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Characterization of D-Xylose Isomerase from *Streptomyces albus*

Youngho KIM and Young Chil HAH

(Department of Microbiology Seoul National University, Seoul, Korea)

*Stleptomyces albus*의 D-Xylose Isomerase의 性質에 관하여

金 泳 浩·河 永 七

(서울大學校·自然科學大學·微生物學科)

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 Co^{2+}, Fe^{2+}, Zn^{2+}, and Cu^{2+} retard or inhibit the cell-growth at the early stages of mycelia propagations, and T-12 strain is especially sensitive to Co^{2+}.

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

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

The Michaelis constant(Km) and V_{max} values of the emzyme for D-glucose and D-xylose were 0.52M, 2.12μmoles/ml·min. and 0.28M, 0.65moles/ml·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 *Streptomycetes* enzyme revealed that it posessed high stability (Takasaki *et al*, 1969; Tsumura *etal*, 1971; Park aud Toma, 1974; Chou *et al*, 1976) which permited 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 denatration(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 to 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 choosen among the several *Streptomycetes*. Additionally, the growth condition for enzyeme 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 *p*H 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, *p*H 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 weght), 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 μmoles of each carbohydrate in 0.6ml of phosphate buffer (*p*H 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 (*p*H 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 μmole 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 mintes. 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 (*p*H 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 (*p*H 7.2). The enzyme was then eluted out with 400ml of 0.01M phosphate buffer(*p*H 7.2) from the Sephadex column and each 4ml aliqact of eluent war collected.

RESULTS

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 prescence 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-xyose was 2.1 times more than in wheat brean 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 1. The activities of the D-xylose isomerase from the various strains isolated in the laboratory

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

Table 2. Effect of carbon sources on enzyme production

Sources	Conc.[1] (%)	Protein (mg/ml)	Activity[2] (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%, $MgSO_4$ $7H_2O$ 0.1%
2. Culture time 60 hours

Table 3. Effect of metal ion in the culture media[1]

Metal salts (1mM)	Protein (mg/ml)	Activity[2] (U/min·ml)	Sp. Activity (U/min·mg protein)
None	3.3	1.40	0.42
$CuSO_4$	N		
$CaCl_2$	2.0	0.62	0.31
$MnCl_2$	2.4	0.78	0.32
$FeSO_4$	I		
$ZnSO_4$	I		
$LiCl_2$	N		
$CoCl_2$	N		

1. Basal medium; D-xylose 1%, peptone 1%, yeast extract 0.5%, malt extract 0.3%, $MgSO_4 \cdot 7H_2O$ 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^{2+}, Zn^{2+}, Co^{2+}. The growth rate, furthermore, was strongly inhibited by Cu^{2+} and Li^{2+}.

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

3. Partial purification of D-xylose isomerase

(1) Heat treatment on enzyme preparations

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

The specifities were measured for cell-free enzyme, obtained from heat-treated mycelia in the absence of Co^{2+} or Mg^{2+} and from mycelia nonheat-treated in the prescence of Co^{2+}/Mg^{2+} were about 0.50 (**Table 5**). The Specific activity of the cell-free enzyme obtained from cells heat-treated

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

Conc. of 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 Co^{2+} or 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 Co^{2+}/Mg^{2+}	0.18	0.36	0.50
Heated in prescence of 0.5mM Co^{2+}	0.76	0.64	1.18
Heated in prescence of 1.0mM Mg^{2+}	1.08	1.06	1.02
Non-heated in prescence of 0.5mM Co^{2+}	0.15	0.31	0.48
Non-heated in prescence of 1.0mM Mg^{2+}	0.48	0.94	0.51

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

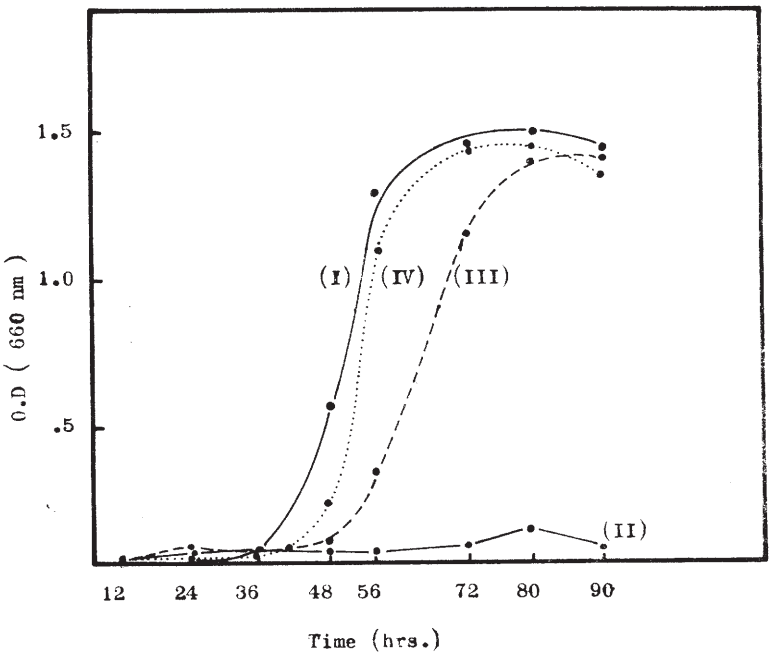

Fig. 1 Effect of Co^{2+} on the mycelium propagation of *Streptomyces albus* T-12. Each media contains a D-xylose, but (Ⅰ) no addition of Co^{2+}, (Ⅱ) in the prescence of Co^{2+} (0.5mM), (Ⅲ) in the prescence of Co^{2+}(0.05mM), and (Ⅳ) 0.005mM of Co^{2+} ions, respectively.

in the prescence of Co^{2+} exhibited a 2.4-fold increase than that of nonheat-treated mycelia in the absence of Co^{2+}/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 prescence of 0.5mM 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 (2.0×70.0cm) equilibrated with 0.01M phosphate buffer at *p*H 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 purfied 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

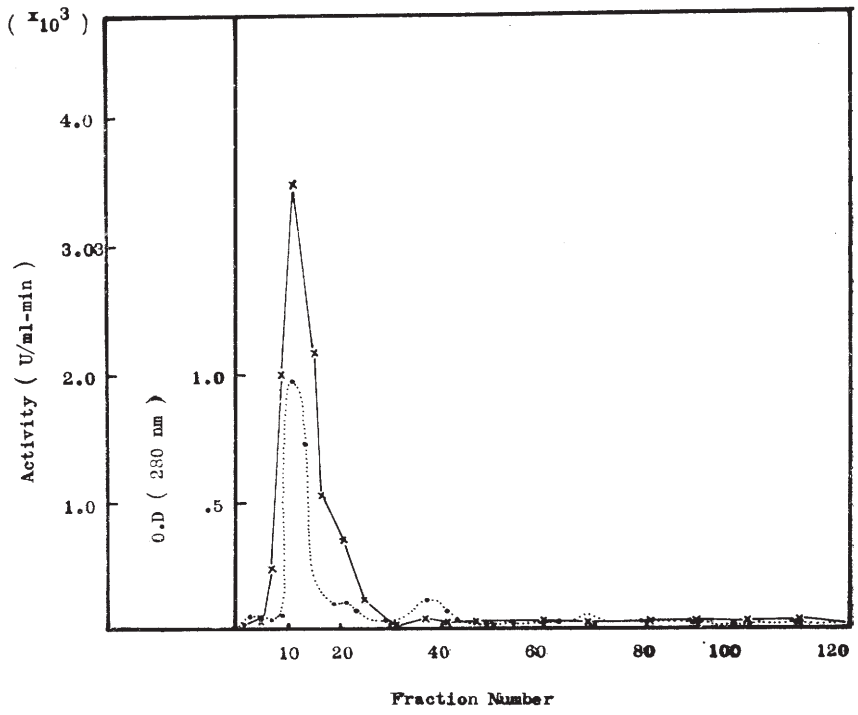

Fig. 2 Chromatogram of D-xylose isomerizing enzymes on Sephadex G-150 column.
The colmn (2.0×70.0cm) was eluted with 0.01M phosphate buffer at pH 7.2. Each fraction volume was 4.0ml. X——X, D-xylose isomerizing activities; protein.

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

	Total Prtein (mg)	Total activity (Sp. activity; U/ml-min-mg protein)			Ratio of		Relative Recovery (%)
		D-gucose	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
$(NH_4)_2SO_4$ 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 $MgSO_4$. As shown on **Table 7**, Mg^{2+} (1.0 mM) either as chloride or as sulfate salt strongly stimulated the enzyme activities. And Co^{2+} ion at the same concentrations was slightly effective. The enzyme furthermore, was activated as by 1.5-fold combined Mg^{2+}(1.0mM) and Co^{2+}(1.0mM) as compared with Mg^{2+} 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 prescence of Mg^{2+} (1.0mM). It was also found that D-mannitol, D-sorbitol and D-glucose-6-phosphate could not isomerized.

(3) Effect of temperature, and *p*H 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
$FeSO_4$	0.0
$CuSO_4$	0.0
$ZnSO_4$	0.0
$CaCl_2$	0.0
Na-arsenate	0.0
$MnCl_2$	0.0
$CoCl_2$	15.7
$MgCl_2$	98.9
$MgSO_4$	100
$Mg^{2+}+CO^{2+}$	145

70°C incubation for 10 minutes

Time coure studies showed the rate of fuctose formation to be nearly constant for one hour at 70°C (**Fig. 3**) in the prescence of 1M of D-glucose and 5mM of Mg^{2+} and 0.01M phoshate buffer(*p*H 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.

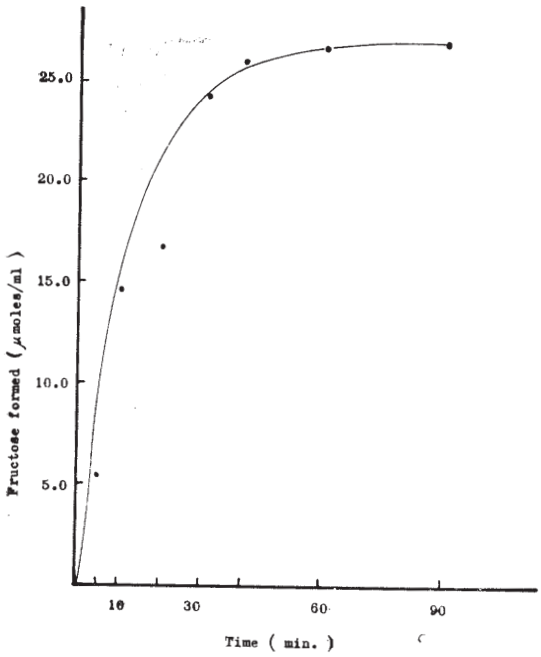

Fig. 3 Time course of D-xylose isomerase activity at 70°C

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 prescence of Mg^{2+} or Mg^{2+} and Co^{2+} combined. The activities in the prescence of lower Mg^{2+} concentrations(0.1mM), furthermore, were lesser than in the prescence of 5.0mM Mg^{2+} concentrations. Especially, the activities were gradually decreased in the absence of metal ion according to increasing. temperature

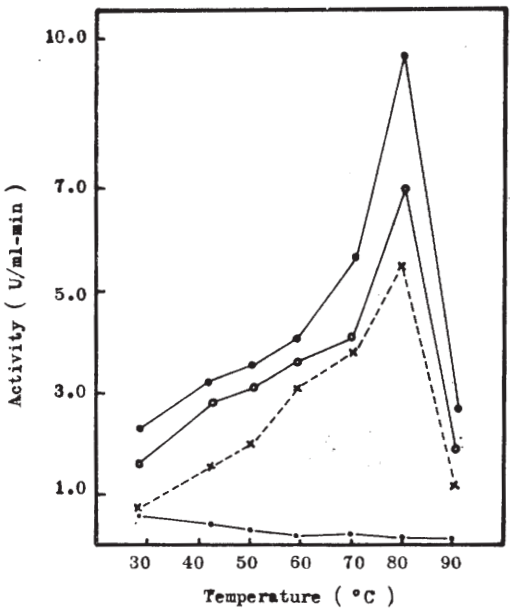

Fig. 4 Effect of Temperatur on D-xylose isomerase activity
The reaction mixtures incubated at each temperatures for 10 min.:•———•, without metal ions; x·····x, with Mg^{2+} (0.1mM);____, with Mg^{2+} (5.0mM);○———○, with Mg^{2+} and Co^{2+} (5.0mM+1.0mM).

When D-xylose isomerase activity was examined in the *p*H 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 *p*H 7.0, as shown in **Fig. 5.**

(4) Effect of thermal tolerence on enzyme activity

The thermal tolerence of D-xylose isom erase was examined in the prescence of metal ions and in the absence of them. In the prescence 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[1] (μmoles 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

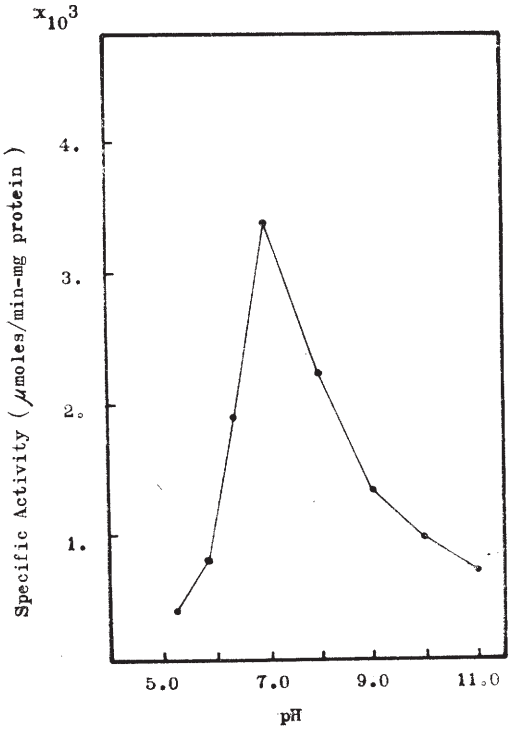

Fig. 5 Effect of *p*H on D-xylose isomerase activity
potassium phosphate buffers (0.01M) were used for *p*H values of 5.3 to 8.0 and carbonate-bicarbonate buffers (0.01M) from *p*H 9.0 to 11.0

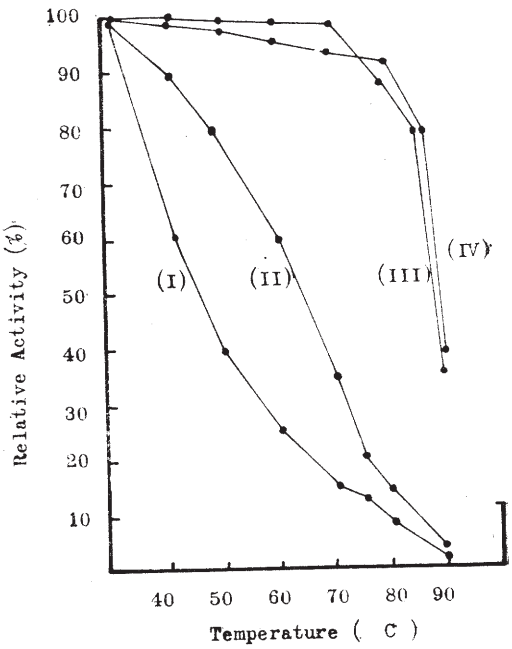

Fig. 6 Thermal tolerence of D-xylose isomerase activity in the prescence of metal ions.
The enzyme was treated for 10 minutes at various temperatures without substrate under the following conditions: without divalent cation (Ⅰ); 0.1mM $MgSO_4$ (Ⅱ); 1.0mM $CoCl_2$ (Ⅲ); and 5.0mM $MgSO_4$ (Ⅳ). After heat treatment, the activity was measured as described in materials and methods.

down rapidly in the absence of metal ionor in the prescence 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 determined 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^{2+} and Co^{2+} are not competitive with each other for binding on the allosteric site of enzyme molecule.

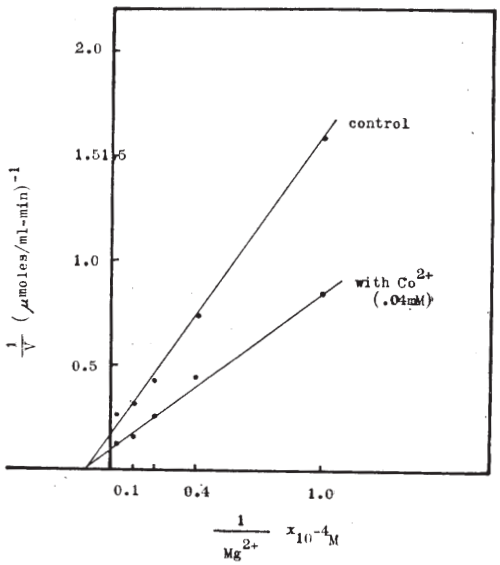

Fig. 7 Difference of the binding site of Mg^{2+} and Co^{2+}

(6) Enzyme kinetics of substrate concentrations

The Michaelis constants(Km) and V_{max} 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), *Paracolobacterium 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-

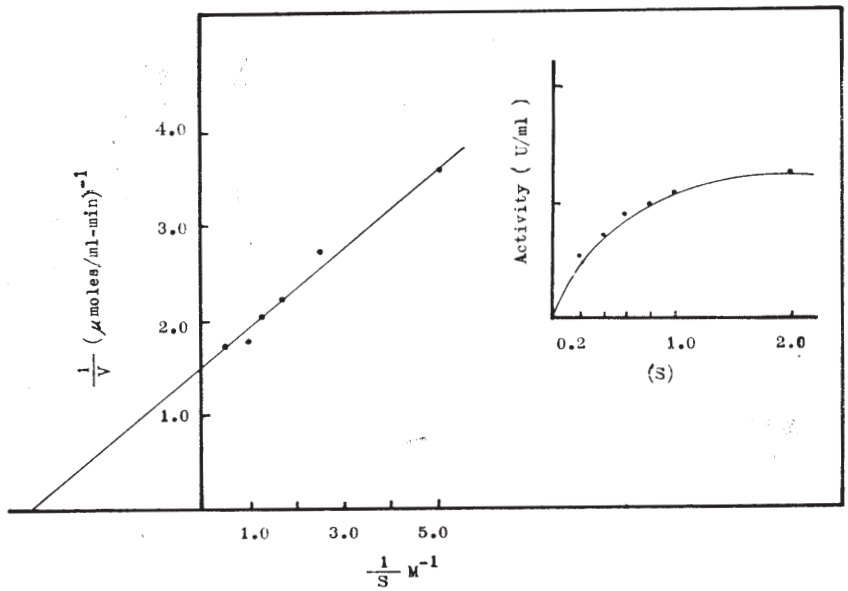

Fig. 8 Double reciprocal plot of the effect of D-glucose concentration on the reaction rate of isomerizing enzyme

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^{2+}, and 200μM. of D-glucose. Incubation was for 10 min. at 70°C.

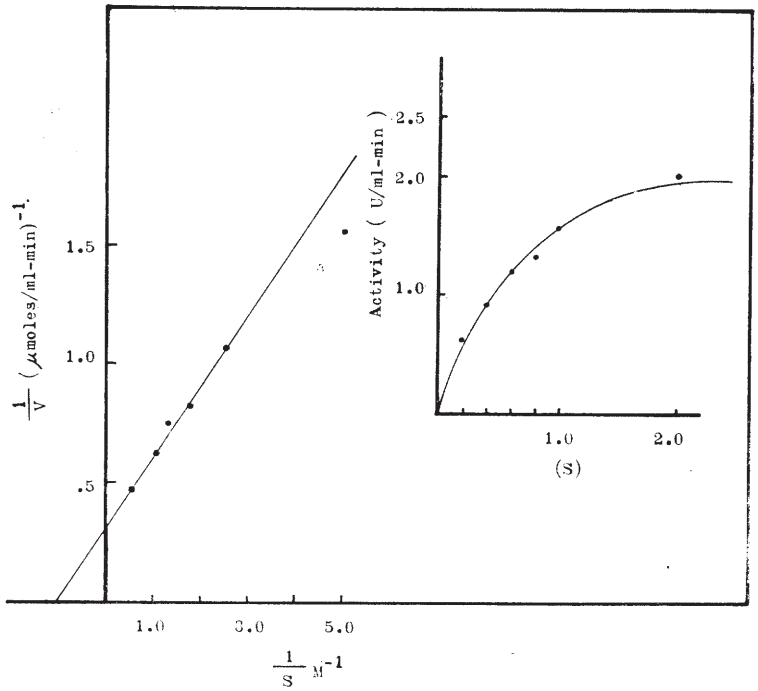

Fig. 9 Double reciprocal plot of the Effect of D-xylose concentration on the reaction rate of isomerizing enzyme

The reaction mixture contained in a total volume of 1.0ml; 3.0μg of the purified enzyme, 0.01M of phosphate buffer *p*H 7.2, 1.0mM of the Mg^{2+}, and 200μm 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; Sanch ez & 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 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 Co^{2+} ions. Obviously, the higher concentrations of Co^{2+} ion retarded the growth rate (Fig. 1). Other metal ions such as Fe^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+} and 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 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 isomeri zation 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°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 acttivity of the enzyme was obtained even if the enzyme was heated at 80° for 30 minutes in the prescence 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 *p*H on the activitiy of the enzyme was studied. The activity of the enzyme showed a maximal at around 70°C and *p*H 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 (Km=0.28M) than **D**-glucose(Km=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 *p*H 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 밀기울 배지보다 D-xylose배지에서 좋았으며 Co^{2+}, Fe^{2+}, Zn^{2+}, Cu^{2+} 같은 2가 금소이온들이 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 크로마토그라피에서 효소 활성이 180배의 증가된 효소를 얻었다.

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

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

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