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A PROTECTIVE EFFECT OF γ-GLOBULIN ON α-GLUCOSIDASES FROM SPINACH SEEDS

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Enzymes are often unstable and lose their activity when diluted, and the loss of activity can be prevented by the addition of serum albumin, SH-reagent and other substances. The activity of many kinds of enzymes, such as β-amylase, β-galactosidase, lipase, acid phosphatase, esterase, lactate dehydrogenase, hexokinase, glycerokinase, cholesterol oxidase, and glutathione reductase has been assayed in the presence of bovine serum albumin[1)], but the mechanism of the protective action of bovine serum albumin has not yet been made clear. During the investigations on the properties of multiple forms of α-glucosidase from spinach seeds, we found that γ-globulin protected these α-glucosidases more effectively than serum albumin. This paper describes the effect of human γ-globulin on the stability of α-glucosidases from the seeds of *Spinacia oleracea* L. cv. Viloflay.

MATERIALS AND METHODS

Seeds of *Spinacia oleracea* L. cv. Viloflay obtained from Yamato Plantation Co. Ltd., Tenri, Nara Prefecture, were pulverized in a Shibata coffee grinder model Ely-3 (Shibata Chemical App. Mfg. Co. Ltd., Tokyo). The powdered spinach seeds (1.5 kg) were extracted with 6 liters of 0.02 M acetate buffer, pH 5.0, containing 10 % sodium chloride at 4 ℃ under stirring. After 24 hr-stirring, the suspension was filtered with a centrifugal dehydrator to remove the residue. The residue was re-extracted with 2 liters of the same buffer containing 10 % sodium chloride, and then filtered. The combined filtrate was used as the crude extract of α-glucosidases. We purified four forms of α-glucosidase from the crude extracts of matured spinach seeds by a procedure including fractionation with ammonium sulfate, column chromatography on CM-cellulose, Bio-Gel P-150, and hydroxyapatite. Figure 1 shows the chromatography of partially purified spinach α-glucosidase on a column of CM-cellulose. Full details of further purification procedure on these peak

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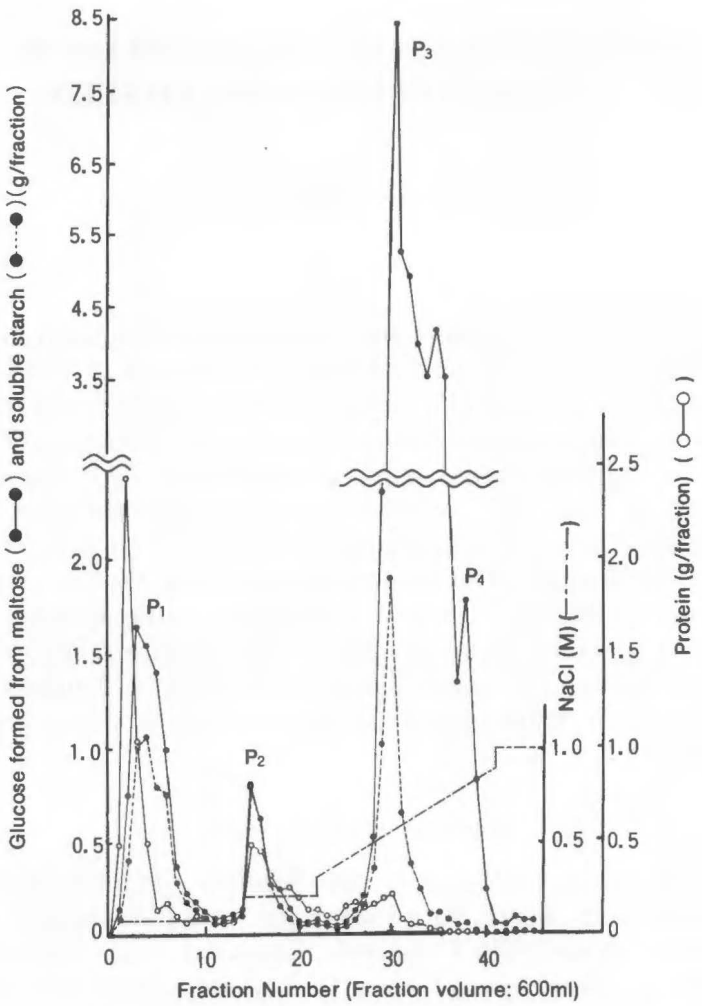

FIG. 1. Elution pattern of α-glucosidases from seeds of *Spinacia oleracea* L. on 1 st CM-cellulose column chromatography.

fractions (P_1, P_2, P_3 and P_4 : α-glucosidases Ⅰ, Ⅱ, Ⅲ and Ⅳ) will be given elsewhere. Each of these purified enzyme preparations was homogeneous on polyacrylamide disc gel electrophoresis at pH 4.0. The molecular weight markers (non-enzymatic proteins) were obtained from Schwarz Bio Research, Orangeburg, N. Y., USA; apo-ferritin from horse (mw 480,000), γ-globulin from human cohn fraction Ⅱ (mw 160,000), albumin from bovine serum (mw 67,000), ovalbumin from chick egg (mw 45,000), chymotrypsinogen A from bovine pancreas (mw 25,000), myoglobin from horse skeletal muscle (mw 17,800), and lysozyme from chick egg white (mw 14,307). These markers were used to examine their effects on

the stability of the enzymes. Maltose (HHH) (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan), Triton X-100 (Nakarai Chemicals, Ltd., Kyoto, Japan) and β-galactosidase from *Esherichia coli* (Boehringer Mannheim GmbH, Mannheim, Germany) were obtained from commercial sources. Other chemicals and reagents used were the same as those described previously[5]. The α-glucosidase activity was assayed in a reaction mixture containing 0.25 ml of 0.1 M acetate buffer at pH 5.0, 0.1 ml of enzyme solution, 0.1 ml of 2 % maltose aqueous solution and distilled water to a final volume of 0.5 ml, and incubation at 37 ℃ for 30 min. After incubation, the reaction was stopped by heating the mixture in a boiling water bath for 10 min. The amount of glucose formed was determined by the method of Papadopoulos and Hess[4], as modified by Dahlqvist[2]. One unit of enzyme activity was defined as the amount of enzyme that produced 1 μmole/min of glucose from maltose in the absence of marker.

RESULTS AND DISCUSSION

During the examination on the properties of multiple forms of α-glucosidases from spinach seeds, these enzymes were found to be inst-

TABLE 1. Effects of molecular weight markers, Triton X-100 and 2-mercaptoethanol on activity of spinach α-glucosidase II.

Exp. No.	I	II
Additions	Glucose formed	
	(μmoles/0.5ml of reaction mixture)	
None	0.367	0.257
Apo-ferritin	0.421	0.406
γ-GIobulin	0.535	0.525
Albumin	0.503	0.484
Ovalbumin	0.472	0.457
Chymotripsinogen A	0.469	0.457
Myoglobin	0.457	0.435
Lysozyme	0.447	0.421
Triton X-100	0.360	0.252
2-Mercaptoethanol	0.358	0.250

Exp. I. The reaction mixture (0.5 ml) containing 0.25 ml of 0.1M acetate buffer, pH 5.0, 0.05 ml of either 0.5 % marker, 0.5 % Triton X-100, 10 mM 2-mercaptoehtanol or distilled water, 0.1 ml of enzyme solution (0.012 unit) diluted 100 times with distilled water just before use, and 0.1 ml of 2 % maltose aqueous solution was incubated at 37℃ for 30 min.

Exp. II. The enzyme (0.1ml, 0.012 unit), immediately after dilution, was preincubated at 0 ℃ for 3 hr with 0.25 ml of 0.1M acetate buffer, pH 5.0. in the presence or absence of 0.05 ml of the addition. Then, the activity remaining was assayed after the addition of 0.1 ml of 2 % maltose aqueous solution.

able. The purified preparations suspended in ammonium sulfate solution were stable after storage for several months in a refrigerator, but were unstable when diluted. As shown in Table 1, α-glucosidase II solution consisting of a 400 times dilution lost about 30 % of its activity in 3 hr at 0 ℃. This loss of activity was prevented by the addition of either of a marker. The relative rates of hydrolysis of maltose in the presence of a marker was higher than that in its absence. The effect of γ-globulin was the most remarkable, and observed even at the low concentration of 25 μg per 0.5 ml of reaction mixture. None of the markers tested had any effect on the determination of glucose by glucose oxidase. The addition of 2-mercaptoethanol, a useful SH-protective reagent, was ineffective in preventing the loss of activity. Matsui et al.[3] reported that the detergent, Triton X-100, contributed to the stability of sweet corn α-glucosidase, but we found no effect of Triton X-100 on spinach α-glucosidases. Both γ-globulin and albumin were effective in preventing the loss of the activities of α-glucosidases I, III, and IV in either incubation at 37 ℃ or preincubation at 0 ℃ and 37 ℃ (Table 2). These results suggest that the markers tested here do not participate in the activation of the α-glucosidase II, but prevent the loss of the enzyme activity during preincubation and incubation after dilution. γ-Globulin protected all four enzymes much more effectively than albumin or other markers. The protec-

TABLE 2. Effects of albumin and γ-globulin on activities of spinach α-glucosidases.

Exp. No.		I	II	III
α-Glucosidases	Additions	Glucose formed		
		(μmoles/0.5ml of reaction mixture)		
I	None	0.467	0.345	0.355
	Albumin	0.591	0.566	0.576
	γ-Globulin	0.640	0.637	0.637
III	None	0.506	0.453	0.406
	Albumin	0.668	0.659	0.651
	γ-Globulin	0.769	0.755	0.753
IV	None	0.359	0.269	0.252
	Albumin	0.458	0.417	0.382
	γ-Globulin	0.547	0.515	0.496

Exp. I: The assay conditions were the same as described in the Exp. I in Table 1, except that human γ-globulin and bovine serum albumin were used. Enzymes used were 0.016 (I), 0.017 (III), and 0.012 units (IV).

Exp. II: The assay conditions were the same as described in the Exp. I, except that the preincubation was carried out at 0 ℃ for 20 hr.

Exp. III: The assay conditions were the same as described in Exp. II, except that the preincubation was carried out at 37℃ for 1 hr.

tive effect of human γ-globulin was also observed to be 1.4–2.2 times in the assay of β-galactosidase from *Esherichia coli*, using the reaction mixture (1.5 ml) containing 0.4 ml of 0.2 M phosphate buffer, pH 7.2, 0.1 ml of enzyme solution (0.15 unit), 0.5 ml of 1.2 % o-nitrophenyl-β-galactopyranoside, 0.15 ml of 0.5 % human γ-globulin and distilled water. The mechanism of the protective effect of γ-globulin is now under investigation.

SUMMARY

γ-Globulin protected all four α-glucosidases from the matured seeds of *Spinacia oleracea* L. cv. Viloflay much more effectively than albumin and other non-enzymatic proteins. γ-Globulin was also a potent protector of β-galactosidase from *Escherichia coli*.

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