS-polyacrylamide gel electrophoresis.*

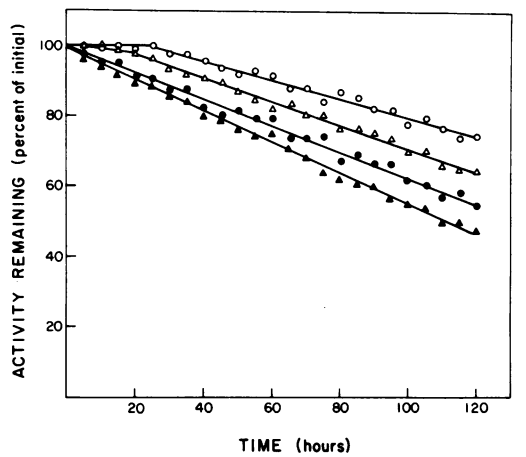

FIG. 8. *Stability of DEAE-cellulose–glucose isomerase and whole cells used for continuous isomerization of 1 M glucose in a plug-flow column. Symbols: column (2.5 by 5 cm) packed with DEAE-cellulose–glucose isomerase providing 5 mM Mg^{2+} and 1 mM Co^{2+} (○); column (2.5 by 9 cm) packed with whole cells providing 5 mM Mg^{2+} and 1 mM Co^{2+} (△); column (2.5 by 5 cm) packed with DEAE-cellulose–glucose isomerase providing 5 mM Mg^{2+} (●); column (2.5 by 9 cm) packed with whole cells providing 5 mM Mg^{2+} (▲).*

commercially by Clinton Corn Processing Co. (22), but no reports were found concerning the effectiveness of benzyl DEAE-cellulose and TEAE-cellulose in the immobilization of glucose isomerase, probably, because these two materials are too expensive for practical use. Therefore, DEAE-cellulose was selected in this study for further investigation. The retention of activity after adsorption to DEAE-cellulose appeared to be lower than that reported for the enzyme of *Bacillus coagulans* adsorbed to DEAE-cellulose (14). Huitron and Limon-Lason (14) also examined the adsorption of the enzyme to aminoethyl cellulose, DEAE-Sephadex A-25, and DEAE-Sephadex A-50. In both the study of Huitron and Limon-Lason and our work described here, immobilization of glucose isomerase showed an increase in specific activity because impurities in the crude enzyme preparations were not retained.

Only glucose and xylose served as substrates for the enzyme of *S. flavogriseus*. Besides glucose and xylose, some glucose isomerases from other microbial sources also catalyze the isomerization of ribose, arabinose, rhamnose, allose, and deoxyglucose (6, 8, 20, 21, 24, 31).

Immobilization of an enzyme on the surface of a carrier usually changes the K_m of that enzyme (2). Some apparent K_m values of immobilized enzymes are larger than the values for soluble enzymes, and others are smaller. Kinetics of immobilized enzymes are generally affected by carrier and substrate charges, pore diameter of the carrier, bulk and pore diffusion rates, and many other parameters. In this study, the apparent K_m of the immobilized enzyme was larger than that for soluble enzyme. Surprisingly, Lloyd and Khaleeluddin (15) reported that both the soluble and immobilized forms (DEAE-cellulose–glucose isomerase complex) of the enzyme prepared from *Streptomyces* sp. ATCC 21175 gave the same K_m for glucose in batch reactions. Later, Huitron and Limon-Lason (14) observed a slight increase in apparent K_m for glucose on adsorption of glucose isomerase to DEAE-cellulose. They believed that this was due to microenvironmental effect.

Most of the glucose isomerases (5, 17, 20, 23, 24, 26) isolated to date showed an optimum temperature around 80°C. Hodge (13) indicated that degradation of ketoses occurs at high temperatures, characterized by a pronounced discoloration of an aqueous sugar solution. Thus, 70°C was used by Chou et al. (5) to isomerize glucose even though the optimum temperature was 80°C. In our study reported here, the use of the enzyme from *S. flavogriseus* was not hampered by its relatively low optimum temperature.

The pH optimum for activity of the enzyme was apparently lower than that of enzymes from other *Streptomyces* species (5, 17, 23, 24, 26). The glucose isomerases prepared from *Actinoplanes missouriensis*, *B. coagulans*, and *Lactobacillus brevis* also have a low pH optimum (6, 21, 31). Under alkaline conditions, a nonmetabolizable sugar, psicose, is produced in hot

glucose and fructose solution (2). Therefore, a low pH optimum is an attractive property for enzyme application because the use of neutral or lower pH in the isomerization of glucose prevents the formation of psicose. In this study, the three enzyme preparations showed the same pH optimum. However, immobilization of the enzyme prepared from *B. coagulans* on DEAE-cellulose shifted pH optimum slightly toward acidic (14). Poulsen and Zittan (P. B. Poulsen and L. Zittan, Enzyme Engineering Conference, Portland, Ore., August, 1975) observed a big shift from pH 7.0 for soluble glucose isomerase to 8.5 for immobilized cell debris of *B. coagulans*.

As in most investigations, the enzyme required Mg^{2+} or Co^{2+} for its activity. It was found that an addition effect resulted from the presence of both Mg^{2+} and Co^{2+}. Tsumura and Sato (27) also demonstrated that Co^{2+} activated glucose isomerase of *Streptomyces phaeochromogenes* in the presence of Mg^{2+} and protected the enzyme against heat denaturation. For the enzyme from *S. albus* (26), the requirements for Mg^{2+} and Co^{2+} were similar to those described above. Both Mg^{2+} and Co^{2+} were required for high activity of glucose isomerase from *A. missouriensis*. Magnesium ion was essential for activity; Co^{2+} enhanced the activity (21). An important feature for the enzyme of *Arthrobacter* sp. was that it did not require Co^{2+} for activity or stability (C. K. Lee, L. E. Hayes, and M. E. Long, U.S. patent 3,645,848, May, 1971). In this study, Mg^{2+} was more effective than Co^{2+}, but the reverse was true for glucose isomerase of *B. coagulans* (8). Like the glucose isomerase from *L. brevis* (31), the enzyme from *B. coagulans* required Co^{2+} to isomerize glucose and ribose, but needed Mn^{2+} to isomerize xylose (6). Danno concluded that the active form of the enzyme was that which contained Co^{2+} or Mn^{2+} and proposed that binding of Co^{2+} and Mn^{2+} caused suitable conformation changes in the catalytic site of the enzyme for glucose and xylose isomerization, respectively (7).

The thermal stability of glucose isomerase from *B. coagulans* was reported to be as good as that of the enzyme from *S. albus* (6, 26). The enzyme from *A. missouriensis* appeared to be the most stable of all the known glucose isomerases because it retained activity at 90°C for 20 min (21). In our study, it was found that almost no difference existed in thermal stability between the soluble and immobilized enzymes; however, the stability of whole cells was less. In many enzyme systems, immobilization increases the stability under conditions of increased temperature (29). Some immobilized enzymes have less stability at high temperature, even in the presence of substrates which usually increase the stability. At present, it is difficult to predict which enzymes will show increased or decreased stability on immobilization. The heat stability of the soluble and immobilized glucose isomerases prepared from *B. coagulans* (adsorbed on DEAE-cellulose) has been studied by Huitron and Limon-Lason (14). For the 15-min periods studied, they found no differences between the preparations. However, Park and Toma (18) reported that DEAE-cellulose-bound enzyme was relatively less heat resistant than semipurified soluble enzyme prepared from *Streptomyces bikiniensis*.

Huitron and Limon-Lason (14) demonstrated a greater operational stability of DEAE-cellulose-glucose isomerase (from *B. coagulans*) complex than this study. They also found that the operational half-life of the immobilized enzyme was a function of the Mg^{2+} concentration and also depended on the geometry of the columns. The operational stability of the immobilized enzyme and whole cells employed in this study could be improved if the operational conditions were investigated in greater detail.

As mentioned in a previous report (3), *S. flavogriseus* produces large quantities of glucose isomerase when grown on straw hemicellulose or H_2SO_4-hydrolysate of ryegrass straw. The organism does not require Co^{2+} for enzyme production. The present report further indicates that glucose isomerase produced from *S. flavogriseus* has several attractive properties, such as low pH optimum for activity and high thermal stability, making it a potentially useful industrial enzyme.

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