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Studies on Invertase from Korean Ginseng, *Panax ginseng* C. A. Meyer

Ⅱ. Purification and Physico-chemical Properties of Ginseng Invertase

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고려인삼 중의 인베르타아제에 관한 연구

제 2 보 : 인삼 인베르타아제의 정제와 물리화학적 성질

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Abstract

An invertase from Korean ginseng (*Panax ginseng* C. A. Meyer) was purified by means of DEAE-cellulose column chromatography and gel-filtration through Sephadex G-75. The homogeneity of the purified invertase was proved by polyacrylamide gel disc electrophoresis. The enzyme was separated into two subunits by SDS-polyacrylamide gel electrophoresis, showing its molecular weights as 48, 000. The enzyme preparation showed a charateristic protein UV-spectra.

Introduction

As described in the preceding paper[1], the crude ginseng invertase was consisted of albumionus materials and shown to be an acid invertase with optimum pH 5.0. The crude ginseng invertase showed some different properties from those of the other source invertase, including the critical temperatures difficult to be ascertained, the weak inhibition effects by heavy metal ions, and the unusual activation of the enzyme by Ca^{2+}, Cu^{2+}, and Fe^{3+}. These specific properties were supposed to be related to the chemical compositions of the enzyme preparation. For the more detailed study on the properties of ginseng invertase, we carried out the purification of the enzyme.

The present paper deals with the purification procedure of the invertase preparation and the physico-chemical properties of the purified enzyme.

Materials and Methods

Materials

Diethyl aminoethyl(DEAE)-cellulose was purchased from Brown Co, USA, Sephadex G-75 from Pharmacia Fine Chemicals, Uppsala, Sweden, acrylamide from Eastman Kodak Co., USA, N, N'-methylene bis acrylamide, N, N, N', N'-tetramethylethylene diamine (TEMED), and amidoblack 10B from E. Merck,

Darmstadt, and sodium lauryl sulfate (SDS) from Ishizu Pharmaceutical Co., Japan. The other reagents were purchased from Nakarai Chemicals Co., Japan.

Assay of invertase

Invertase was assayed according to the method of Pressey[2] with a slight modification as described in the previous paper[1].

Determination of protein

The amount of protein was determined by the method of Lowry *et. al.*[3]

Purification of crude invertase

The crude invertase(protein 58.2 *mg*, spec. act. 4.062) prepared from ginseng extracts as described in the previous paper[1] was applied into DEAE-cellulose column chromatography which was carried out stepwise with 0.01, 0.1, and 0.2*M* acetate buffer(pH 4.7), and then with 0.2*M* acetate buffer(pH 4.7) containing 1.0*M* NaCl. C_2-fraction obtained from DEAE-cellulose column chromatography was gel-filtrated through Sephadex G-75 with 0.01*M* acetate buffer(pH 4.7). DG_2-fraction obtained from gel-filtration through Sephadex G-75 was regel-filtrated through Sephadex G-75 in the same way as above. DRG-fraction obtained from regel-filtration through Sephadex G-75 was used as a purified ginseng invertase.

Electrophoresis

Polyacrylamide gel disc electrophoresis with or without sodium lauryl sulfate(SDS) was carried out according to the method of Ornstein and Davis[4,5].

Measurement of UV absorption spectra

The ultraviolet absorption spectra of the purified invertase was measured by Beckman Model DU-2 Spectrophotometer.

Results and Discussion

Purification of crude invertase

1. DEAE-cellulose chromatography

The chromatographic patterns of crude invertase on DEAE-cellulose column are shown in Fig. 1. As shown in Fig. 1, the crude invertase was fractionated into four fractions. The activities of invertase in each fraction are shown in Table 1. As shown in Table 1, the invertase activities were dispersed into all fractions, showing the highest specific activity and recovery in D_2-fraction.

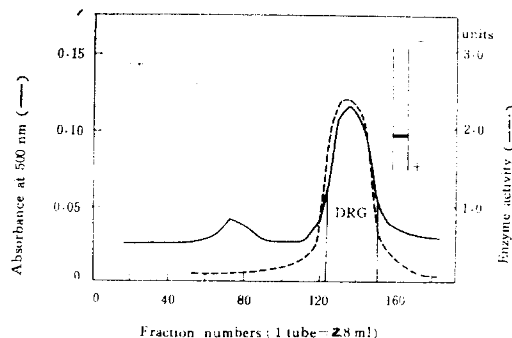

Fig. 1. DEAE-cellulose column chromatography of crude invertase

Size of column: 3.0×39.0 *cm*, flow rate: 12.6 *ml*/hr, activity zone: |—|

Table 1. Invertase activities of each fraction in DEAE-cellulose chromatography

Fractions	Total protein (*mg*)	Specifie Activity (units/*mg* protein)	Total Activity (units)	Recovery (%)
D_1	8.15	1.767	14.4	6.1
D_2	11.95	9.941	118.8	50.3
D_3	10.30	3.350	34.5	14.6
D_4	12.70	4.780	60.7	25.7
Crude invertase	58.20	4.062	236.4	100.0

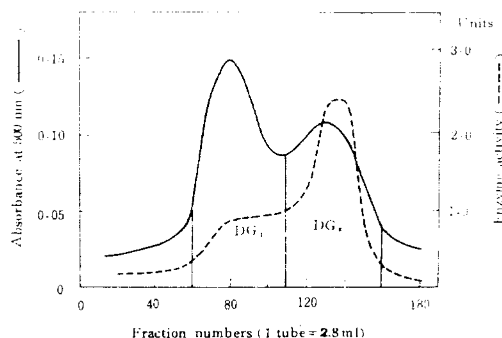

Fig. 2. Gel-filtration of invertase through Sephadex G-75

Size of column: 2×90 *cm*, flow rate: 2.5 *ml*/hr

For the next purification step, the D_2-fraction was concentrated and dialyzed against 0.01*M* acetate buffer (pH 4.7) for 3 days.

2. Gel-filtration through Sephadex G-75

The gel-filtration pattern of D_2-fraction through Sephadex G-75 with 0.01*M* acetate buffer(pH4.7) is shown Fig. 2. As shown in Fig. 2, the invertase activities were eluted into the fractions of DG_1 and DG_2. These two fractions were pooled separately and their invertase activities were measured. The recoveries of invertase activity in the fractions of DG_1 and DG_2 were 37.5 and 61.1%. The specific activities of DG_1- and DG_2-fractions increased 1.1 and 1.7 folds of that of applied D_2-fraction, respectively.

3. Regel-filtration through Sephadex G-75

The regel-filtration pattern of DG_2-fraction through Sephadex G-75 with 0.01*M* acetate buffer(pH 4.7) is shown in Fig. 3. As shown in Eig. 3, the invertase activity was recovered in the fractions between 340 and 420 *ml*. The invertase in this fraction migrated as a single band on 7.5% polyacrylamide gel disc electrophoresis at pH 8.3. This fraction was pooled, concentrated, and dialyzed against deionized water for 3 days. This final fraction, DRG-fraction, was purified about 64.7 folds of crude ginseng extracts and the recovery of invertase activity was about 19.5% as shown in Table 2.

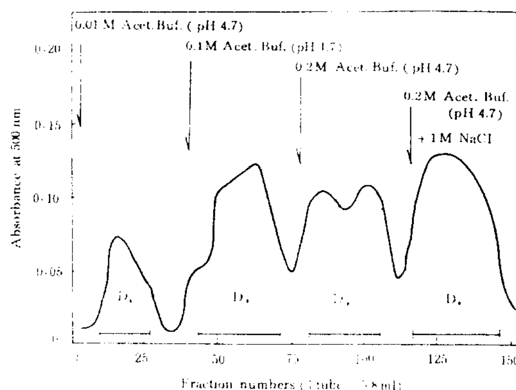

Fig. 3. Regel-tiltration of invertase through Sephadex G-75.

Size of column: 2 90*cm*, flow rate: 2.5*ml*/hr

Table 2. Summary of separation and purification procedures

Fractions	Total protein (*mg*)	Specific activity (units/*mg* protein)	Total activity (units)	Recovery (%)	Purification fold
Crude extract	1352.42	0.272	367.5	100	1
Crude invertase	58.20	4.062	236.4	64.3	14.9
D_2-fraction	11.95	9.941	118.8	32.3	36.5
DG_2-fraction	4.46	17.063	76.1	20.7	62.7
DRG-fraction	4.06	17.611	71.5	19.5	64.7

Polyacrylamide gel disc electrophoresis of the purified invertase

The purified invertase migrated as a defined band on 7.5% polyacrylamide gel column as shown in Fig. 4. From this result, it was inferred that the purified invertase was electrophoretically homogenous.

SDS-polyacrylamide gel electrophoresis of the purified invertase

The electrophoretic patterns of the purified invertase in polyacrylamide gel containing SDS are shown in Fig. 5. As shown in Fig. 5, both of inverases with or without 2-mercaptoethanol migrated as two bands in SDS-polyacrylamide gel electrophoresis,

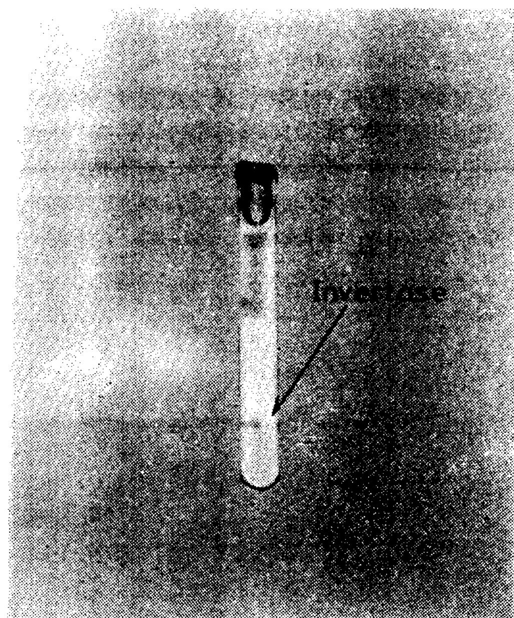

Fig. 4. Polyacrylamide gel disc electrophoresis pattern of purified invertase

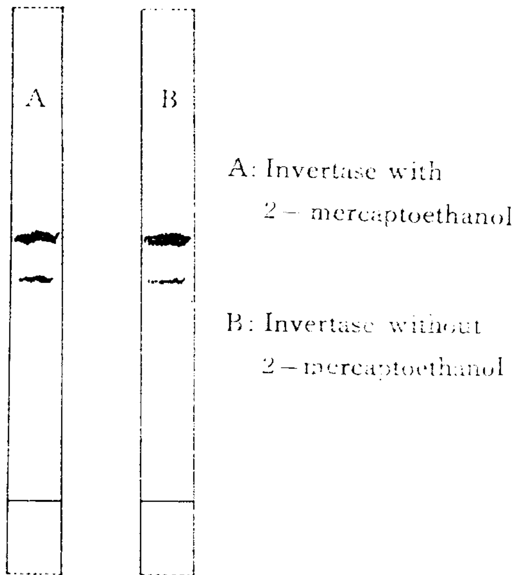

Fig. 5. SDS-polyacrylamide gel electrophoretic patterns of purified invertase

respectively. This means that the purified invertase protein was divided into two subunits by SDS.

The ralative migrations of standard proteins and invertase subuits were plotted against the log of molecular weights in Fig. 6. Form the plot in Fig. 6, the molecular weights of two invertase subunits were assumed to be 28,000 and 20,000, respectively. From these results, the molecular weights of the purified invertase was calculated to be 48,000.

UV absorption spectra of purified invertase

The ultraviolet absorption spectra of the purified invertase are shown in Fig. 7, the purified invertase gave a characteristic protein ultraviolet spectrum with a maximum at 278 *nm*, showing a ratio of

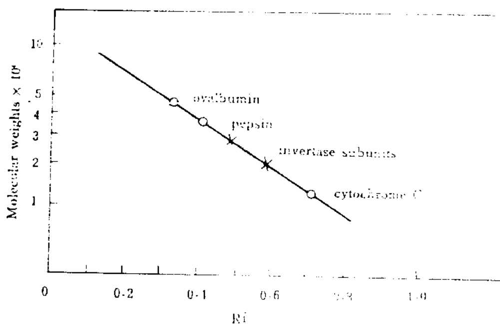

Fig. 6. Plot of Rf against log molecular weights of protein

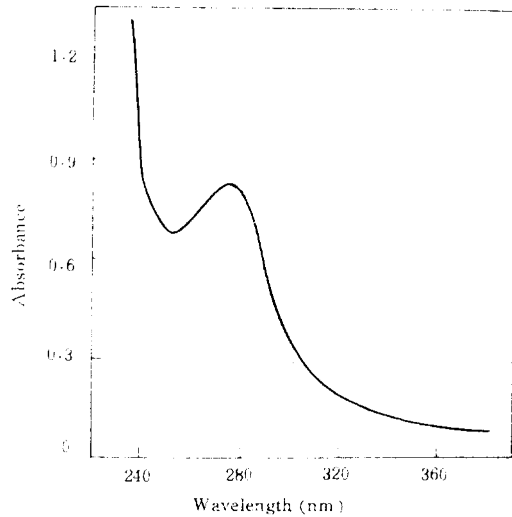

Fig. 7. UV absorption spectra of purified invertase

absorbances at 280 and 260 *nm* of 1.26

In general, the specific activities of plant invertases are not so greatly increased through purification procedure. Cooper and Greenshields[6] reported 110-fold increasing of the specific activity for the dwarf French bean invertase, and Nakagawa *et al*[7] only 9-fold and 20-fold increasing of it for the tomato fruit invertase(β-fructofuranosidase) E and L, respectively. In the present work, the specific activity of purified ginseng invertase was increased 64.7 times of that of crude extracts. This increment is too smaller than that(1130-fold) for yeast invertase in the report of Gascon and Lampen[8]. With respect to these specific phenomena on plant invertase, it could be supposed that the carbohydrate moiety of enzyme preparation was related to that.

In data not presented here, the purified ginseng invertase contains about 6.2% of sugar. Nakagawa *et al*[7] reported that the highly purified tomato invertase E and L contained carbohydrate of about 5.3 and 9.1%, respectively. On this problem, Kaya[9] had recommended to remove sugar components to increase the specific activity of invertase. However, we needed further investigations since there had also been reported the unrelated effects of carbohydrate moiety on the activity of invertase[9~11].

The purified ginseng invertase was homogeneous by polyacrylamide gel electrophoresis, but the enzyme

was divided into two subunits by SDS and 2-mercaptoethanol. The molecular weights of purified invertase was calculated to be 48,000. These properties of ginseng invertase are similar to those of tomato fruit invertase[7], of which molecular weights are 75,000. In comparison, molecular weights of yeast invertase and *Neurospora* invertase are 112,000~270,000[8,12,13] and 210,000[14], respectively.

Lastly, the purified ginseng invertase gave a characteristic protein ultraviolet spectrum. The ratio (1.26) of absorbances at 280 and 260 *nm* was smaller than that(1.75) of yeast invertase reported by Gascon and Lampen[8], or than that(1.80) of yeast invertase in the report of Neumann and Lampen[13].

There is no evidence, however, of the presence of nucleotides and nucleic acids in the purified ginseng invertase preparation.

요 약

고려인삼(*Panax ginseng* C. A. Meyer)중의 인베르타아제(invertase)를 연구하기 위하여 조(粗)인삼 인베르타아제를 분리 조제한 후 DEAE-cellulose 관크로마트 그래피, Sephadex G-75를 통한 겔 여과 등의 방법에 의하여 정제하였다.

정제된 인베르타아제는 specific activity가 粗인베르타아제에 비해 64.7배 증가되었으며 효소활성의 recovery는 약 19.5%였다. 정제된 인베르타아제는 polyacrylamide gel electrophoresis에 의해 균일성(homogeneous)을 나타내었고 SDS-polyacrylamide gel electrophoresis에서는 2개의 subunits로 분리되었다. 두 subunits의 분자량은 각각 28,000과 20,000이었고 따라서 인삼 인베르타아제의 분자량은 48,000으로 계산되었다.

정제된 인베르타아제는 전형적인 단백질의 UV 흡수 스펙트럼을 나타내었다.

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