The Presence of A New Peptide in A Brown Algae, Undaria Pinnatifida (1)

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미역에서 分離된 新페프타이드 (1)

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要 約

二次元 페이퍼크로마토그레피와 이온交換樹脂크로마토그레피를 使用하여 미역의 알콜抽出物에서 新 페프타이드를 分離하고 그 構成은 글루타민酸, 아스파라긴酸, 알라닌의 三種 아미노酸으로 되어 있음을 밝혔다. 各 아미노酸 結合順序는 次報에 發表하기로 한다.

A new peptide was isolated from an edible brown algae "*Undaria pinnatifida*" by both two dimensional and ion-exchange resin chromatographies. The peptide was composed of three amino acids, glutamic acid, alanine and aspartic acid.

Introduction

Naturally occurring peptides are of great interest, since some of these often exhibit the potent biological activity of antibiotic, hormone, toxin, growth factor, or the like. The formation of certain peptides such as glutathione has been considered to provide model reactions in the biosynthesis of protein.[1] Some may be important metabolites.

A few information on the peptides obtained from marine algae are available. Hass et al. isolated glutamic octapeptide[2] from *Pelvetic canaliculata* in 1931 and later aspartic acid pentapeptide and others.[3] A peptide like substance, eisenine, was isolated by Ohira from *Eisenic bicyelis Setchell* and formulated as pyrrolidonyl glutamylalanine.[4] Another peptide was isolated from aqueous extracts of a sea weed *Pelvetia fastigiata* and formulated as L-pyrrolidonyl α-L-glutamine by Dekker et al. in 1959, Hass described on some peptides isolated from *Griffitnisic flosculose* and *Carollina squamata*.[6]

A new peptide has been isolated from *Undaria* at this laboratory during thee investigation of free amino acids in the above sea weed.[7] A part of the results is presented in this paper.

Experimental

Sample preparation: *Undaria Pinnatifida* collected at the east coast of Korea was air-dried, cut, and further dried in an oven at 70°C overnight. The material was ground into fine powder by waring blender. The composition of the sample was: H_2O 8.29%, Fat 2.15%, Nitrogen 1.47%, Ash 33.4%.

Extraction: 500 grams of the powder were extracted with one liter of 95% ethanol on the steam bath about 3 hours with occasional stirring. Two more successive extractions were made and the combined extracts were kept at room temperature overnight. The extract, after removal of mannite that was crystalized out by filtration, was concentrated under a reduced pressure down

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to about 200 ml. The concentrate was filtered to remove inorganic salts and again concentrated to 50 ml. After the addition of 200 ml. of warm water to the final concentrate, chlorophyll, tannin, and resin like substances were removed by filtration. The filtrate was then concentrated in vacuo to a syrup which was dissolved in 200 ml. of warm water and chilled in an ice box overnight. After the filtration a yellowish clear solution resulted. To this filtrate was added a sufficient amount of the saturated basic lead acetate with vigorous shaking. The mixture was filtered and the filtrate was freed of excessive lead by the addition of a dilute sulfuric acid. The filtrate free of lead sulfate was treated with barium hydroxide, filtered and washed with water. The combined filtrate and washings were evaporated in vacuo to about 50 ml.

Fractionation of peptide: Applying an ascending two-dimensional paper chromatography with the use of phenol-water(100 : 39 w/v) (A)[8] as the first solvent and butanol-acetic acid-water(4:1:1 v/v) (B)[9] as the second it was possible to attain a satisfactory fractionation of a peptide from amino acids. Approximately 5 ml of the extract prepared as described above was spotted at one corner (1.2 cm away from both edges) of the Whatman No. 1 filter paper(24 cm × 24 cm) that was washed with ditilled water before the operation. The samples were allowed to run parallel in four sheets. One of the papers was sprayed with 0.2% ninhydrin solution[10] in ethanol and used as the guide to locate the peptide area on the other chromatograms. With the aid of the guide chromatogram the peptide area was located, cut out and eluted with water. The eluate was evaporated in to dryness under the reduced pressure.

Identification of amino acids in peptide: In order to identify the constituent amino acids the residue was completely hydrolyzed with 0.5 ml of 5.7 N-HCl at 110°C for 18 hours.[12] The hydrolyzate was then taken up in water and evaporated under a reduced pressure. This treatment was repeated twice to remove HCl. The final residue was dissolved in 0.05 ml of 10% isopropanol. The solution so obtained was analyzed chromatographically[13] and the constituent amino acids were identified.

A sample of peptide the constituents of which are known was hydrolyzed in 6 N-HCl at 105°C for 18 hours. The solution was evaporated to dryness twice with water and the residue was subjected to the two-dimensional paper chromatography with the solvents (A) and (B). After the first dimensional test the quantitative markers of the constituent amino acids in an appropriate range of concentrations were applied to the paper. After the second dimensional run the concentrations of amino acids of the peptide were determined by comparing with those of the standard markers after the method of Mendelstam and Rogers.[14]

For the determination of the peptide in *Undaria pinnatifida* ion exchange chromatographic method[15][16] using Amberite IR-120 was employed. Ten grams of powdered sample was extracted with 100 ml portion of 80% ethanol overnight and filtered. The residue was washed thoroughly with 300 ml of 80% ethanol. The extract and washings were combined and evaporated over water bath to about 50 ml. The resulting solution was filtered to rid of impurities like chlorophyll and inorganic salts. The filtrate was reevaporated again to dryness and the residue was dissolved in a small amount of water, filtered and made up to 25 ml with distilled water. One ml of the preparation was introduced to the 150 cm column of Amberite IR-120. The pH of initial buffer was 3.25 and the temperature of the column was maintained at 50°C throughout the analysis. The effluent was collected in 2 ml fraction and analyzed photometrically with one ml. of the modified ninhydrin reagent[17].

Results and Discussion

The alcoholic extract of the sea weed was positive to the Biuret reaction and readily separated the peptide from concomitant amino acids by

the two-dimensional paper chromatography as shown in Figure 1. The eluate of the cut peptide area was subjected to one dimensional paper chromatography and indicated Rf values as follows.

Solvent system	Rf
Butanol-pyridine-water(7:7:4 v/v)	0.13
Butanol-acetic acid-water(4:1:1 v/v)	0.12
Phenol-water(100:39 w/v)	0.66

The isolated peptide, although limited in quantity, was enough to identify the amino acids therein.

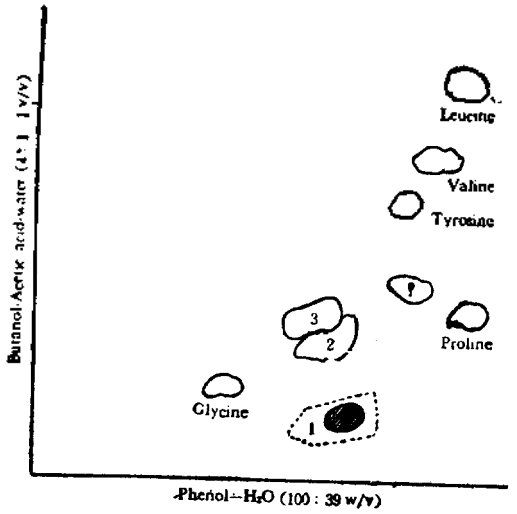

Fig. 1—Two-dimensional chromatograms of the peptide and amino acids in alcoholic extract of *Undaria pinnatifida:* spot 1 sky blue color, peptide; spot 2 violet color, unknown; spot 3 yellow color, unknown.

The hydrolyzate of the peptide was chromatographed in two dimensions using the above mentioned solvent systems. The separated spots corresponded to glutamic acid, aspartic acid, and alanine(Figure 2). Those amino acids of known concentrations were used as standards in parallel with the unknown. Investigation on the sequence of those amino acids has been in progress at this laboratory using dinitrophenylation method[18] for the N-terminal assay and acetylation method[19] for the C-terminal assay.

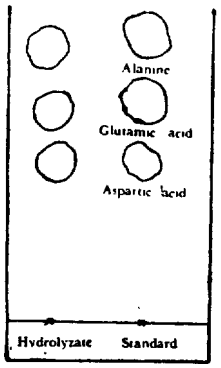

Fig 2—Chromatogram of the peptide hydrolyzate. Solvent; Butanol-acetic acid-water(4:1:1)

An attempt to isolate a sufficient amount of the peptide by the formation of mercuric complex failed to give the crystalline product of peptide after removal of Hg The failure may be due to the nature of the peptide. However, the mercuric complex gave the same pattern of amino acids on hydrolysis.

The determination of the peptide by the ion

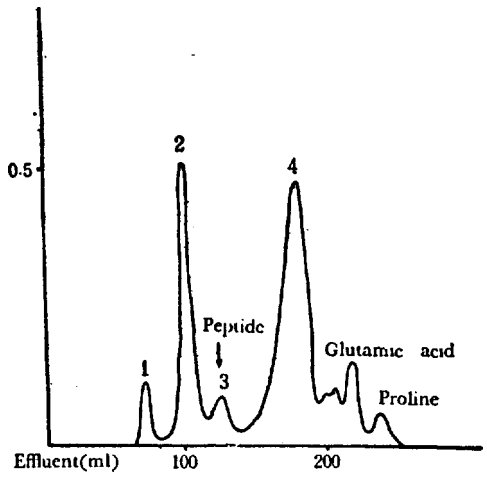

Fig. 3—Chromatographic fractionation of the peptide on the column of Amberite IR-120. Load on 1.0× 150 cm column was one ml of the preparation, obtained by elution of some amino acids and peptide at 50°C from the column at the flow rate of 14 ml per hour. Effluent collected in 2-ml fraction. Peak No. 3 cerresponds the peptide isolated in this experiment.

exchange chromatography developed by Moore and Stein[15] gave the result as shown in Figure 3. The peak of the peptide emerged between the 118 ml and 140 ml effluents with the aid of 0.2 N sodium citrate buffer(pH 3.25). The effluent fraction was desalted with Dowex $2^{(20)}$ and paper chromatographed. Rf values of the spots in the different solvent systems gave excellent agreement with those of the peptide isolated by the two-demensional paper chromatography. The color of the spot developed by 0.2% alcoholic ninhydrin solution was also sky blue.

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