KOREAN J. FOOD & NUTRITION
Vol. 4. No. 2, 155-160(1991)

Purification of Glucose Isomerase from *Arthrobacter* sp. L-3

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Arthrobacter sp. L-30이 생성하는 Glucose Isomerase의 정제

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요 약

Arthrobacter sp. L-3이 생성하는 glucose isomerase를 DEAE-cellulose column chromatography법으로 2단계 NaCl농도 구배로 용출함으로서 순수분리하였다. 이것이 SDS-acrylamide gel electrophoresis상에서 단일띠를 보임으로서 매우 잘 분리되었음을 알 수 있었다.

Glucose isomerase의 K_m값과 V_{max}값이 각각 0.175 M, 0.29(mg/ml/min)로 얻어졌다. 한편, SDS-acrylamide gel electrophoresis와 Sephadex-G100-50에 의한 gel filtration으로부터 분자량이 각각 42,000과 180,000으로 얻어져, 이 효소는 분자량이 42,500인 4개의 subunit로 구성되었음을 알 수 있었다.

I. Introduction

Glucose isomerase(G.I. : D-xylose- ketol-isomerase, E.C. 5.3.1.5) is one of the valuable enzymes in food industries which is used in production of high fructose syrup (HFS)[1~5].

Many papers have been published on the glucose isomerases of the *Pediococcus* sp.[6], *Lactobacillus* sp.[7], *Bacillus* sp.[8], and *Streptomyces* sp.[9].

Arthrobacter sp. is a good source for glucose isomerase production and it has several advantages in fermentation, but was not well studied. Generally, xylose is not demanded as an inducer for glucose isomerase production in *Arthrobacter* sp. and magnesium ion is only required for growth and in enzyme reaction[10,11].

The present paper describes the purification of glucose isomerase from *Arthrobacter* sp. L-3.

II. Materials and Methods

1. Microorganism and cell culture

The microorganism for production of glucose

isomerase was *Arthrobacter* sp. L-3.

The cells for seed culture were incubated at 30℃ in LB broth(tryptone 1%, yeast extract 0.5%, NaCl 0.5%, glucose 1%, pH 7.0~7.5) for 18~24hrs with shaking(rpm 100). Main cultures were grown in the fermentation medium (yeast extract 0.25%, peptone 1%, NaCl 0.5%, $MgSO_4 \cdot 7H_2O$ 0.05%, glucose 1%, pH 7.0~7.5) with jar fermentor(Marubishi MD 250~2.6L, Japan, work volume 11, rpm 200, airflow rate 1.0vvm). The inoculum size was 1%(v/v) with fresh LB grown seed cultures. After 40hr incubation, the cells were harvested and washed twice with 0.85% NaCl solution. The collected cells were preserved at 4℃ and used as an enzyme source.

2. Chemicals

Sodium dodecyl sulfate, acrylamide, dithiothretol, Sephadex G-100-50 and molecular weight standard marker protein were purchased from Sigma(U.S.A.). DEAE-cellulose was obtained from Merck(Germany). Yeast extract, peptone and other medium compounds were purchased from Difco(Detroit, U.S.A.).

3. Enzyme assay

The glucose isomerase activity was assayed by measuring the amounts of fructose converted from glucose by glucose isomerase[12].

The reaction mixture was contained 1.0 ml of substrate solution(1 M glucose in 50mM potassium phosphate buffer pH 7.2 plus 30 mM $MgSO_4 \cdot 7H_2O$) and equal volume of cells suspension(150mg of wet cells/ml).

The reaction was carried out at 60℃ for 1 hr and stopped by the addition of 2 ml of 0.5 M perchloric acid($HClO_4$). The D-fructose produced was determined by the resorcinol method, that is, to 0.5ml reactant solution were added 0.5 ml of D.W. and 0.5 ml of resorcinol reagent(glacial acetic acid 100 ml+thiourea 0.25g+resorcinol 0.1g) and mixed throughly.

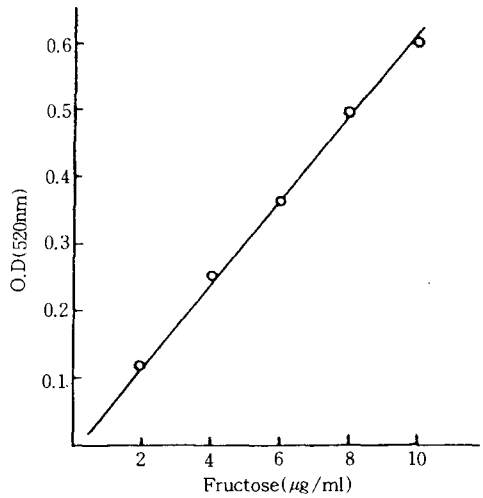

Fig. 1. Standard curve for determination of fructose by resorcinol method.

The reaction mixture was incubated at 80℃ for 10 min and cooled to room temperature. The optical density was estimated at 520 nm. A linear relationship was obtained between concentration of fructose and optical density in the range 2 μg to 10 μg per ml(Fig. 1).

Ⅲ. Results and Discussions

1. Purification of glucose isomerase by DEAE-cellulose column chromatography

The crude enzyme which was disrupted by sonication, percipitated by 70% ammonium sulfate and dialyzed with 50mM phosphate buffer solution (pH 7.2) was applied to DEAE-cellulose column(35×450mm) which was equilibrated with same buffer solution. Elution was

carried out by NaCl gradient elution(Fig. 2). Each fraction volume was 7.5ml and all fraction was tested glucose isomerase activity. Quantity of protein was also determined by spectrophotometer(Shimazu, Japan) at 280nm. With the two-stage of NaCl gradient elution, the enzyme was purified by one step. The purified enzyme preparation was showed a single band on SDS-acrylamide gel electrophoresis as shown in Fig. 4. This means that the enzyme purification was completely performed. The fractions No. 112 to No. 120 were collected, dialyzed and lyophilized for the determination of molecular weight and estimation of K_m value and V_{max} of the enzyme.

2. K_m value and V_{max} determination

Fig. 3 shows the effect of D-glucose concentration on the enzyme reaction. The Michaelis-Menten constant(K_m) for glucose as a substrate was determined by Lineweaver-Burk plot. The estimated K_m value was 0.175M and the V_{max} was 0.29(mg of product/ml/min).

3. Molecular weight determination

The molecular weight of glucose isomerase was also determined by SDS-gel electrophoresis and gel filtration with Sephadex G-100-50.

In the SDS-gel electrophoresis, the molecular weight was estimated as about 42,500 from the standard curve(Fig. 4).

In the gel-filtration with Sephadex G-100-50 for native protein form, the V_e/V_o value was 1.6 and the molecular weight was assumed to be 180,000(Fig. 5). From the above results, the glucose isomerase from *Arthrobacter* sp. L-3 was composed of four identical subunits.

Ⅳ. Abstract

D-glucose isomerase was purified from *Arthrobacter* sp. L-3 by DEAE-cellulose column chromatography with a linear gradient increase in NaCl and purified enzyme preparation was showed a single band on SDS-acrylamide gel electrophoresis.

The K_m value and V_{amx} was 0.175M and 0.2g(mg of protein/min), respectively. Molecular weight by SDS-acrylamide gel electrophoresis was estimated to be 42,000 and by Sephadex G-100-50 gel filtration, the molecular weight was assumed to be 180,000. From the results, the glucose isomerase from *Arthrobacter* sp. L-3 was composed of four identical subunits.

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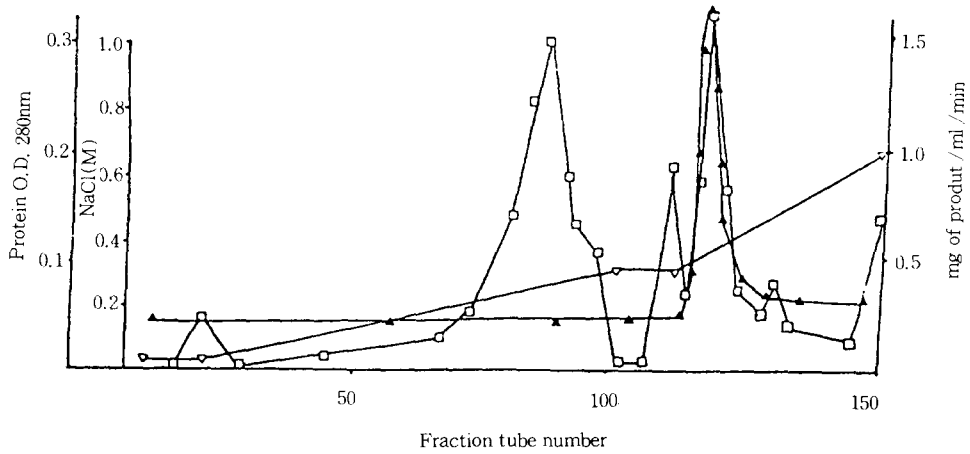

Fig. 2. DEAE-cellulose column chromatography pattern of glucose isomerase.

(Column size: 35 × 450mm, One fraction: 7.5ml, protein: □ – □, glucose isomerase activity: ▲ – ▲
NaCl concentration: ▽ – ▽)

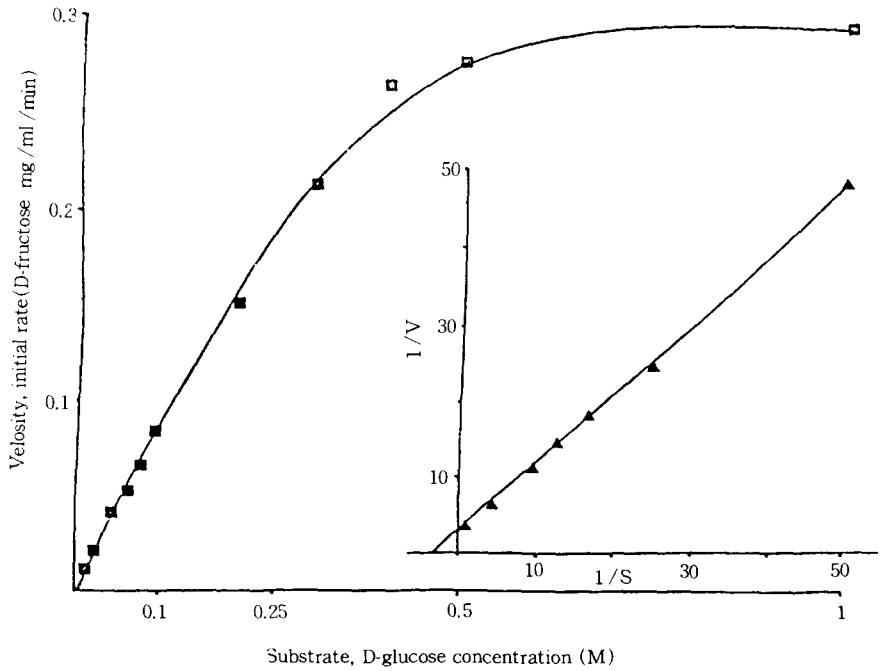

Fig. 3. Effect of substrate(D-glucose) concentration on glucose isomerase activity.

The composition of reaction mixture was 50mM phosphate buffer(pH 7.2),
30mM $MgSO_4$, various concentration of D-glucose as indicated and the enzyme solution.

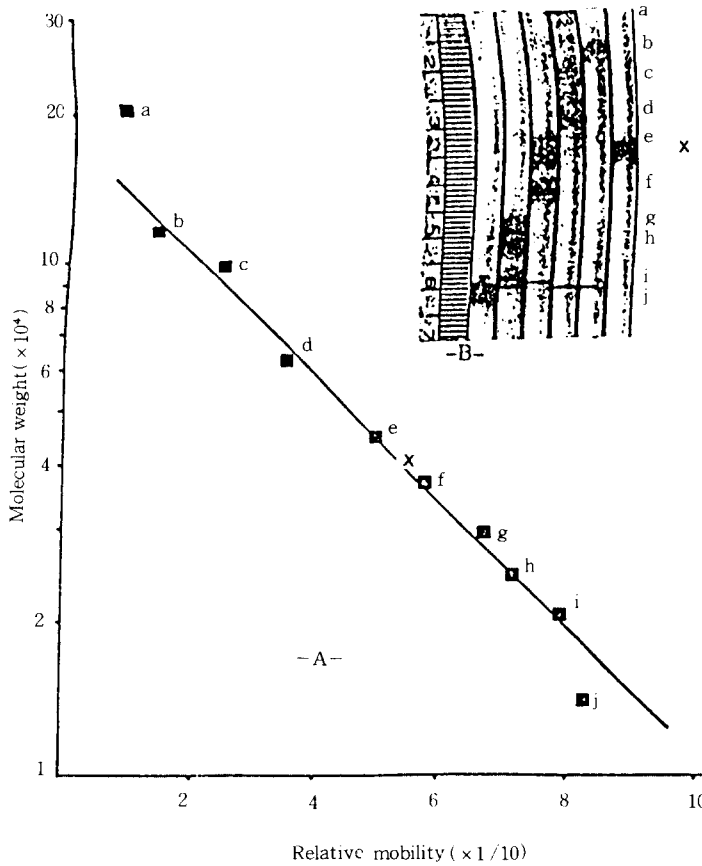

Fig. 4. Calibration curve(A) and separation pattern(B) for molecular weight determination of the glucose isomerase by SDS-acrylamide gel electrophoresis.

The standard proteins used and their molecular weight were:

a; myosin(205,000), b; β-galactosidase (116,000),

c; phosphorylase(97,400), d; albumin, bovine (66,000),

e; albumin, egg(45,000), f; glyceraldehyde-3-phosphate dehydrogenase(36,000),

g; carbonic anhydrase (29,000), h; trysinogen(24,000),

i; trypsin inhibitor(20,100) j; β-lactoalbumin(14,200)

Glucose isomerase (X): relative mobility= 0.51, molecular weight=42,500

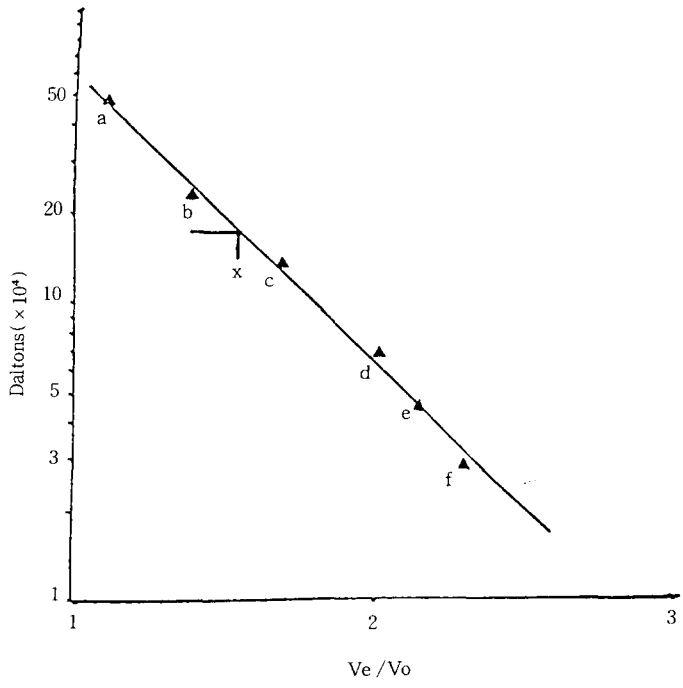

Fig.5. Molecular weight determination of the glucose isomerase by Gel filtration using Sephadex G-100-5

The standard proteins used and their molecular weight were:
a:Urease-jack beans(tetramer, 480,000), b:Urease-jack beans(dimer, 240,000),
c:albumin, bovine serum(132,000), d:albumin, bovine serum(monomer, 66,000),
e:albumin, egg(45,000), f:carbonic anhydrase(29,000).
X:Glucose isomerase Ve /Vo=1.6 ,
molecular weight=180,000

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(1991년 8월 30일 수리)