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

I. Separation and Properties of Crude Invertase

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고려 인삼 중의 Invertase에 관한 연구

제 1 보 : 粗 Invertase의 분리와 성질

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Abstract

Crude invertase was obtained from the water extracts of Korean ginseng, *Panax ginseng C. A. Meyer*, by fractionation with 0.8~1.0 saturation of ammonium sulfate. The properties of the crude invertase were as follows: Crude invertase was stable in the pH range between 5 and 9, and at the temperature below 35°C. Crude invertase showed the optimum pH at 5.0 and the optimum temperature at 50°C. The activity of the crude invertase was inhibited by Ag^{+}, Mn^{++}, Hg^{++}, Zn^{++}, and Rb^{+}, while Ca^{++}, Cu^{++}, and Fe^{3+} demonstrated remarkable increasing effects on the enzyme activity.

Introduction

Korean ginseng (*Panax ginseng C. A. Meyer*) has been used as a mysterious cure-all medicine in Asia for several thousand years. In spite of using it as a potent medicine for very long time ago, scientific studies of ginseng have been carried out only during the last 130 years. Garriques[1] isolated glycoside from American ginseng (*Panax quinquefolium L.*) and named it as panaquilon. Panaquilon was the first substance reported to be a available component of ginseng. Fujutani[2] isolated panaquilon, a glycoside, from Korean ginseng and examined its effect on the central nervous system, finding out its sedative action. Sakai[3] isolated panacene, fragrant non-glycoside, from the same plant and reported its pharmacological effect on the central nervous system.

Since then, many investigators who examined the tonic effect of ginseng on each organ of the human body emphasized the action of ginseng on the central nervous system, and latter they presumed that ginseng would also act on the metabolism of a living body.

The comprehensive and detailed explanation of the tonic effect of ginseng in modern scientific terms

was given by two separate research groups, Brekhman group[4] and Oura group[5]. Brekhman[4] explained, from a pharmacological point of view, that the tonic effect of ginseng was an effect which increased the non-specific resistibility of a living body, so called adaptogenic activity. Oura[5] explained in modern biochemical terms that ginseng extract facilitated the protein synthesis and metabolism of living body.

Based on these reports, it has been recently recommended to study comprehensively the biological effects of ginseng, especially in the field of endogenous enzymology. By enzymological studies, it is also expected to elucidate the ecological characteristics of ginseng such as high sensitivity to a soil-sick, shade-endurance, and high susceptibility to a rot, etc. as well as the metabolism of the efficient components in ginseng plant.

It is, however, a very unfortunate thing that we have only few reports on ginseng enzymes including Yamakuchi's work[6] on diastase and phenolase and Sato's work[7] on amylase. Author[8] found that ginseng contained peculiarly glycosidases such as α-amylase, β-amylase and invertase, but not protease, lipase, or catalase.

This paper describes the separation and some properties of crude ginseng invertase.

Materials and Methods

Materials

The samples used in this experiment were four-year-old roots of raw Korean ginseng which were harvested at Kanghwa-do, Korea, on July, 1977. All chemicals were obtained from commercial sources and were of analytical grade.

Extraction of crude invertase

In order to separate invertase, raw ginsengs were cleansed, peeled off, and then homogenized with the same volume of distilled water. The homogenates were incubated at 30°C for 30 min and then strained through cheese cloths. The filtrate obtained was centrifuged at 3,000 r.p.m. for 10 min and the resulting supernatant was used as a crude extract of invertase.

Separation of crude invertase

The crude extract was saturated with ammonium sulfate to 0.8 and centrifuged at 8,000 r.p.m. and 5°C for 20 min. The resulting supernatant was brought to saturation with ammonium sulfate and centrifuged at 8,000 r.p.m. and 5°C for 20 min. Invertase was yielded in the resulting precipitate by about 64 % (Table 1). The precipitate was dissolved in a small amount of deionized water and dialyzed against deionized water using Visking tube #30/32. After centrifuging to remove some insoluble materials, the dialyzed solution was used as crude invertase preparations throughout this experiment.

Assay of invertase

Invertase was assayed according to the method of Pressey[9] with a slight modification. An aliquot of the enzyme was incubated, in a final volume of 1.0 *ml*, with 0.5 *ml* of 0.2 *M* sodium actate buffer (pH 4.7) and 100 *μmoles* of sucrose at 37°C for 30 min. The reactions were terminated by addition of 1.0 *ml* of 0.5 *M* Na_2HPO_4 and heating at 100°C for 2 min. The solution (0.5 *ml*) was then analyzed for reducing sugars by the method of Nelson and Somogyi[10~12].

One unit of invertase was defined as the amount of enzyme liberating 1 *μmole* of reducing sugar/min at 37°C[13].

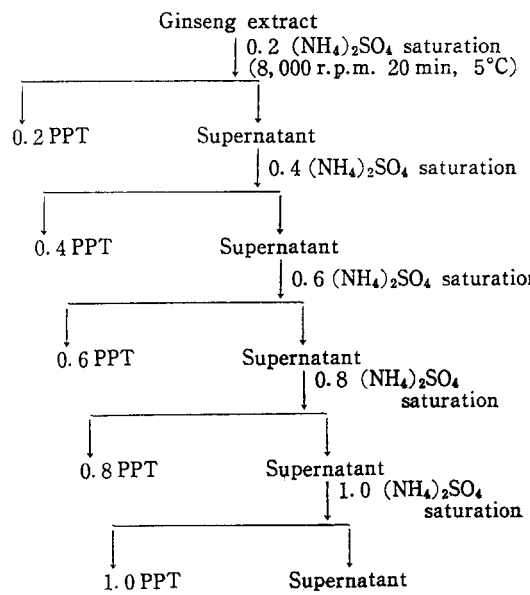

Fig. 1. Fraction of the crude extract

Table 1. Invertase activities in fractions of crude extract

Fractions	Total protein (*mg*)	Spec. act. (units/ *mg* protein)	Total act. (units)
0.2	39.5	—	—
0.4	20.5	—	—
0.6	88.5	0.176	15.60
0.8	94.4	0.200	18.90
1.0	14.8	4.101	68.70
Super.	86.1	0.049	4.20

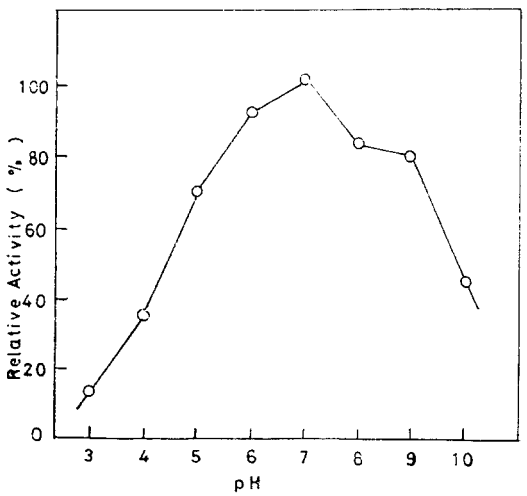

Fig. 2. pH stabilities of crude ginseng invertase

Determination of protein

The amount of protein was determined by the method of Lowry *et al.*[14].

Results

Fractionation of the crude extracts with ammonium sulfate

The crude extract was fractionated by the procedure as shown in Fig. 1. The activities of invertase contained in each fraction are shown in Table 1. As shown in Table 1, about 64% of invertase activity were contained in 1.0-fraction which was used as crude invertase.

Some properties of crude invertase

a. pH stabilities

The crude invertase was adjusted to pH 3 and 4 with 0.01 *M* acetate buffer, 5, 6, and 7 with 0.01 *M* phosphate buffer, and 8, 9, and 10 with 0.01 *M* carbonate buffer, respectively and then kept for 24 hr in a low temperature room (about 5°C). After kept, each solution was dialyzed against deionized water for 3 days and the remaining invertase was assayed. The results obtained are shown in Fig. 2. As shown in Fig. 2, the crude invertase was stable at pH range between 5 and 9.

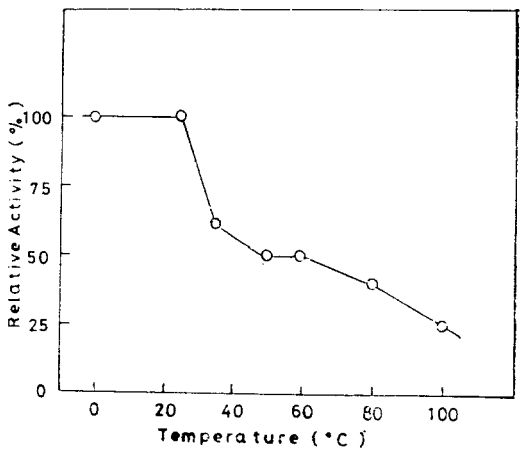

Fig. 3. Thermal stabilities of crude ginseng invertase

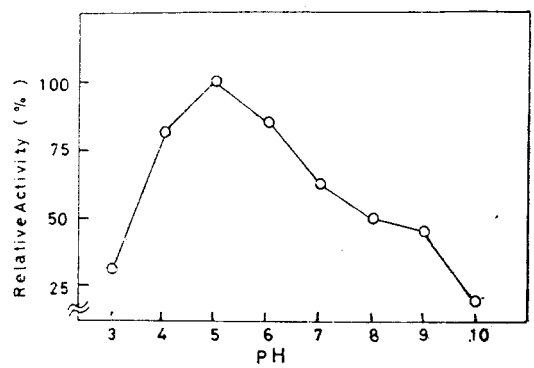

Fig. 4. Effects of pH on the activities of crude ginseng invertase

b. Thermal stabilities

The crude invertase was mixed with the double volumes of 0.01 *M* acetate buffer (pH 4.7) and then incubated for 30 min at 0, 15, 25, 35, 50, 60, 80, and 100°C, respectively. After removing the precipitates, if any, the remaining invertase was assayed. The results obtained are shown in Fig. 3. As shown in Fig. 3, the crude invertase was stable at the temperature below 35°C for 30 min.

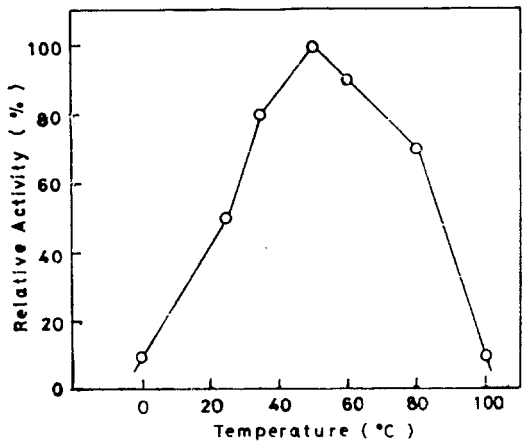

Fig. 5. Effects of temperature on the activities of crude ginseng invertase

c. pH dependences

The crude invertase was assayed at pH adjusted to 3 and 4 with 0.01 *M* acetate buffer, 5, 6, and 7 with 0.01 *M* phosphate buffer, and 8, 9, and 10 with 0.01 *M* carbonate buffer, respectively. The results obtained are shown in Fig. 4. As shown in Fig. 4, the crude invertase shows maximum activity at pH, 5.0, showing more significant decreasing of activity in the acid conditions than at the alkali conditions.

d. Temperature dependences

The crude invertase was assayed at 0, 25, 35, 50, 60, 80, and 100°C, respectively. The results obtained are shown in Fig. 5. As shown in Fig. 5, the crude invertase shows an optimum temperature at 50°C.

e. Effects of metal ions

In order to study the effects of metal ions on the activity of crude invertase, several inorganic salts (1×10^{-5} *moles*) were added to the reaction mixtures, respectively and then the remaining activities of invertase were measured. The results are shown in Table 3. As shown in Table 3, the activity of crude invertase was inhibited by Ag^{+}, Mn^{++}, Hg^{++}, Zn^{++}, and Rb^{+}, while Ca^{++}, Cu^{++}, and Fe^{3+} demonstrated remarkable increasing effects on the enzyme activity. The differences between inhibitions of the enzyme activity by heavy metal ions and by common metal ions are insignificant. K^{+}, Na^{+}, Sn^{++}, and Co^{++} did not affect on the activity of crude invertase.

Table 3. Effects of metal ions on invertase activities

Metals	Relative activity(%)
None	100.0
KCl	98.5
NaCl	100.6
$AgNO_3$	76.4
$CaCl_2$	127.5
$MnCl_2$	72.0
$SnCl_2$	100.0
$CoCl_2$	101.3
$HgCl_2$	61.8
$CuCl_2$	135.7
$FeCl_3$	185.7
$Zn(NO_3)_2$	80.9
Rb_2CO_3	93.6

Discussion

The invertase in the extract of Korean ginseng came mostly to the fraction precipitated with 1.0 saturation of ammonium sulfate, and the separated invertase was easily dissolved in water as well as in inorganic salt solution. From these phenomena, it appeared that the crude ginseng invertase was consisted of albuminous materials.

The crude ginseng invertase was shown to be an acid-invertase with optimum pH 5.0, being similar to those obtained by Hatch *et al.*[15] from sugar cane (optimum pH 5.0~5.5), and by Pridham and Walter[16] from *Vicia faba* seeds (optimum pH 5.1), and by Cooper and Greenshields[17,18] from bean (optimum pH 4.8~5.2). Although the crude ginseng invertase has the optimum at pH 5.0, the enzyme showed a sharp decrease in activity below pH 5.0. The similar results were obtained by Kuhn[19] with the bean β-fructofuranosidase.

The optimum temperature(50°C) of the crude ginseng invertase was almost accorded with that (55°C) of yeast invertase for dilute sucrose solutions[20]. On the other hand, the critical temperature of the enzyme was difficult to be ascertained, because the decrasing curve of enzyme activity at the temperature above 35°C was very slow. This is supposed to be related to the chemical properties(especially

on the sugar component) of enzyme preparation.

Inhibitions of crude ginseng invertase by metal ions, peculiarly even by Ag^+ or Hg^{++}, were not strong as compared with those of yeast invertase reported by Myrback[21~23], Manchester[24], and Semenza *et al.*[25]. Activations of the enzyme by Ca^{++}, Cu^{++}, and Fe^{3+} are very unusual because there were no reports of any similar results with the other source invertases. It also suggests that the crude ginseng invertase has some specific properties related to the chemical compositions of the enzyme preparation.

요 약

고려 인삼(*Panax ginseng C. A. Meyer*)중의 invertase를 연구하기 위하여 조(粗) 인삼 invertase를 분리 조제하여 그 성질을 조사해 본 결과 다음과 같았다.

1. 조 인삼 invertase는 ammonium sulfate 0.8~1.0 포화 분획에 의하여 효과적으로 조제되었다.

2. 조 인삼 invertase는 pH 5~9, 35°C 이하의 조건에서 안정성을 나타내었다.

3. 조 인삼 invertase는 최적 pH 5.0, 최적 온도 50°C를 나타내었다.

4. 조 인삼 invertase는 Ag^+, Mn^{++}, Hg^{++}, Zn^{++}, Rb^+등의 금속 이온에 의하여 저해되었으나 그 저해 정도는 크지 못하였다. 한편 Ca^{++}, Cu^{++}, Fe^{3+}등에 의한 효소 활성 증대 효과는 특이적이었다.

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