

## Characterization of D-Xylose Isomerase from *Streptomyces albus*

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### *Streptomyces albus*의 D-Xylose Isomerase의 性質에 관하여

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#### ABSTRACT

*Streptomyces albus* T-12 which had been isolated and identified in the laboratory, was selected for the studies on the cultural conditions on the production of D-xylose isomerase and the enzymological characteristics using the partially purified enzyme.

The best results in the enzyme production came from D-xylose medium than wheat bran. The divalent metal ions such as  $\text{Co}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Cu}^{2+}$  retard or inhibit the cell-growth at the early stages of mycelia propagations, and T-12 strain is especially sensitive to  $\text{Co}^{2+}$ .

After 60 hours of shaking cultivation at 30°C and 200 rpm, a maximum enzyme activity, 0.49 enzyme units, was obtained. Cell-free enzyme obtained from mycelia heat-treated in the presence of 0.5mM  $\text{Co}^{2+}$ , showed a 2.4-fold increase in specific activity than the enzyme from untreated mycelia. The specific activity of the purified enzyme through Sephadex G-150 column showed 180 fold to the crude enzyme.

The effective activators of the enzyme appeared to be  $\text{Mg}^{2+}$  and  $\text{Co}^{2+}$  ions, and it exhibited the maximal enzyme activity showed at pH 7.0 and at temperature around 80°C when  $\text{Mg}^{2+}$  and  $\text{Co}^{2+}$  ions were added. The enzyme isomerized D-glucose, D-xylose, D-ribose, L-arabinose, D-mannose, and L-rhamnose in the presence of  $\text{Mg}^{2+}$  and  $\text{Co}^{2+}$  ions as an activators.  $\text{Mg}^{2+}$  and  $\text{Co}^{2+}$  ions were non-competitively bound at different allosteric sites of enzyme molecule.  $\text{Mg}^{2+}$  (5mM) or  $\text{Co}^{2+}$  (1.0mM) protected against the thermal denaturations of the enzyme activities.

The Michaelis constant ( $K_m$ ) and  $V_{max}$  values of the enzyme for D-glucose and D-xylose were 0.52M, 2.12  $\mu\text{moles/ml}\cdot\text{min.}$  and 0.28M, 0.65  $\mu\text{moles/ml}\cdot\text{min.}$ , respectively.

## INTRODUCTION

D-xylose isomerase (D-xylose ketol-isomerase, EC 5.3.1.5), also known as "D-glucose isomerase" or "sugar isomerizing enzyme", catalyzes the isomerization of D-xylose to D-xylulose and of D-glucose to D-fructose or vice versa in the divalent metal ions for the activity.

The enzymic interconversion between D-glucose and D-fructose from *Pseudomonas hydrophilla* was initially reported by Marshall and Kooi (1957). The enzyme is an intracellular enzymes induced by D-xylose as a sole carbon source.

After the discovery in 1961 of a D-glucose isomerase from *Aerobacter cloacae* (Tsumura and Sato, 1961), other microbial enzyme sources, including *Escherichia intermedia* (Natake and Yoshimura, 1964), *Lactobacillus brevis* (Yamanaka, 1968), *Bacillus congulaas* (Danno *et al*, 1966; Danno, 1970, 1971, 1973), and several *Streptomyces* species (Tsumura and Sato, 1965; Tsumura *et al*, 1967; Takasaki, 1966, 1971; Strandberg *et al*, 1971; Park *et al*, 1974, 1976; Sanchez *et al*, 1975), have been studied. Especially, Tsumura and Sato (1965) and Takasaki *et al* (1967) have intensively studied on the inducible intracellular enzyme with D-xylose from *Streptomyces* species, since these enzymes had a more stable and more active form than *Aerobacter cloacae* (Tsumura *et al*, 1965).

Subsequent studies on the *Streptomyces* enzyme revealed that it possessed high stability (Takasaki *et al*, 1969; Tsumura *et al*, 1971; Park and Toma, 1974; Chou *et al*, 1976) which permitted its extraction and manipulation for a long times at room temperatures. Additionally, it was in

general strongly activated by a specific divalent cations such as  $Mg^{2+}$ , and/or  $Co^{2+}$  and  $Mn^{2+}$  for a functioning catalytic action (Yamanaka, 1963; Danno, 1970; Yoshimura *et al*, 1966). And it exhibited optimum activity at temperatures around 70—80°C; the high temperature prevents the further metabolism of the product by undesirable enzymes when crude extracts are used as an enzyme reaction sources. And these divalent cations also protected the enzyme function from thermal denaturation (Tsumura *et al*, 1965; Takasaki *et al* 1969; Sanchez *et al*, 1975; Chou *et al*, 1976; Park *et al*, 1974, 1976). The catalytic effect of D-xylose isomerase was inhibited by  $Cu^{2+}$ ,  $Hg^{2+}$ , and  $Zn^{2+}$ , and to some extent by  $Ca^{2+}$ . Other inhibitors were xylitol and D-sorbitol etc.

The enzyme, furthermore, showed a diverse substrate specificity according to their origin of enzyme sources. The isomerizing enzyme found in *Lactobacillus brevis* (Yamanaka, 1968), *Bacillus coagulans* (Danno, 1970) catalyzed D-xylose and D-ribose as well as D-glucose to their respective ketoses, but D-ribose isomerizing activity was not detected in *Streptomyces* sp. (Takasaki *et al*, 1969; Tsumura *et al*, 1965). *Streptomyces albus* (Sanchez *et al*, 1975) enzyme, however, had a specific activity for D-xylose, D-glucose, D-ribose, D-allose, L-arabinose and L-rhamnose.

The functional role of activator such as divalent cation altered the substrate specificity of the enzyme (Sanchez *et al*, 1975). After all, D-xylose isomerase was detected in a various substrate specificity.

It seems, therefore, very interesting to characterize a D-xylose isomerase concerning the substrate affinity and the metal ion effect as a cofactor.

In order to study these enzymatical

characteristics, *Streptomyces albus* T-12 which had been isolated from soil and identified in our laboratory was chosen among the several *Streptomyces*. Additionally, the growth condition for enzyme production, the partial purification and some related enzymatic properties are investigated.

## MATERIALS AND METHODS

### 1. Organism and cultural conditions

*Streptomyces albus* T-12 was selected from the strains which were identified in this department.

All cultures were grown on the modified RM medium containing 1.0% carbon source, 1.0% peptone, 0.5% yeast extract, 0.3% malt extract, 0.5% NaCl, and 0.1%  $MgSO_4 \cdot 7H_2O$ , and adjusted to pH 7.2. The cultures were incubated at 30°C on the reciprocal rotary shaker at 200rpm for 60 hours.

This strain was cultured under various cultural conditions, the different carbon sources and inorganic metal ions.

### 2. Enzyme preparation

After incubation for 60 hours, the hyphal mycelia were harvested by filtering method with Whatman Filter Paper No. 2, washing three times with 0.01M phosphate buffer, pH 7.2, and resuspended in a appropriate volume of the same buffer containing 5mM  $Co^{2+}$  or  $Mg^{2+}$  ion.

The mycelial suspensions were heated at 70°C for 30 minutes, and treated with lysozymes (11mg/g of wet cell weight), and then incubated at 40°C for about 24 hrs.

Whole cell and cell-debris were removed by centrifugation, Sorvall SS-3 centrifuger, at 15,000g for 20 minutes. Resulting

supernatant was used in following experiment as a crude enzyme preparation. The enzyme preparation was stored at 4°C.

### 3. Determination of protein concentration

Protein concentration was determined by the method of lowry *et al* (195) using eggs albumin (Wako Chemical Ind., Ltd) as a standard. For the determination of protein concentration in the elutes from the column chromatography, optical density by Hitachi Recording UV Spectrophotometer Model EPS-3T at 280nm was measured.

### 4. Assay of enzyme

The incubation mixture containing 0.2ml of enzyme solution and 0.2ml of 5mM  $Mg^{2+}$  solution was preincubated for 5 min. at 70°C. To start the enzyme reaction, 200  $\mu$ moles of each carbohydrate in 0.6ml of phosphate buffer (pH 7.2) was added to the preincubated mixture. After this enzyme reaction system was incubated at 70°C for 10 minutes, the ketoses formed were assayed by modified cystein-carbazole method (Dische *et al*, 1951).

In a control system, phosphate buffer solutions (pH 7.2) were added in place of carbohydrates solution. Color developed at room temperature for 10 minutes was detected at 560nm for D-fructose and 530nm for the others by Turner Spectrophotometer Model 350.

One unit of these enzymic activities was defined as the enzyme amount which produced one  $\mu$ mole of ketoses per one minute under the condition specified above.

### 5. Microbial cell growth

The mycelial growth was determined by measuring the absorbance of the cell suspension at 660nm on spectrophotometer.

## 6. Partial purification of isomerizing enzymes

### (1) Ammonium sulfate fractionation

To the above crude enzyme solutions ammonium sulfate powder was added by slow stirring, until 30% saturation and then removed the precipitate by centrifugation at 15,000g for 10 minutes. Next fractionation was carried out by salting out with 80% of saturation ammonium sulfate for 24 hours at 4°C. The supernatant, which had no activities, was removed by centrifugation at 15,000g for 20 minutes. After centrifugation, the precipitate obtained was dissolved in 5ml 0.01M phosphate buffer (pH 7.2), and dialyzed against tertiary distilled water for 2 days. The precipitate resulted was removed by filtering through the Glass Filter.

### (2) Column chromatography on Sephadex G-150

After dialysis against tertiary distilled water, 5ml of enzyme solution was applied to a column (2.0×70.0cm) of Sephadex G-150 which had been previously equilibrated with 0.01M phosphate buffer (pH 7.2). The enzyme was then eluted out with 400ml of 0.01M phosphate buffer (pH 7.2) from the Sephadex column and each 4ml aliquot of eluent was collected.

## RESULTS

**Table 1.** The activities of the D-xylose isomerase from the various strains isolated in the laboratory

Strains	Species	Activities (U/ml)
T-12	<i>S. albus</i>	2.79
KW	<i>S. chrysomallus fumingatus</i>	2.04
K-16	<i>S. olivaceus</i> or <i>coelicolor</i>	1.23
S-48	<i>S. pilosus</i>	0.76
K-2	<i>S. catenulae</i>	0.48

## 1. Selection of strains

The isomerizing activities of D-xylose isomerases from 16 *Streptomyces* spp. which had been isolated in the laboratory were detected. One of them, *Streptomyces albus* T-12, showed the highest activity of isomerization (Table 1). This species was used for the enzymatic studies in this experiment.

## 2. Cultural conditions

### (1) Effect of carbon sources

*Streptomyces* D-xylose isomerase is an inducible enzyme requiring the presence of D-xylose or xylan as a carbon source and inducer in the culture medium for its production. Table 2 shows the production of D-xylose isomerase by D-xylose or various xylan in wheat bran and D-xylose plus D-glucose, and D-glucose alone. The enzyme activity of the cells grown on the medium containing D-xylose was 2.1 times more than in wheat bran extracts, and 3.5 times more than when D-xylose was supplemented with 0.3% D-glucose, and its activity of grown only in D-glucose was not nearly detected.

### (2) Effect of metal ion culture media

Stimulation of D-xylose isomerase production by metal ions was reported by Danno (1970), Tsumura (1966), Takasaki (1969), Chou *et al* (1976).

**Table 2.** Effect of carbon sources on enzyme production

Sources	Conc. <sup>1</sup> (%)	Protein (mg/ml)	Activity <sup>2</sup> (U/min·ml)	Sp·Activity (U/min·mg Protein)
D-xylose	1.0	3.30	1.40	0.42
Wheat bran extracts	2.0	1.73	0.35	0.20
D-glucose D-xylose	0.3-0.7	1.94	0.23	0.12
D-glucose	1.0	1.76	0.07	0.04

1. Basal medium; Peptone 1%, yeast extract 0.5%, malt extract 0.3%, MgSO<sub>4</sub> 7H<sub>2</sub>O 0.1%
2. Culture time 60 hours

**Table 3.** Effect of metal ion in the culture media<sup>1</sup>

Metal salts (1mM)	Protein (mg/ml)	Activity <sup>2</sup> (U/min·ml)	Sp. Activity (U/min·mg protein)
None	3.3	1.40	0.42
CuSO <sub>4</sub>	N		
CaCl <sub>2</sub>	2.0	0.62	0.31
MnCl <sub>2</sub>	2.4	0.78	0.32
FeSO <sub>4</sub>	I		
ZnSO <sub>4</sub>	I		
LiCl <sub>2</sub>	N		
CoCl <sub>2</sub>	N		

1. Basal medium; D-xylose 1%, peptone 1%, yeast extract 0.5%, malt extract 0.3%, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.1%

2. Culture time; 60 hours

N; Growth rate was very poor or negligible.

I; 1mM of metal concentrations was inhibitory.

**Table 3** shows the inhibitory effect of the growth of mycelium by 1mM of the metal ions such as Fe<sup>2+</sup>, Zn<sup>2+</sup>, Co<sup>2+</sup>. The growth rate, furthermore, was strongly inhibited by Cu<sup>2+</sup> and Li<sup>2+</sup>.

**Fig. 1** shows the effect of Co<sup>2+</sup> on the mycelium propagation according to the various concentrations of Co<sup>2+</sup>. The low concentrations of Co<sup>2+</sup> caused retardation of the growth rate, whereas the absence of Co<sup>2+</sup> gave the best results (**Table 4**). A maximum specific activity was obtained after 70 hours of cultivation in the media without Co<sup>2+</sup>.

### 3. Partial purification of D-xylose

#### isomerase

- (1) Heat treatment on enzyme preparations

Heat treatment to the harvested mycelial suspension in the presence of 0.5mM Co<sup>2+</sup> or 1.0mM Mg<sup>2+</sup> at 70°C for 30 minutes was found to be effective partial purification technique for D-xylose isomerase.

The specificities were measured for cell-free enzyme, obtained from heat-treated mycelia in the absence of Co<sup>2+</sup> or Mg<sup>2+</sup> and from mycelia nonheat-treated in the presence of Co<sup>2+</sup>/Mg<sup>2+</sup> were about 0.50 (**Table 5**). The Specific activity of the cell-free enzyme obtained from cells heat-treated

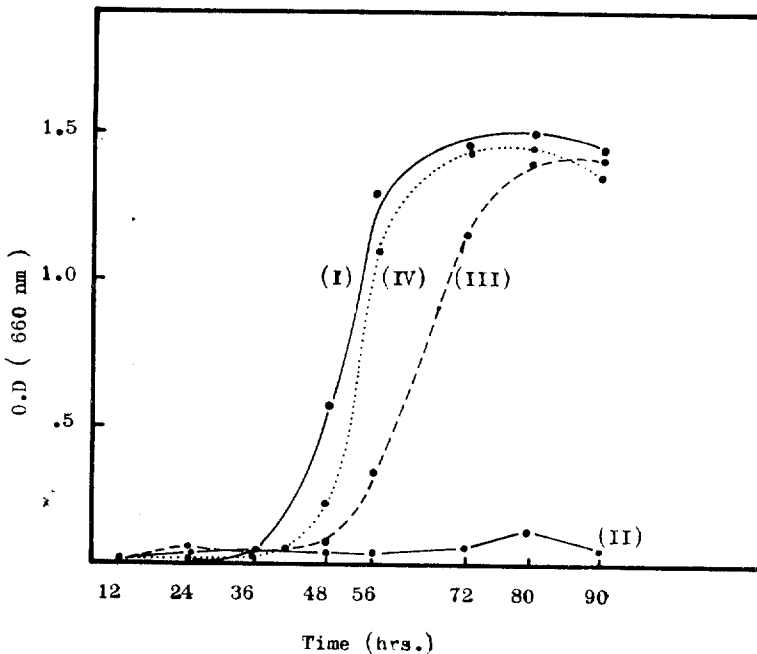
**Table 4.** Effect of  $\text{Co}^{2+}$  ion on the growth course of D-xylose isomerase activity

Conc. of $\text{Co}^{2+}$ ion (mM)	Sp. activity (U/ml·min·mg of pro.)		
	48hrs	72hrs	90hrs
None	0.38	0.53	0.26
.005	0.36	0.40	0.26
.05	Negligible	0.28	0.18
.5	Negligible	Negligible	Negligible

**Table 5.** Effect of heat treatment and  $\text{Co}^{2+}$  or  $\text{Mg}^{2+}$  on the enzyme activity

Treatment on mycelium suspension	Cell-free enzymes		
	Activity (U/ml/min)	Protein (mg/ml)	Sp. activity (U/min·mg pro.)
None	0.36	0.74	0.49
Heated in absence of $\text{Co}^{2+}/\text{Mg}^{2+}$	0.18	0.36	0.50
Heated in presence of 0.5mM $\text{Co}^{2+}$	0.76	0.64	1.18
Heated in presence of 1.0mM $\text{Mg}^{2+}$	1.08	1.06	1.02
Non-heated in presence of 0.5mM $\text{Co}^{2+}$	0.15	0.31	0.48
Non-heated in presence of 1.0mM $\text{Mg}^{2+}$	0.48	0.94	0.51

Heated at 70°C for 30 minutes on harvested mycelial suspension



**Fig. 1** Effect of  $\text{Co}^{2+}$  on the mycelium propagation of *Streptomyces albus* T-12. Each media contains a D-xylose, but (I) no addition of  $\text{Co}^{2+}$ , (II) in the presence of  $\text{Co}^{2+}$  (0.5mM), (III) in the presence of  $\text{Co}^{2+}$  (0.05mM), and (IV) 0.005mM of  $\text{Co}^{2+}$  ions, respectively.

in the presence of  $\text{Co}^{2+}$  exhibited a 2.4-fold increase than that of nonheat-treated mycelia in the absence of  $\text{Co}^{2+}/\text{Mg}^{2+}$  preparations.

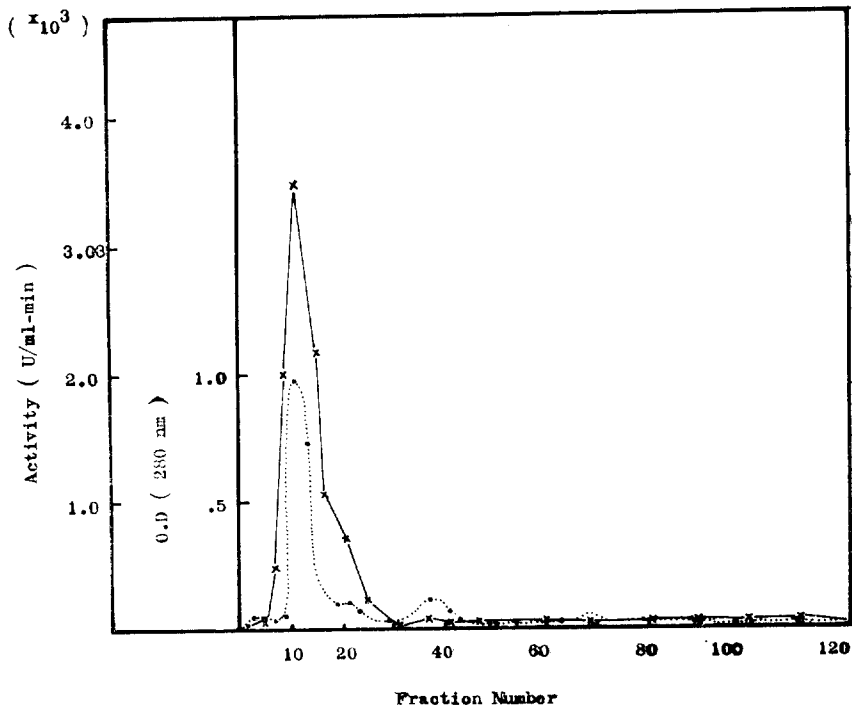
For the further purification of enzymes, fractionation of the enzyme by salting out with ammonium sulfate from the cell-free extract which was treated with heat was carried out. After centrifugation, the precipitate was re-heated at the same temperature described above in the presence of  $0.5\text{mM Co}^{2+}$ . This preparations showed a very effective purification for this enzyme.

#### (2) Column chromatography on Sephadex G-150

After dialysis of precipitated protein against tertiary distilled water for two days, 5ml of enzyme solutions were

applied on a Sephadex G-150 column ( $2.0 \times 70.0\text{cm}$ ) equilibrated with  $0.01\text{M}$  phosphate buffer at  $\text{pH } 7.2$ . As shown on Fig. 2, each 4ml aliquot of eluent was collected in one of the tubes of a fraction collector. One major peak and two minor peaks were appeared with regard to protein. D-glucose isomerizing activities were largely shown on chromatogram from fraction number's 9 to 25, (Fig. 2).

The purified enzyme activity on Sephadex G-150 column chromatography shows 180-fold than that of crude enzyme. The specific activities of D-glucose, D-xylose, and D-ribose isomerization are summerized together with the ratio of D-xylose, D-ribose isomerizing activities over D-glucose isomerizing activities, which were 1.7



**Fig. 2** Chromatogram of D-xylose isomerizing enzymes on Sephadex G-150 column.

The column ( $2.0 \times 70.0\text{cm}$ ) was eluted with  $0.01\text{M}$  phosphate buffer at  $\text{pH } 7.2$ . Each fraction volume was  $4.0\text{ml}$ . X—X, D-xylose isomerizing activities; protein.

**Table 6.** Partial purification of D-xylose isomerase by heating, ammonium sulfate and column chromatography on Sephadex G-150

	Total Protein (mg)	Total activity (Sp. activity; U/ml-min-mg protein)			Ratio of		Relative Recovery (%)
		D-glucose	D-xylose	D-ribose	X/G	R/G	
Heated cell-free enzyme	358.3	404.1 (1.10)	673.6 (1.88)	365.5 (1.02)	1.71	0.93	100
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> I, II. Sat.	89.8	143.6 (1.59)	239.0 (2.66)	132.3 (1.47)	1.67	0.92	25.1
Heat treated supernatant	44.8	7,456.8 (166.5)	12,900 (288.0)	6,958.0 (155.4)	1.73	0.93	12.5
Sephadex G-150	27.2	5,412.8 (199.0)	9,201.8 (338.3)	5,032.0 (185.1)	1.70	0.92	7.6

and 0.9, respectively (Table 6).

#### 4. Enzymic properties of D-xylose isomerase

##### (1) Effect of metal ion on the activities

The various kinds of metal ions used in this experiment are listed on Table 7. The effects of metal ions on the activation of D-xylose isomerase were expressed by relative activities compared with that of MgSO<sub>4</sub>. As shown on Table 7, Mg<sup>2+</sup> (1.0 mM) either as chloride or as sulfate salt strongly stimulated the enzyme activities. And Co<sup>2+</sup> ion at the same concentrations was slightly effective. The enzyme furtherm-

ore, was activated as by 1.5-fold combined Mg<sup>2+</sup>(1.0mM) and Co<sup>2+</sup>(1.0mM) as compared with Mg<sup>2+</sup> alone.

##### (2) Substrate specificity

As shown on Table 8, the enzyme efficiently isomerized D-xylose, D-glucose and D-ribose to their respective ketoses and a lesser extent L-arabinose D-mannose, D-galactose and L-rhamnose in the presence of Mg<sup>2+</sup> (1.0mM). It was also found that D-mannitol, D-sorbitol and D-glucose-6-phosphate could not isomerized.

##### (3) Effect of temperature, and pH on the enzyme activity

**Table 7.** Activation of D-xylose isomerase from *Streptomyces albus* T-12 by divalent cation

Metal salts (5mM)	Relative activity (%)
None	0.09
FeSO <sub>4</sub>	0.0
CuSO <sub>4</sub>	0.0
ZnSO <sub>4</sub>	0.0
CaCl <sub>2</sub>	0.0
Na-arsenate	0.0
MnCl <sub>2</sub>	0.0
CoCl <sub>2</sub>	15.7
MgCl <sub>2</sub>	98.9
MgSO <sub>4</sub>	100
Mg <sup>2+</sup> +CO <sup>2+</sup>	145

70°C incubation for 10 minutes



Time course studies showed the rate of fructose formation to be nearly constant for one hour at 70°C (Fig. 3) in the presence of 1M of D-glucose and 5mM of  $Mg^{2+}$  and 0.01M phosphate buffer (pH 7.2). The fructose formed reached at maximal quantity after 30 minutes. The time of half activities of maximum is about 10 minutes. So it would be determined the enzyme reaction time as 10 minutes.

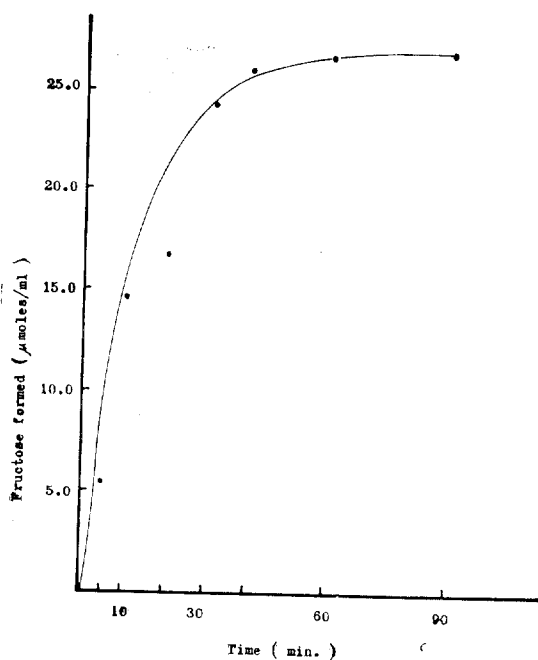


Fig. 3 Time course of D-xylose isomerase activity at 70°C

The reaction mixtures were incubated at each temperatures indicated in Fig. 4 for 10 minutes. The activity functioned optimally at 70 to 80°C when the reaction mixtures were in the presence of  $Mg^{2+}$  or  $Mg^{2+}$  and  $Co^{2+}$  combined. The activities in the presence of lower  $Mg^{2+}$  concentrations (0.1mM), furthermore, were lesser than in the presence of 5.0mM  $Mg^{2+}$  concentrations. Especially, the activ-

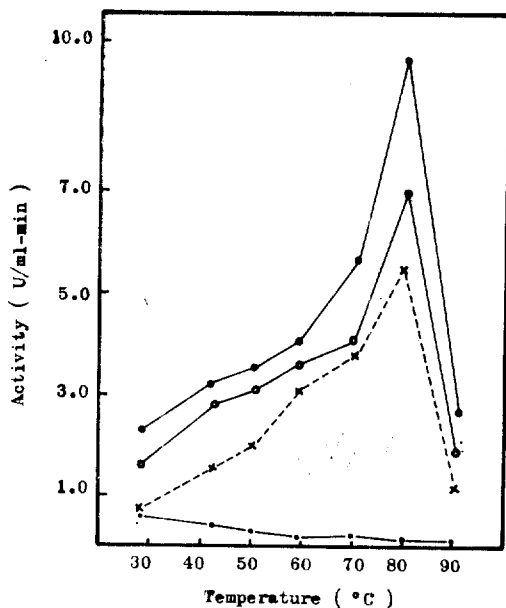


Fig. 4 Effect of Temperature on D-xylose isomerase activity

The reaction mixtures incubated at each temperatures for 10 min.: —•—, without metal ions; x-----x, with  $Mg^{2+}$  (0.1mM); —, with  $Mg^{2+}$  (5.0mM); o—o, with  $Mg^{2+}$  and  $Co^{2+}$  (5.0mM + 1.0mM).

ities were gradually decreased in the absence of metal ion according to increasing temperature

When D-xylose isomerase activity was examined in the pH range of 5.3 to 11.0 using potassium-phosphate buffers (0.01M) and carbonate-bicarbonate buffers (0.01M), the maximal activity observed at around pH 7.0, as shown in Fig. 5.

#### (4) Effect of thermal tolerance on enzyme activity

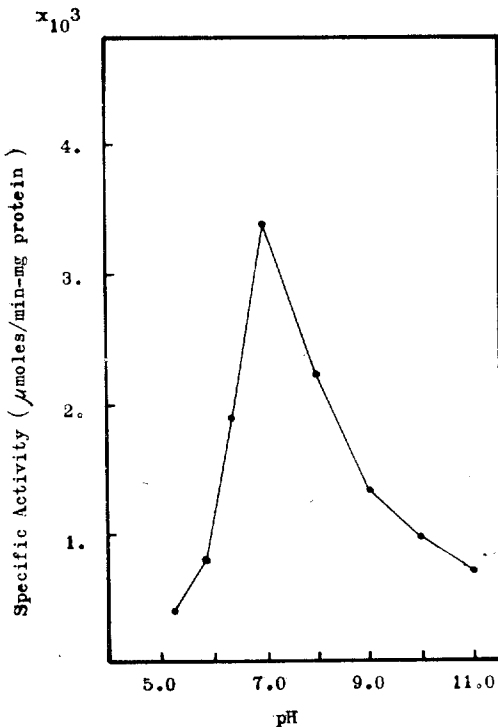
The thermal tolerance of D-xylose isomerase was examined in the presence of metal ions and in the absence of them. In the presence of  $Mg^{2+}$  (5.0mM) and  $Co^{2+}$  (1.0mM), the activities were kept nearly 100% from 30 to 80°C, but dropped

**Table 8.** Substrate specificity of isomerizing enzymes, in *Streptomyces albus* T-12

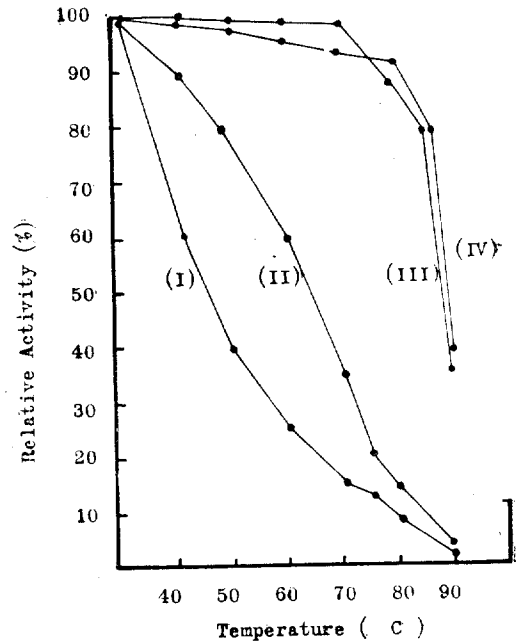
Substrates (0.1M)	Activity <sup>1</sup> ( $\mu$ moles of ketose formed/min per mg of pro.)
D-glucose	1.42
D-xylose	1.53
D-ribose	1.05
L-arabinose	.44
D-mannose	.23
D-galactose	.22
L-rhamnose	.21
D-mannitol	.0
D-sorbitol	.0
D-sorbitol	.0
Glucose-6-phosphate	.0

1. The activity was measured as described in materials and methods.

Reaction Time; 10 minutes at 70°C

**Fig. 5** Effect of pH on D-xylose isomerase activity

potassium phosphate buffers (0.01M) were used for pH values of 5.3 to 8.0 and carbonate-bicarbonate buffers (0.01M) from pH 9.0 to 11.0

**Fig. 6** Thermal tolerance of D-xylose isomerase activity in the presence of metal ions.

The enzyme was treated for 10 minutes at various temperatures without substrate under the following conditions: without divalent cation (I); 0.1mM  $MgSO_4$  (II); 1.0mM  $CoCl_2$  (III); and 5.0mM  $MgSO_4$  (IV). After heat treatment, the activity was measured as described in materials and methods.

down rapidly in the absence of metal ion in the presence of lower concentration  $Mg^{2+}$  (0.1mM) in spite of lower temperatures (Fig.6).

(5) The  $Mg^{2+}$  and  $Co^{2+}$  binding site on the enzyme

The experiment designed in Fig. 7 was to determine whether the binding site of  $Mg^{2+}$  and  $Co^{2+}$  was the same or not. Fig. 7 is the double reciprocal plot of the reaction rate as a function of various  $Mg^{2+}$  concentrations and  $Mg^{2+}$ - $Co^{2+}$  (0.04mM) under the fixed concentrations. D-glucose

Mg<sup>2+</sup> and Co<sup>2+</sup> are not competitive with each other for binding on the allosteric site of enzyme molecule.

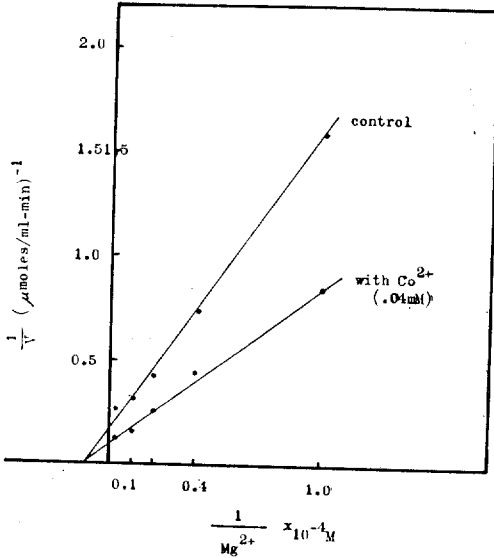


Fig. 7 Difference of the binding site of Mg<sup>2+</sup> and Co<sup>2+</sup>

(6) Enzyme kinetics of substrate concentrations

The Michaelis constants(K<sub>m</sub>) and V<sub>max</sub> values for D-xylose isomerizing enzyme calculated from the plots of Lineweaver-Burk were 0.52M, 2.12 μmoles/ml.min. for D-glucose and 0.28M, 0.65 μmoles/ml.min. for D-xylose, respectively (Figs. 8 and 9).

### DISCUSSION

Most reported microorganisms such as *Bacillus megaterium* (Takasaki & Tanabe, 1962), *Pseudomonas hydrophilla*(Marshall & Kooi, 1957), *Aerobacter aerogenes* (Natake & Yoshimura, 1963), *Paracolo-bacterium aerogenoides*(Takasaki & Tanabe, 1964), *Streptomyces phaeochromogenes* (Tsumura & Sato, 1965), *B. coagulans* (Danno *et al.*, 1967) and *Lactobacillus brevis*(Yamanaka, 1968), require D-xylose for the induction of D-xylose isomerase. *Streptomyces albus*, however, could prod-

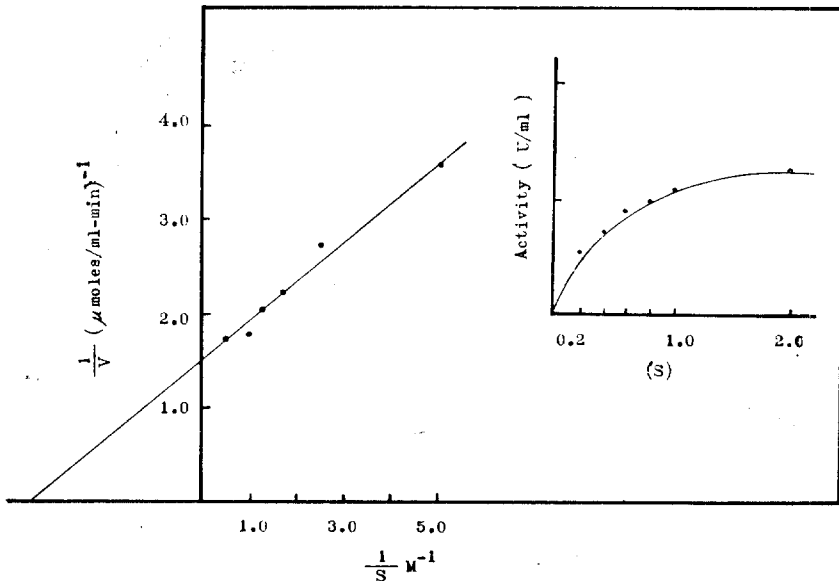
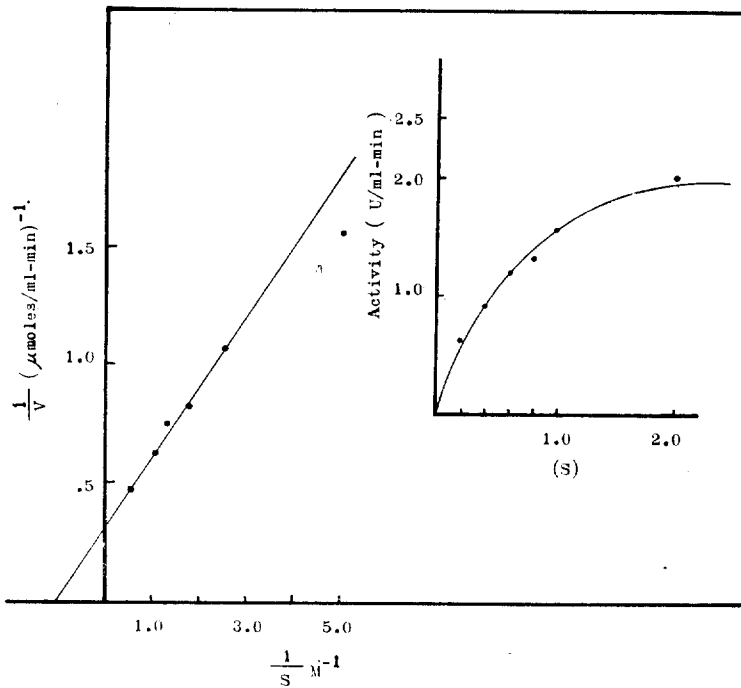


Fig. 8 Double reciprocal plot of the effect of D-glucose concentration on the reaction rate of isomerizing enzyme

The reaction mixture contained in a total volume of 1.0ml; 3.0μg of the purified enzyme, 0.01M of phosphate buffer (pH 7.2,) 1.0mM of the Mg<sup>2+</sup>, and 200μM. of D-glucose. Incubation was for 10 min. at 70°C.



**Fig. 9** Double reciprocal plot of the Effect of D-xylose concentration on the reaction rate of isomerizing enzyme

The reaction mixture contained in a total volume of 1.0ml; 3.0 $\mu\text{g}$  of the purified enzyme, 0.01M of phosphate buffer pH 7.2, 1.0mM of the  $\text{Mg}^{2+}$ , and 200 $\mu\text{m}$  of D-xylose. Incubation was for 10 min. at 70°C.

uce the enzyme in the growth medium containing either D-xylose or xylan(Takasaki *et al.*, 1969; Park *et al.*, 1974; Sanchez & Smiley, 1975; Chou *et al.*, 1976). The newly isolated strain of *Streptomyces albus* T-12 has highly produced D-xylose isomerase in the D-xylose medium(Tables 1 and 2). The fact that D-glucose did not induce detectable amount of isomerase may be due to catabolic repression by D-glucose(Magasanik, 1961).

In the requirement of metal ions for the enzyme productions, 1mM of  $\text{Co}^{2+}$  ions, especially, inhibited and retarded the growth in the early stage Takasaki *et al.*(1969) reported that *Streptomyces* sp. has produced D-xylose isomerase when it was cultured in a modified RM medium containing 1mM of  $\text{Co}^{2+}$  ions. Obviously,

the higher concentrations of  $\text{Co}^{2+}$  ion retarded the growth rate (Fig. 1). Other metal ions such as  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ca}^{2+}$  and  $\text{Cu}^{2+}$ , also, did not stimulated the growth rate.

The catalytic conversion of D-glucose to D-fructose was studied by using a partially purified enzyme obtained from *S. albus* T-12. Tsumura and Sato(1965) noted that the effect of  $\text{Co}^{2+}$  enhanced the heat stability of the enzymes. The heat stability of the enzyme might be depended on the prevention of the thermal denaturation by metal inos. In this experiments, the isomerizing enzymes, which were partially purified with column chromatography through Sephadex G-150 as shown in Fig. 2 and on Tables 5 and 6. As shown on Table 6, heat treatment

after ammonium sulfate fractionation, also, was a very effective partial purification technique.

It seems now probable that a single enzyme (D-xylose isomerase) is responsible for isomerizations of the three substrates on Table 6, since only D-xylose serves as inducer of isomerase biosynthesis and the ratios of D-xylose and D-ribose isomerization of the enzyme were constant as 1.7 and 0.9 through the whole purification procedures.

The catalytic function is strongly stimulated by  $Mg^{2+}$  at  $70^{\circ}C$ , but  $Co^{2+}$  alone at the same concentrations is about 15% of  $Mg^{2+}$  activation. When  $Mg^{2+}$  and  $Co^{2+}$  were combined, the maximal activity of the enzyme was obtained even if the enzyme was heated at  $80^{\circ}$  for 30 minutes in the presence of  $Mg^{2+}$  and  $Ca^{2+}$  ions, the activity did not change. This suggests that metal ion cause the conformational change of enzyme, which may resist the thermal denaturation.  $Mg^{2+}$  and  $Co^{2+}$  ions were non-competitively bound at different site of enzyme molecule (Fig. 7).

As shown on Table 8 and other papers the substrate specificity of the enzyme is various.

The effect of temperatures and pH on the activity of the enzyme was studied. The activity of the enzyme showed a maximal at around  $70^{\circ}C$  and pH 7.0 when the activators, such as  $Mg^{2+}$  and  $Co^{2+}$  combined or singly, were in the enzyme reaction systems. It is easy to use

the crude-enzyme in the laboratory, since the high temperature prevents the further metabolism by the other undesirable enzymes.

It is interesting to note that previously reported Km values of this enzyme from either *Streptomyces* (Tsumura and Sato, 1965) or other microbial sources (Danno, 1970; Yamanaka, 1968) showed higher affinity for D-xylose than D-glucose, except of Sanchez *et al* (1975). The significance of the low affinity for D-xylose ( $K_m=0.28M$ ) than D-glucose ( $K_m=0.52M$ ) is not understood

In the studies of the reaction mechanism of D-xylose isomerase, Keith and Mildvan (1972) studied the binary and tertiary complexes system with  $Mn^{2+}$ , substrates, and inhibitors. That is, they studied the mechanism of the enzyme conformational changes. On the contrary, Ramchander *et al* (1977) studied the mechanism of an intramolecular hydrogen transfer of each substrate.

In view of the properties of *Streptomyces albus* T-12 studied by us seems similar to those reported by other investigations with respect to metal requirement (Tsumura *et al*, 1966; 1967; Takasaki, 1966; 1967; 1974), temperature (Danno, 1970) and pH optima for enzyme activity, but is not similar in sensitivity to  $Co^{2+}$ .

In order to further study on the mechanism of isomerization of isomerizing enzymes, it must be carried out by further purified enzymes.

## 적 요

본 실험실에서 분리 동정된 여러가지 *Streptomyces*중에서 *Streptomyces albus* T-12를 선별하였다. 이 균주를 써서 D-xylose isomerase 효소를 생성키 위한 배양조건을 결정하였으며, 부분 정제된 효소액을 써서 효소학적 특성을 고찰하였다.

효소 생성은 밀기를 배지보다 D-xylose배지에서 좋았으며  $Co^{2+}$ ,  $Fe^{2+}$ ,  $Zn^{2+}$ ,  $Cu^{2+}$  같은 2가 금속이온들이 균사생장을 초기에 지연 또는 억제하였으며, 특히  $Co^{2+}$  이온에 민감하였다.

30°C에서 60시간 배양한 결과 최고의 효소능(0.49)을 얻었다  $Co^{2+}$  0.5mM 농도의 buffer용액에서 균사를 70°C, 30분간 열처리하였더니 효소의 활성이 2.4배 증가하였다. Sephadex G-150 column 크로마토그래피에서 효소 활성이 180배의 증가된 효소를 얻었다.

$Mg^{2+}$ 와  $Co^{2+}$ 이온이 가장 효과적인 활성제로 두가지 금속 이온이 있을 때 pH 7.0, 80°C에서 최적 활성도가 나타났다. 그리고 이 효소는 D-glucose, D-xylose, D-ribose, L-arabinose, D-mannose L-rhamnose 등의 기질을 isomerization하였다.  $Mg^{2+}$ 와  $Co^{2+}$  이온은 효소분자의 활성부위에 따로 결합하여 높은 온도에 의한 열변성을 억제하였다.

효소의 기질 반응도속도(Km)는 D-glucose와 D-xylose에서 각각 0.52M, 0.28M로 나타났다.

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