Note

Pyroglutamyl-arginine, N-Terminal Tryptic Peptide of Wheat Germ Agglutinin

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Wheat germ agglutinin (WGA) has been used extensively as a tool in studies on the characterization and isolation of cell surface components.¹⁾ WGA is one of the most extensively studied plant lectins: Early physico-chemical studies on WGA^{2~4)} have revealed that WGA consists of two identical subunits which have a molecular weight of approximately 17,000 and a four-fold structural domain repeat. The amino acid composition of WGA indicates an unusually high content of cystine and glycine. The total number of amino acid residues is estimated to be 165. The N-terminal residue of WGA is blocked. Two to

four isolectins are present depending on the source of the wheat germ. This is presumably due to the polyploid nature of wheat.

Although the primary⁵⁾ as well as the three-dimensional structure^{6,7)} of WGA has been elucidated, no precise characterization of the N-terminal residue has been reported yet.⁵⁾ In this communication, isolation and characterization of the N-terminal tryptic peptide of WGA is described.

WGA was isolated from raw wheat germ (kindly supplied by Nippon Flour Mills Co., Ltd.) as described previously⁸⁾ and further purified by chitin-affinity chromatography.⁹⁾ A tryptic digest of WGA was obtained by treating performic acid oxidized WGA with TPCK-trypsin (Sigma) at 37°C for 16 hr. The tryptic digest was fractionated by ion-exchange chromatography using AG 50W × 2. As shown in Fig. 1, seven peaks were obtained. Identification of the N-terminal residue was done for each peak fraction. Since no PTH-amino acid was detected for fraction 4 by Edman degradation, this fraction must contain the N-terminal tryptic peptide of WGA. Yield of the peptide T-4 was 84%.

In order to characterize this peptide (T-4), it was treated with *Bacillus amyloliquefaciens* pyroglutamyl peptidase (PG peptidase: EC 3.4.19.3; a kind gift from Dr. D. Tsuru).¹⁰⁾ In Table I, results of amino acid analysis and PG peptidase treatment of this peptide together with authentic L-pyroglutamyl-L-alanine (pGlu-Ala) (Cyclo Chemical Corp.) as a control are shown. As seen in the Table, after acid hydrolysis, equi-molar amounts of Glu

FIG 1. Separation of Tryptic Digest of WGA by Ion Exchange Chromatography.

The tryptic digest of performic acid oxidized WGA was applied to an AG 50W × 2 column (0.8 × 70 cm), equilibrated with 0.1% pyridine-acetate, pH 3.80. A gradient elution with increasing pH and ionic strength was established with a nine-chamber apparatus, and the composition of buffers was the same as described by Canfield.¹⁴⁾ The elution was carried out at 37°C at a flow rate of 20 ml per hr and fractions of 3 ml were collected. Aliquots from alternate tubes were assayed by the ninhydrin reaction.

TABLE I. AMINO ACID COMPOSITION AND PG PEPTIDASE TREATMENT WITH PEPTIDE T-4^a

	pGlu-Ala		Peptide T-4	
	Glu (nmol)	Ala (nmol)	Glu (nmol)	Arg (nmol)
A. Amino acid composition	79.68 (0.99)	80.12 (1.00)	70.32 (1.03)	68.06 (1.00)
B. Pyroglutamyl peptidase	<0.01 (0)	76.77 (0.96)	<0.01 (0)	65.43 (0.96)

^a A: Approximately eighty nmol each of peptide T-4 and authentic pGlu-Ala were hydrolyzed with 6N HCl in a sealed, evacuated tube at 105°C for 24 hr and analyzed in a JEOL amino acid analyzer JLC 6AH.

B: The same amount of the samples was treated with PG peptidase in 50 mM phosphate buffer, pH 7.3, containing 30 mM 2-mercaptoethanol and 1 mM EDTA for 16 hr at 37°C⁽¹²⁾ and the digest was assayed by the amino acid analyzer.

and Arg from the peptide T-4 were detected. By treatment with PG peptidase, only Arg from the peptide T-4 was quantitatively liberated. These results strongly suggest that the peptide exists in the form of pGlu-Arg. To confirm this, reduced and carboxymethylated WGA was treated with PG peptidase and directly sequenced by the automated procedure using a JEOL Sequence Analyzer under the conditions described previously.⁽¹¹⁾ A new N-terminal residue, Arg, was detected by an amino acid analyzer JLC 6AH (JEOL) after back hydrolysis of the thiazolinone derivative from the first cycle of sequence analysis with 5.7N HCl containing 0.1% SnCl₂ at 150°C for 4 hr.⁽¹²⁾ Yield of Arg was estimated to be approximately 30%. No other amino acids in significant amounts were detected. The new N-terminal residue was followed by Cys(Cm)-Gly-Glx-Glx-Gly-.

Specificity of PG peptidase for amino acids penultimate

to a terminal pyroglutamic acid residue has been investigated.⁽¹³⁾ The results presented in this communication have revealed that the N-terminal residue of WGA is pGlu and the enzyme is able to remove this residue from S-carboxymethylated WGA.

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