Properties of d-Xylose Isomerase from Streptomyces albus

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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 $\mu$mol/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 0.06% MgSO$_4$·7H$_2$O, 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/in$^2$. The final enzyme preparation had a specific activity for d-glucose of 1.085 $\mu$mol/min per mg of protein, corresponding to a two-fold purification over the crude extracts. The enzyme preparation was stored at -5 C.
Enzyme assays. A 1-ml mixture containing 16 μmol of MgSO₄·7H₂O, 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 CoCl₂·6H₂O 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-β-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 aldonoitrile 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 albicoides 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.

Mg²⁺ (1.0 mM) either as chloride or as sulfate strongly stimulated D-glucose isomerase activity extracted from the microorganism under study (Table 1); Co²⁺ and Mn²⁺ at the same concentrations were weak activators. When the activity was tested in several Mg²⁺ or Co²⁺ concentrations (Fig. 2), the maximal activity obtained in the presence of Co²⁺ was 25% of that with Mg²⁺. Respective affinity constants for Mg²⁺ and Co²⁺ of 0.3 and 0.003 mM (Fig. 3) were graphically determined (13).

An additive effect resulted from the presence of both Mg²⁺ and Co²⁺. 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 Co²⁺ at different Mg²⁺ levels. Lineweaver-Burk treatment of the data demonstrated that although Co²⁺ does not alter the Kₘ for Mg²⁺ it increases Vₘₐₓ (Fig. 4). This observation suggests that the cations do not compete for a common binding site on the enzyme molecule. Mg²⁺ and Co²⁺ either singly

**TABLE 1. Activation of D-glucose isomerase from S. albicoides NRRL 5778 by divalent cations**

| Metal salt (1 mM) | Relative activity (%) |
|------------------|----------------------|
| None             | 9.85                 |
| FeSO₄            | 0.0                  |
| CuSO₄            | 0.0                  |
| CaCl₂            | 0.0                  |
| NiSO₄            | 5.32                 |
| MnCl₂            | 13.15                |
| CoCl₂            | 28.5                 |
| MgCl₂            | 100.0                |
| MgSO₄            | 100.0*               |
| MgSO₄ plus CoCl₂ | 137.5                |

*The enzyme was dialyzed overnight against de-mineralized distilled water to remove metallic ions.

*The activity was assayed as described in Materials and Methods but omitting the MgSO₄.

*The activity in the presence of MgSO₄ was set as 100%.
PROPERTIES OF D-XYLOSE ISOMERASE FROM S. ALBUS

![Graph](image.png)

**Fig. 2.** Effect of Co²⁺ (Δ) and Mg²⁺ (O) concentrations on d-glucose isomerase activity. Conditions as described in Materials and Methods but varying Mg²⁺ and Co²⁺ concentrations.

**Fig. 3.** Plots of the reaction rate of D-glucose isomerase with varying Co²⁺ and Mg²⁺ 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 aldono-nitrile 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...
reported on an isomerase from a *Streptomyces* sp. which is active at 35 °C and specific only for D-ribosel.

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-ribosel) 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* values of 5.5 mM which also tends to confirm the single-enzyme hypothesis. Sorbitol inhibition of L-ribosel and L-rhamnosel 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.

**DISCUSSION**

The catalytic conversion of D-glucose to D-fructose was studied using a partially purified enzyme with isomerizing activity obtained from *S. albus* NRRL 5778. In addition to D-glucose, this enzyme also isomerizes D-xylose, D-ribose, D-allose, L-ribosel, and L-rhamnosel. Steric correlations were found in these carbohydrates since, with the exception of L-rhamnosel, all of 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-ribosel further reduces the amount of isomerizations by the enzyme.

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

| Substrate (80 mM) | Activitya (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-ribosel        | 0.0369                                                 |
| D-Arabinose      | 0.0                                                    |
| D-Lylose         | 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 (%) | Relative isomerization (%)a |
|-------------------|-----------------------------|
|                   | D-Xylose | L-ribose | 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     | 4.88     | 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*         | 100.0    | 100.0    | 100.0    | 100.0     | 100.0      |

a The activity obtained with D-xylose as inducer was set as 100%.

Hexoses or methylpentoses 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*), 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

| Metal addition | l-Arabinose | l-Rhamnose | D-Glucose | D-Ribose | D-Xylose |
|----------------|-------------|------------|-----------|----------|----------|
| None           | 100.0       | 100.0      | 100.0     | 100.0    | 100.0    |
| MgSO₄         | 100.0       | 100.0      | 9.85      | 6.75     | 36.5     |
| CoCl₂         | 100.0       | 100.0      | 100.0     | 100.0    | 100.0    |

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

Mg²⁺ alone has been reported, Co²⁺ is apparently required to obtain maximum glucose isomerase formation (20-22). Even though Co²⁺ is an essential trace metal for human nutrition (18), it is toxic at higher levels (8, 19). The Co²⁺ 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 Co²⁺ content to acceptable levels. From the standpoint of human health, it would be desirable to eliminate Co²⁺.
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 and 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.

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