APPLIED MICROBIOLOGY, June 1975, p. 745-750
Vol. 29, No. 6
Printed in U.S.A.

Properties of D-Xylose Isomerase from *Streptomyces albus*

SERGIO SANCHEZ[1] AND KARL L. SMILEY*

Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604

Received for publication 6 January 1975

A partially purified D-xylose isomerase has been isolated from cells of *Streptomyces albus* NRRL 5778 and some of its properties have been determined. D-Glucose, D-xylose, D-ribose, L-arabinose, and L-rhamnose served as substrates for the enzyme with respective K_m values of 86, 93, 350, 153, and 312 mM and V_{max} values measuring 1.23, 2.9, 2.63, 0.153, and 0.048 μmol/min per mg of protein. The hexose D-allose was also isomerized. The enzyme was strongly activated by 1.0 mM Mg^{2+} but only partially activated by 1.0 mM Co^{2+}. The respective K_m values for Mg^{2+} and Co^{2+} were 0.3 and 0.003 mM. Mg^{2+} and Co^{2+} appear to have separate binding sites on the isomerase. These cations also protect the enzyme from thermal denaturation and from D-sorbitol inhibition. The optimum temperature for ketose formation was 70 to 80 C at pH values ranging from 7 to 9. D-Sorbitol acts as a competitive inhibitor with a K_i of 5.5 mM against D-glucose, D-xylose, and D-ribose. Induction experiments, Mg^{2+} activation, and D-sorbitol inhibition indicated that a single enzyme (D-xylose isomerase) was responsible for the isomerization of the pentoses, methyl pentose, and glucose.

The conversion of D-glucose to D-fructose by microbial enzymes was first described in cell-free extracts of *Pseudomonas hydrophila* by Marshall and Kooi (16). This activity in the genus *Streptomyces* was initially reported by Tsumura and Sato (23). Subsequent studies of the enzyme from this genus, involving purification, crystallization, and kinetic characterization, demonstrated that the inducible enzyme D-xylose keto isomerase (EC 5.3.1.5) converted D-xylose as well as D-glucose to their respective ketoses (21). In addition to these carbohydrates, the isomerases found in *Lactobacillus brevis* (24) and in *Bacillus coagulans* (3) also catalyzed D-ribulose synthesis from D-ribose. The latter activity, however, was not detected in *Streptomyces* (21). This paper characterizes a D-xylose isomerase from *Streptomyces albus* which utilizes, in addition to D-glucose, the following carbohydrates: D-xylose, D-ribose, D-allose, L-arabinose, and L-rhamnose. Furthermore, it describes the functional role of Mg^{2+} as an activator of isomerization and discusses the practical importance of the cation.

MATERIALS AND METHODS

Organism and cultural conditions. *Streptomyces albus* (Rossi Doria) Waksman and Henrici NRRL 5778 was isolated by the authors from a local garden soil by suspending 1 g of soil in 100 ml of sterile distilled water and plating from dilutions of the suspension on an inorganic salts-agar medium (17) containing 0.5% D-xylose as sole carbon source.

All cultures were grown in a medium (RM) containing 0.4% yeast extract, 0.3% malt extract, 0.5% NaCl, and 0.05% $MgSO_4 \cdot 7H_2O$, and adjusted to pH 7.3. The cultures were incubated at 29 C on a rotary shaker at 250 rpm. Stock cultures were maintained on RM medium solidified with 1.5% agar.

For mycelium propagation, a 250-ml Erlenmeyer flask with 100 ml of RM was inoculated with spores from 2-day-old slants and incubated for 24 h. The total content of the flask was subsequently added to a 2.8-liter Fernbach flask containing 800 ml of RM supplemented with 0.5% D-xylose. After a 24-h incubation, the mycelium was harvested by filtration through Whatman no. 41 paper, washed twice with distilled water, and resuspended in 0.2 M potassium phosphate buffer (pH 7.2) at room temperature, maintaining a ratio of 2 g of mycelium (wet weight) per 5 ml of buffer.

Cobalt was not included in RM medium since it does not enhance isomerase formation. Cobalt has been reported to stimulate isomerase formation by other strains of *Streptomyces* (10, 21, 22).

Enzyme preparation. Enzyme solutions were prepared according to Strandberg and Smiley (20) with the modification that the mycelial suspension was disrupted at 4 C by passage through a French pressure cell at 8,000 lb/in². The final enzyme preparation had a specific activity for D-glucose of 1.085 μmol/min per mg of protein, corresponding to a two-fold purification over the crude extracts. The enzyme preparation was stored at −5 C.

[1]Present address: Dept. of Nutrition and Food Science, M.I.T., Cambridge, Mass. 02139.

Enzyme assays. A 1-ml mixture containing 16 μmol of $MgSO_4 \cdot 7H_2O$, 100 μmol of potassium phosphate buffer (0.2 M), pH 7.2, and 2 to 4 mg of protein with isomerizing activity was preincubated for 5 min at 70 C. To start the reaction, 160 μmol of each carbohydrate in 1 ml of buffer were added to the preincubated mixture. When required, 0.08 μmol of $CoCl_2 \cdot 6H_2O$ was used. The reaction was incubated at 70 C for 15 min and stopped with 1 ml of 5% trichloroacetic acid. Unless otherwise specified, the enzyme activity was measured under these conditions. Fructose and ketopentoses formed were determined by the cysteine-carbazole method (5), in which color was developed at room temperature for 15 min. The optical density values were read at 540 nm in a Gilford 300-N spectrophotometer. All ketopentoses were determined by comparison with a standard curve prepared with D-ribulose-o-nitrophenylhydrazone (1) whereas ketohexoses were determined by comparison to a standard curve prepared with D-fructose. Enzyme activities were expressed as micromoles of ketose formed per minute per milligram of protein.

The carbohydrates used as substrates were shown to be at least 99% pure by gas-chromatographic analysis of their peracetylated aldononitrile derivatives (7) and by paper chromatography (9).

Protein was determined by the method of Lowry et al. (14), using bovine serum albumin as standard.

Chemicals. All the carbohydrates with the exception of D-allose were purchased from Sigma Chemical Co. D-Allose was prepared at Northern Regional Research Laboratory, Peoria, Ill., by W. Dick essentially as described by Kawana et al. (11).

RESULTS

The enzymatic conversion of D-glucose to D-fructose, using cell-free extracts of *Streptomyces albus* NRRL 5778, functioned optimally at 70 to 80 C (Fig. 1a) which is in agreement with other *Streptomyces* species (20–22). Enzyme activity was optimal between pH 7 to 9 (Fig. 1b) and is in accord with previously reported values for other *Streptomyces* strains (20, 21) but lower than pH 9.5 reported for *Streptomyces phaeochromogenes* strain SK (22). An Arrhenius plot of the temperature activity values revealed an activation energy of 47,300 J per mol. Since D-fructose can be converted to D-glucose and other sugars through enolization reactions at pH 8 or higher, the present work was done at pH 7.2 and 70 C.

Mg^{2+} (1.0 mM) either as chloride or as sulfate strongly stimulated D-glucose isomerase activity extracted from the microorganism under study (Table 1); Co^{2+} and Mn^{2+} at the same concentrations were weak activators. When the activity was tested in several Mg^{2+} or Co^{2+} concentrations (Fig. 2), the maximal activity obtained in the presence of Co^{2+} was 25% of that with Mg^{2+}. Respective affinity constants for

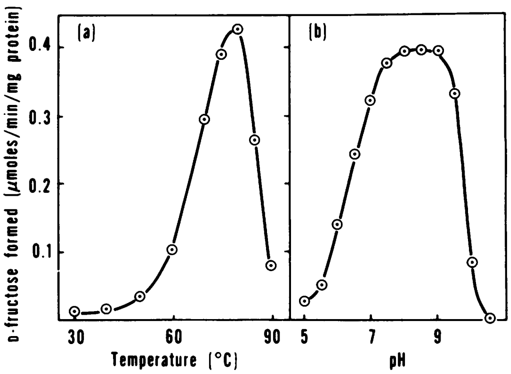

FIG. 1. *Effect of temperature (a) and pH (b) on D-glucose isomerase activity. Activity was measured as described in Materials and Methods, except that the temperature and pH values were varied as indicated. Potassium phosphate buffers (0.05 M) were used for pH values of 5.0 to 8.5 and carbonate-bicarbonate buffers (0.05 M) from pH 8.5 to 11.0. All results were corrected for alkaline isomerization.*

TABLE 1. *Activation of D-glucose isomerase[a] from S. albus NRRL 5778 by divalent cations*

Metal salt (1 mM)	Relative activity[b] (%)
None	9.85
$FeSO_4$	0.0
$CuSO_4$	0.0
$CaCl_2$	0.0
$NiSO_4$	5.32
$MnCl_2$	13.15
$CoCl_2$	28.5
$MgCl_2$	100.0
$MgSO_4$	100.0[c]
$MgSO_4$ plus $CoCl_2$	137.5

[a] The enzyme was dialyzed overnight against demineralized distilled water to remove metallic ions.

[b] The activity was assayed as described in Materials and Methods but omitting the $MgSO_4$.

[c] The activity in the presence of $MgSO_4$ was set as 100%.

Mg^{2+} and Co^{2+} of 0.3 and 0.003 mM (Fig. 3) were graphically determined (13).

An additive effect resulted from the presence of both Mg^{2+} and Co^{2+}. To learn more about the additive effect, glucose isomerizing rates were assayed either in the absence or in the presence of a fixed amount of Co^{2+} at different Mg^{2+} levels. Lineweaver-Burk treatment of the data demonstrated that although Co^{2+} does not alter the K_m for Mg^{2+} it increases V_{max} (Fig. 4). This observation suggests that the cations do not compete for a common binding site on the enzyme molecule. Mg^{2+} and Co^{2+} either singly

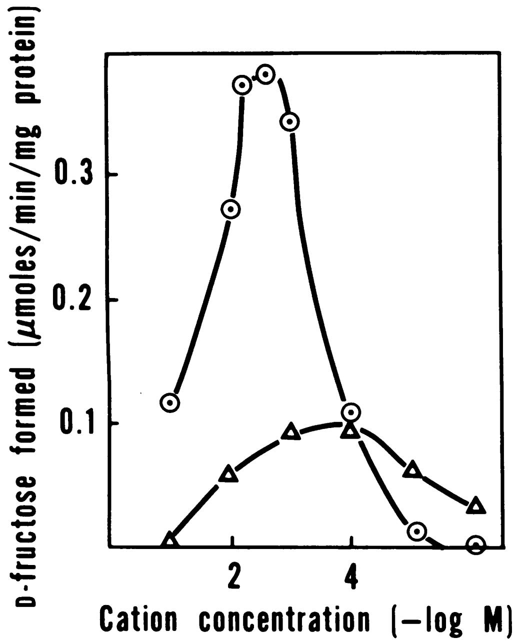

FIG. 2. *Effect of* Co^{2+} (Δ) *and* Mg^{2+} (O) *concentrations on* D-*glucose isomerase activity. Conditions as described in Materials and Methods but varying* Mg^{2+} *and* Co^{2+} *concentrations.*

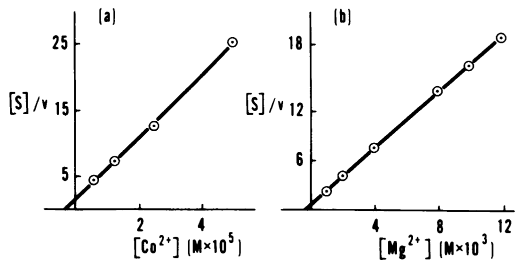

FIG. 3. *Plots of the reaction rate of* D-*glucose isomerase with varying* Co^{2+} *and* Mg^{2+} *concentrations. The reactions were carried out for 10 min under conditions described in Materials and Methods. The initial velocity was expressed as micromoles of* D-*fructose formed per minute per milligram of protein at 70 C.*

or combined inhibited thermal denaturation of the enzyme (Fig. 5a). Consequently, a 24-h preincubation at 70 C did not significantly alter enzyme activity when both cations were present, even though substrate was absent (Fig. 5b).

Surprisingly, the enzyme isomerized an interesting array of substrates. As shown in Table 2, in addition to isomerizing D-glucose and D-xylose, as has been reported for other *Streptomyces* species (20, 21, 23), the enzyme from strain NRRL 5778 also isomerized D-ribose, D-allose, L-arabinose, and L-rhamnose to their respective ketoses. Because sufficient amounts of D-allose were not available, further work with this sugar could not be done. Gas-chromatographic analyses of the peracetylated aldononitrile derivatives (7) and paper chromatography (9) of all the substrates showed them to be >99% pure, thus eliminating contamination of the substrates as the source of ketose formation. Respective K_m values for D-glucose and D-xylose of 86 and 93 mM and V_{max} values of 1.23 and 2.9 μmol/min per mg of protein were graphically determined (13). Additionally, it was found that K_m values for D-ribose, L-arabinose, and L-rhamnose were 350, 153, and 312 mM, respectively, whereas V_{max} values were 2.63, 0.153, and 0.048 μmol/min per mg of protein. Yamanaka and Isumori (25) recently

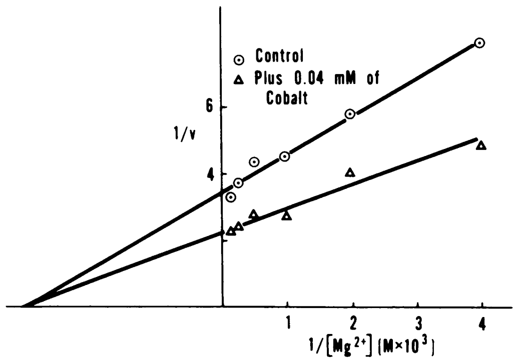

FIG. 4. *Double reciprocal plots of the velocity of* D-*glucose isomerase activity versus varying* Mg^{2+} *concentrations in the absence* (O) *or presence* (Δ) *of a fixed* Co^{2+} *concentration. Conditions as in Fig. 3.*

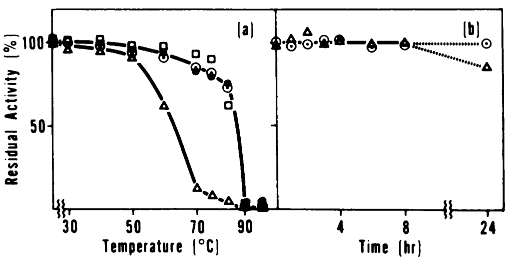

FIG. 5. (*a*) *Thermal tolerance of* D-*glucose isomerase activity. The enzyme was treated for 10 min in the absence of substrate at the indicated temperatures under the following conditions: no cation* (Δ); 4×10^{-5} *M* Co^{2+} (●); 8×10^{-3} *M* Mg^{2+} (O); *and* Co^{2+} *plus* Mg^{2+} (□). *After treatment, the activity was measured as described in Materials and Methods.* (*b*) *Heat stability of* D-*glucose isomerase activity. The enzyme was treated at 70 C during the indicated time in the presence of* 4×10^{-5} *M* Co^{2+} *plus* 8×10^{-3} *M* Mg^{2+} (Δ). *Control was at room temperature* (O). *After treatment the activity was measured as described in Materials and Methods.*

reported on an isomerase from a *Streptomyces* sp. which is active at 35 C and specific only for L-arabinose.

Whether the five activities result from a single isomerase or from five different enzymes could be determined by examining the ability of each substrate utilized to induce isomerizing activity. Only D-xylose effectively induced enzyme synthesis (Table 3). This observation suggests that a single enzyme (D-xylose isomerase) catalyzed the isomerization of the aforementioned carbohydrates. The fact that D-glucose induced no detectable isomerase activity may be due to catabolic repression by D-glucose (15). Table 4 presents further evidence supporting the existence of only one isomerase. Isomerization of the five substrates was markedly activated by Mg^{2+} and (with the exception of L-arabinose) partially by Co^{2+}. Dixon plots (6) in Fig. 6 show that D-sorbitol competitively inhibited D-glucose, D-xylose, and D-ribose isomerization with identical K_i values of 5.5 mM which also tends to confirm the single-enzyme hypothesis. Sorbitol inhibition of L-arabinose and L-rhamnose isomerization was not tested. Interestingly, D-sorbitol inhibited only when Co^{2+} and Mg^{2+} were not present in the reaction system (Fig. 7). These cations prevented inhibition of D-xylose isomerization even by high D-sorbitol concentrations. Similar results were obtained in the presence of either Mg^{2+} or Co^{2+} alone. Sorbitol also failed to inhibit isomerization in the presence of Co^{2+} and Mg^{2+} when D-xylose was substituted by either D-glucose or D-ribose.

TABLE 2. *Substrate specificity of S. albus NRRL 5778 isomerase*

Substrate (80 mM)	Activity[a] (μmol of ketose formed/min per mg of protein)
D-Glucose	1.085
D-Xylose	0.666
D-Ribose	0.488
D-Allose	0.250
L-Rhamnose	0.086
L-Arabinose	0.0369
D-Arabinose	0.0
D-Lyxose	0.0
L-Fucose	0.0
D-Fucose	0.0
D-Mannose	0.0
D-Galactose	0.0

[a] The activity was measured as described in Materials and Methods. Reaction time, 10 min at 70 C.

TABLE 3. *Effects of carbon source in growth medium on isomerizing activity of S. albus NRRL 5778*

Carbon source (1%)	Relative isomerization (%)[a]				
	D-Xylose	L-Arabinose	D-Ribose	D-Glucose	L-Rhamnose
No addition	14.0	15.45	13.0	10.25	6.55
D-Glucose	0.0	0.0	1.56	0.0	0.0
D-Fructose	5.41	3.8	3.34	1.25	3.12
D-Ribose	7.3	2.67	3.55	2.9	1.11
L-Arabinose	7.6	7.02	4.68	1.93	1.25
L-Rhamnose	4.78	8.3	6.92	2.64	1.49
Glycerol	10.15	10.12	9.6	3.29	3.79
D-Xylose[b]	100.0	100.0	100.0	100.0	100.0

[a] After 24 h of growth in RM medium supplemented with inducer, the isomerization was measured as described in Materials and Methods.

[b] The activity obtained with D-xylose as inducer was set as 100%.

DISCUSSION

The catalytic conversion of D-glucose to D-fructose was studied using a partially purified enzyme with isomerizing activity obtained from *Streptomyces albus* NRRL 5778. In addition to D-glucose, this enzyme also isomerizes D-xylose, D-ribose, D-allose, L-arabinose, and L-rhamnose. Steric correlations were found in these carbohydrates since, with the exception of L-rhamnose, these sugars have a C1 conformation and the hydroxyl groups on carbon 2 are in equatorial position. If the OH is in an axial position as in D-lyxose or D-mannose, they are not isomerized by the enzyme. When the OH groups of carbon 3 and 4 are equatorial as in D-glucose and D-xylose, maximum isomerization is obtained. However when the OH groups are axial at carbon 3 as in D-ribose and D-allose, the rate of isomerization is diminished. Axial OH at carbon 4 in the pentose L-arabinose further reduces the amount of isomerizations by the enzyme. Hexoses or methyl pentoses with axial OH on carbon 4 such as D-galactose or D-fucose are not isomerized. The utilization of L-rhamnose which has a 1C conformation is not understood at present.

It seems probable that a single enzyme (D-xylose isomerase) is responsible for isomerizations of all the aforementioned substrates, since only D-xylose serves as inducer of isomerase biosynthesis and because all five isomerase activities are uniformly stimulated by Mg^{2+} and to a lesser extent by Co^{2+} cations. D-Sorbitol competitively inhibits isomerization of D-glucose, D-xylose, and D-ribose with identical inhibition constants (K_i) which also suggests that all isomerizations occur on the same active center of a single enzyme.

TABLE 4. *Effect of magnesium and cobalt on S. albus NRRL 5778 isomerizing activities*[a]

Metal addition	Relative activity (%)[b]				
	L-Arabinose	L-Rhamnose	D-Glucose	D-Ribose	D-Xylose
None	0.0	8.87	9.85	6.75	36.5
$MgSO_4$	100.0	100.0	100.0	100.0	100.0
$CoCl_2$	0.0	11.3	28.5	40.3	41.0

[a] The activities were assayed using the same concentrations of cations and carbohydrates described in Materials and Methods.

[b] The activity obtained in the presence of Mg^{2+} was set to 100%.

This enzyme exhibits a fairly high K_m value for D-xylose (93 mM). In contrast, a relatively high affinity for D-glucose is measured (K_m = 86 mM) when compared to reported values for similar enzymes (K_m = 250 to 920 mM) from several microbial sources including *Streptomyces* (16, 20, 23, 24). It is interesting to note that previously reported K_m values of this enzyme from either *Streptomyces* (21, 23) or other microbial sources (3, 24) all show higher affinity for D-xylose than D-glucose. The significance of the low affinity for D-xylose is not understood since it alone induces isomerase formation and is therefore presumed to be the natural substrate.

An additional characteristic of the enzyme is that the catalytic function is strongly stimulated by Mg^{2+}, but only partially by either Co^{2+} or by Mn^{2+}. A more stable conformation appears to result from the enzyme-Mg^{2+} complex since it has high resistance to thermal degradation. Furthermore, the elimination of D-sorbitol inhibition (competitive) either by Mg^{2+} or Co^{2+} suggests that the conformational stabilization of the enzyme molecule no longer permits binding of the hexitol; i.e., the cations alter the substrate specificity of the enzyme. Since Co^{2+} increases V_{max} without altering the K_m for Mg^{2+}, we conclude that these cations do not compete for a common binding site on the enzyme molecule. A similar example for isomerizing enzymes was described by Danno for Co^{2+} and Mn^{2+} in *Bacillus coagulans* (4).

Because the isomerase from *Streptomyces albus* NRRL 5778 is highly stimulated by Mg^{2+} and poorly by Co^{2+}, it may be of interest for producing corn sugar syrups containing high D-fructose concentrations. D-Glucose isomerase from other microbial sources including *Streptomyces* are preferentially activated by Co^{2+} (4, 10, 16). Additionally, in other *Streptomyces* species, even though activation by Mg^{2+} alone has been reported, Co^{2+} is apparently required to obtain maximum glucose isomerase formation (20–22). Even though Co^{2+} is an essential trace metal for human nutrition (18), it is toxic at higher levels (8, 19). The Co^{2+} content of fructose syrups is about 1 mM (2) and this concentration is known to cause toxic effects in rats (12). Consequently fructose syrups must be treated with ion exchanges to reduce the Co^{2+} content to acceptable levels. From the standpoint of human health, it would be desirable to eliminate Co^{2+}

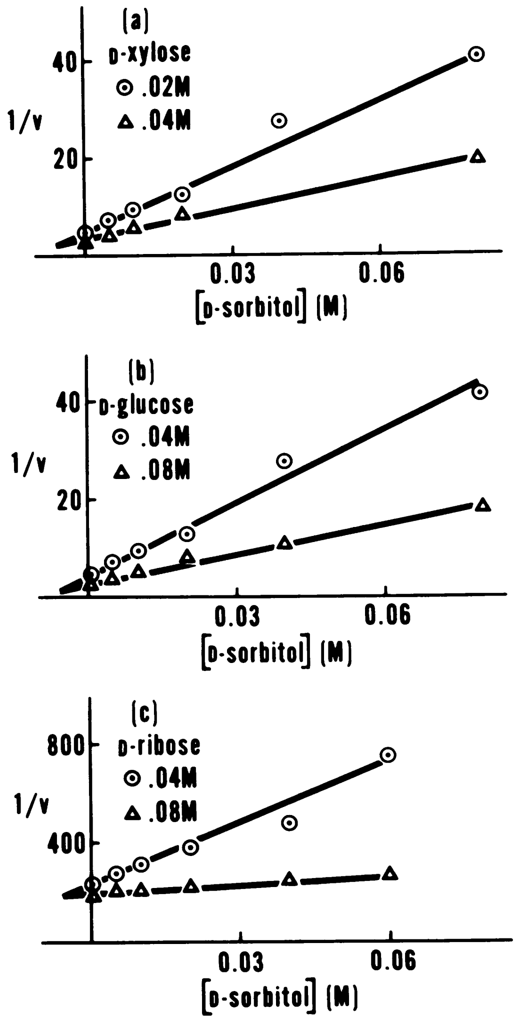

FIG. 6. *Dixon's plots for D-sorbitol inhibition of isomerizing activity where D-xylose (a), D-glucose (b), and D-ribose (c) are substrates. The reaction was carried out for 10 min in the absence of cations as described in Materials and Methods. The K_i value obtained for all three substrates corresponds to 5.5 mM.*

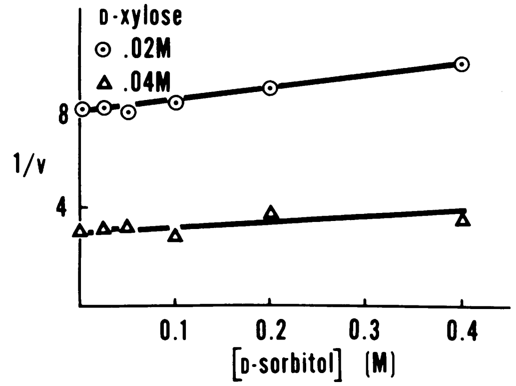

FIG. 7. *Prevention of D-sorbitol inhibition of D-xylose isomerization by Mg^{2+} plus Co^{2+}. The reaction was measured after 10 min in the presence of 8×10^{-3} M Mg^{2+} plus 4×10^{-5} M Co^{2+} by the procedure described in Materials and Methods.*

from the process. It seems probable that this could be accomplished by using the isomerase produced by NRRL 5778. Finally since the activity occurs between 70 to 80 C, either the use of mutants with altered D-fructokinase or complicated and expensive enzyme purification methods to prevent further metabolism of D-fructose are avoided. These characteristics, together with good affinity for glucose, make this isomerase a potentially useful industrial enzyme.

ACKNOWLEDGMENTS

We are indebted to M. Slodki for the gas-chromatographic analyses, to T. G. Pridham who kindly identified our organism, and to L. Nakamura, M. Slodki, and G. W. Strandberg for critical reading of the manuscript and for helpful suggestions.

S.S. was supported by a postdoctoral fellowship from the Consejo Nacional de Ciencia y Tecnologia and Asociacion Nacional de Universidades e Institutos de Ensenanza Superior, Mexico.

LITERATURE CITED

1. Anderson, R. L. 1966. D-Lyxose isomerase, p. 593-596. *In* W. A. Wood (ed.), Methods in enzymology, vol. 9. Academic Press Inc., New York.
2. Cotter, W. P., N. E. Lloyd, and C. W. Hinman. 1971. Method for isomerizing glucose syrups. U.S. Patent 3,623,953.
3. Danno, G. 1970. Studies on D-glucose-isomerizing enzyme from *Bacillus coagulans*, strain HN-68. Part V. Comparative study on the three activities of D-glucose, D-xylose, and D-ribose isomerization of the crystalline enzyme. Agric. Biol. Chem. **34**:1805-1814.
4. Danno, G. 1971. Studies on D-glucose-isomerizing enzyme from *Bacillus coagulans*, strain HN-68. Part VI. The role of metal ions on the isomerization of D-glucose and D-xylose by the enzyme. Agric. Biol. Chem. **35**:997-1006.
5. Dische, Z., and E. J. Borenfreund. 1951. A new spectrophotometric method for the detection and determination of keto sugar and trioses. J. Biol. Chem. **192**:583-587.
6. Dixon, M. 1953. The determination of enzyme inhibition constants. Biochem. J. **55**:170-171.
7. Dmitriev, B. A., L. V. Backinowsky, O. S. Chizhov, B. M. Zolotarev, and N. K. Kochetkov. 1971. Gas-liquid chromatography and mass spectrometry of aldononitrile acetates and partially methylated aldononitrile acetates. Carbohydr. Res. **19**:432-435.
8. Jacobziner, H., and H. W. Raybin. 1961. Accidental cobalt poisoning. Arch. Pediat. **78**:200-205.
9. Jeanes, A., C. S. Wise, and R. J. Dimler. 1951. Improved techniques in paper chromatography of carbohydrates. Anal. Chem. **23**:415-420.
10. Kasumi, T., K. Kawashima, and N. Tsumura. 1974. Immobilization of glucose isomerase by entrapping in cross-linked polyacrylamide gel. J. Ferment. Technol. **52**:321-327.
11. Kawana, M., H. Ohriu, and S. Emoto. 1968. A facile synthesis of 1,2:5,6 di-O-cyclohexylidene-α-D-allose. Bull. Chem. Soc. (Japan) **41**:2199-2201.
12. Levy, H., V. Levison, and A. Shade. 1950. The effect of cobalt on the activity of certain enzymes in homogenates of rat tissue. Arch. Biochem. **27**:34-40.
13. Lineweaver, H., and O. Burk. 1934. The determination of enzyme dissociation constants. J. Am. Chem. Soc. **56**:658-666.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
15. Magasanik, B. 1961. Catabolite repression. Cold Spring Harbor Symp. Quant. Biol. **26**:249-256.
16. Marshall, R. O., and E. R. Kooi. 1957. Enzymatic conversion of D-glucose to D-fructose. Science **125**:648-649.
17. Pridham, T. G., P. Anderson, C. Foley, L. A. Lindenfelser, C. W. Hesseltine, and R. G. Benedict. 1957. A selection of media for maintenance and taxonomic study of *Streptomyces*. Antibiot. Ann. **57**:947-953.
18. Schroeder, H. A., A. P. Nason, and I. H. Tipton. 1967. Essential trace elements in man: cobalt. J. Chronic Dis. **20**:869-870.
19. Somers, E. 1974. The toxic potential of trace metals in foods. A review. J. Food Sci. **39**:215-217.
20. Strandberg, G. W., and K. L. Smiley. 1971. Free and immobilized glucose isomerase from *Streptomyces phaeochromogenes*. Appl. Microbiol. **21**:588-593.
21. Takasaki, Y., and Y. Kosugi. 1969. *Streptomyces* glucose isomerase, p. 561-570. *In* D. Perlman (ed.), Fermentation advances. Academic Press Inc., New York.
22. Tsumura, N., M. Hagi, and T. Sato. 1967. Enzymatic conversion of D-glucose to D-fructose. Part VIII. Propagation of *Streptomyces phaeochromogenes* in the presence of cobaltous ion. Agric. Biol. Chem. **31**:902-907.
23. Tsumura, N., and T. Sato. 1965. Enzymatic conversion of D-glucose to D-fructose. VI. Properties of the enzyme from *Streptomyces phaeochromogenes*. Agric. Biol. Chem. **29**:1129-1134.
24. Yamanaka, K. 1968. Purification, crystallization and properties of the D-xylose isomerase from *Lactobacillus brevis*. Biochim. Biophys. Acta **151**:670-680.
25. Yamanaka, K., and K. Isumori. 1973. Purification and properties of L-arabinose isomerase from *Streptomyces* sp. Agric. Biol. Chem. **37**:521-526.