Chromatographic Determination of Amino Acids in Nonprotein and Protein Fraction Of Undaria Pinnatifida

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An edible brown alga, "Undaria pinnatifida" which occurs along the eastern seaboard of Korea, has been one of the popular soup materials especially as a traditional food item of the nursing mother in Korea.

Although a few analytical data on the contents of iodine, mannite. alginic acid and fatty acids of the alga have been reported by Japanese workers, little is known on the nature of the protein of the alga.

In the present study, an attempt was made to determine the amino acid composition of botn protein and nonprotein fractions* of the alga quantitatively, by the use of the ion eschange resin chromatography developed by Moore and Stein. (2). (3).

EXPERIMENTAL

1. Materials. The air dried sea weed "*Undaria Pinnatifida*" under investigation-was purchased in the market in Seoul.

After drying the alga at the temperature below 60°C., it was pulverized with waring blendor and the powdered alga was stored in a desiccator until used.

The composition of the sample was fouud to be of 1.47 per cent of total nitrogen, 2.15 per cent of ether extractable fraction, 8.29 per cent of moisture and 33.43 per cent of ash contents.

2. Fxtraction of the Nonprotein Fraction. Ten grams of powdered sample were extracted with 100 ml. of 80 per cent ethanol over night at room temperature. Removed from the extract by filtration, the residue was washed thoroughly with

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* In this paper nonprotein (soluble) nitrogen implies nitrogenous compounds the extracted by 80 per cent ethanol; protein nitrogen implies that which remains insoluble in this solvent (1).

300 ml. of 80 per cent ethanol. The extract and the washings were combined and evaporated on a water bath at 50°C.

The extract was concentrated down to about 50 ml., allowed to cool and separated from inorganic salts, waxes, pigments, and the other precipitates by filtration. The filtrate was re-evaporated to dryness, then the residue was dissolved in 25 ml. of 80 per cent ethanol and solution was filtered, evaporated again until no more alcoholic odor remained. The solution was kept in an ice-box over night and light yellowish solution was optained after filtration.

It was made up to the final volume of 25 ml. with distilled water. The total nitrogen of the solution was equilvalent to 13.3 mg. and 1.25 ml. of the sampie solution was taken as a sample of each ion exchange chromatography. 3. **Hydrolysis of the Protein Fraction.**

The residue of 80 per cent ethanol extraction of the alga obtained in the preceding procedure was dried at the room temperature and kept in a desiccator until its analysis.

The nitrogen content of the sample was 2.17 per cent.

Three hundred and sixty nine milligrams of the sample, epuivalent to 8 mg. of total nitrogen were, equivalent to 8 mg. of total nitrogen were introduced into a 300-ml. round bottomed flask with a reflux condenser and hydrolyzed with 200 ml. of 6 N hydrochloric acld for 24 hours at 110°C. (4).

The hydrolyzate was evaporated to dryness under a reduced pressure, addition of distilled water and evaporation to dryness under a reduced pressure were repeated until the removal of the excess hydrochloric acid was completed.

After dissolving the residue with the small amount of distilled weter, the solution was carefully filtered and then the filtrate was adjusted to the final volume of 25 ml.

The total nitrogen in the solution was 7.98 mg. and 1 ml. of the solution was taken for the ion exchange chromatographic analysis.

4. **Analysis if amius acids.**

The amino acids were analysed by use of the ion exchuge resin column chromatography developed moore aud Stein [2], [3].

RESULTS AND DISCUSSION

The amino acid compositions of both protein and nonprotein fractions optained from "*Undaria pinnatifida*", were in good contrast with each other as shown in

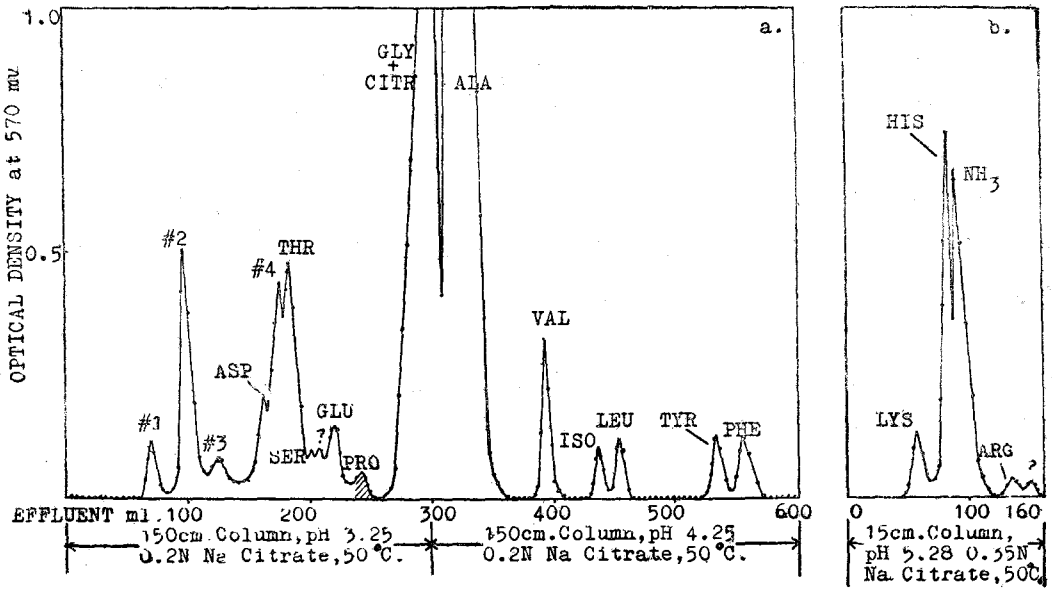

Fig 1 Chromatographic Fractionation of the Nonprotein Fraction of the 'Undaria Pinnatifida' on Column of Amberlite IR-120.
a. Obtained by elution of neutral and acidic amino acids at 50°C. from a 1.0 X 150 cm. column at flow rate of 8ml. per hour.
b Elution of the basic amino acids from a 1.0 X 15 cm. column of the resin at 50°C

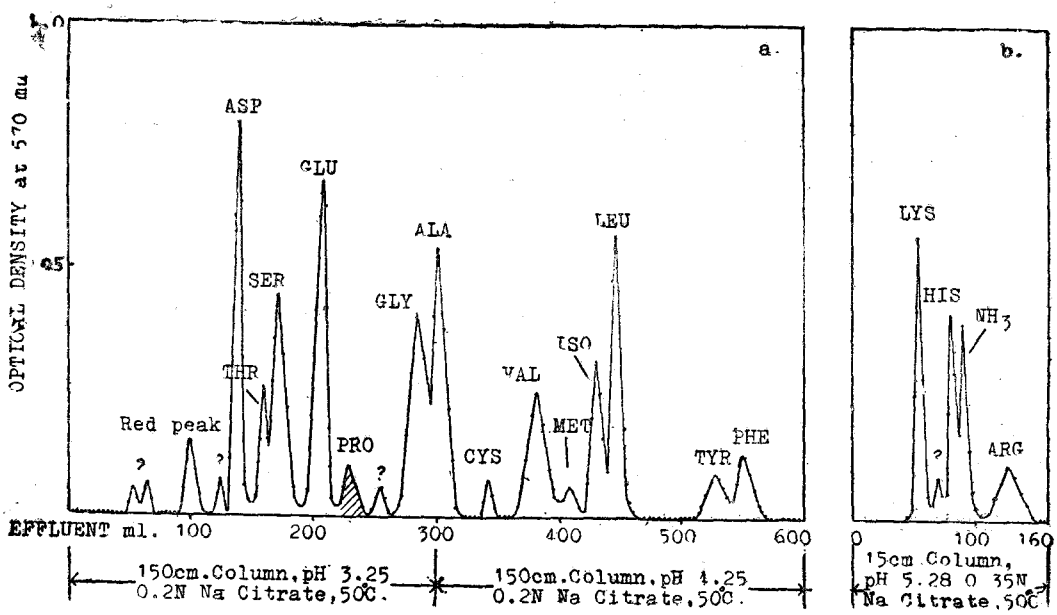

Fig. 2. The Amino Acid Composition of the Protein Fraction of the 'Undaria Pinnatifida'.
a. Obtained by elution of neutral and acidic amino acids at 50°C. from a 1.0 X 150 cm. column at flow rate of 8 ml. per hour.
b. Elution of the basic amino acids from a 1.0 X 15 cm column of the resin at 50°C.

Table 1 and 2.

The nitrogen content of the nonprotein fraction (soluble in 80 per cent ethanol) extracted from 10 g of the dry alga, was about 13 mg. while the protein fraction, the resulting residue of above extraction, contained about 130 mg. of nitrogen, so the ratio of nitrogent of both fraction was one to ten.

The observed amino acid composition of the protein fraction is indicative of fairly even distribution of the essential amino acids therein. The amounts of essential amino acids in this fraction are rather higher than those found in the soybean protein.

In the nonprotein fraction there were two peaks which occupy over 30 per cent of the total nitrogen in the fraction. One of them was alanine and the other was found to be the mixture of citrulline and glycine. The later peak was fractionated at 260 ml. to 300 ml. with pH 3.25 sodium citrate buffer from 150-cm. column and still overlappedewith glycine peak. Stein and Moore[5]. [6]. described that citrulline is overlapped with glycine at the position between alanine and proline from the 0.9X 100-cm. Dowen-50 column. Also this nitrogen containing substance was I locate very close to glutamine[7] at two dimensional paper chromatogram, using phenol-water (100:39 w/v)[3] and butanol acetic acid-water (4:1:1 v/v) (9) as solvent system, and resisted the acid hydrolysis. If this substance were glutmaic acid should have appeared on the two dimensional paper chromatogram after its hydrolysis, but the original spot was still unchanged. In addition, this substance was positive to the Ehrlich reagent (dissolved 2 g. of p-dimethyl-aminobenzaldehyde in 100 ml. of 2 per cent HCl) and had same Rf values with the authentic citrulline on the one dimensional paperchromatogram using the following three solvent system : phenol-water (100:39 w/v), butanol-aceti cacidwater (4:1:1 v/v) and butanol-pyridine-water (7:7:4 v/v)[7].

Although most of the usual amino acids were found in the nonprotein fraction, there were some unidentified ninhydrin positive peaks, as shown in Figure 3. One of them, the third peak in Figure 3, was identified as a new tripeptied. This naturally occuring peptide was confirmed to be composed of

Table 1. Composition of the Nonprotein Fraption and the Protein Fraction of "Undaria pinnatifida"

	Nonprotein Nitrogen Fraction			Protein Nitrogen Fraction		
	mM/16gNN	g/16gN	N%	mM/16gN	g/16gN	N%
Aspartic acid	14.9	1.98	1.32	9.70	12.91	8.52
Threonine	32.4	3.86	2.83	21.0	2.50	1.88

Serine	3.5	0.37	0.31	99.6	10.47	8.77
Glutamic acid	11.7	1.72	1.01	102.6	15.09	9.06
Proline	18.1	2.08	1.57	59.6	6.86	5.26
Glycine	(+Citrulline) 175.6	—	30.97	39.6	6.73	7.98
Alanine	316.8	28.23	27.89	95.6	88.52	8.39
Valine	15.4	1.80	1.38	71.0	8.32	6.26
Isoleucine	5.5	0.72	0.50	44.0	5.77	3.88
Leucine	6.9	0.91	0.63	74.6	9.71	6.51
Tyrosine	7.9	1.43	0.69	20.6	3.72	1.75
Phenyl-alanine	11.2	1.85	1.01	25.6	4.23	2.26
Lysine	12.0	1.75	2.14	47.6	6.96	8.39
Histidine	32.2	5.00	8.48	29.1	4.51	7.64
Ammonia	91.4	1.55	8.04	24.6	0.42	2.13
Arginine	3.7	0.65	1.32	18.3	3.19	6.39
Cystine	—	—	—	6.6	1.59	1.12
Methionine	—	—	—	7.6	1.13	0.63
A peptide	9.2	3.40	2.45	—	—	—

Table 2. Comparison of Amino Acid Composition of Nonprotein Fraction and Protein Fraction in 10 g. of "*Undaria pinnatifida*"

	Nonprotein Nitrogen Fraction			Protein Nitrotein Fraction		
	μM	mg.	mg. N	μM	mg.	mg. N
Aspartic acid	12.3	1.63	0.17	814.8	108.46	11.42
Threonine	26.9	3.20	0.38	176.4	31.96	2.52
Serine	2.9	0.30	0.04	836.6	87.93	11.76
Glutamic acid	9.7	1.43	0.14	861.8	126.80	12.10
Proline	15.0	1.73	0.21	500.6	57.63	7.06
Glycine	(+Citrulline) 146.0	—	4.12	752.6	56.50	10.58
Alanine	263.4	23.47	3.69	803.0	71.55	11.26
Valine	12.8	1.50	0.18	596.4	69.87	8.40
Isoleucine	4.6	0.60	0.06	369.6	48.48	5.21
Leucine	5.7	0.75	0.08	626.6	82.19	8.74
Tyrosine	6.5	1.18	0.09	173.0	31.35	2.35
Phenyl alanine	9.3	1.54	0.13	215.0	41.46	3.02
Lysine	10.0	1.46	0.28	399.8	58.45	11.26
Histidine	26.8	4.16	1.13	244.1	37.88	10.25
Ammonia	76.0	1.29	1.07	206.6	3.51	2.86
Arginine	3.1	0.53	0.17	153.7	26.78	8.57
Cystine	—	—	—	55.4	13.31	1.51
Methionine	—	—	—	63.8	9.52	0.84
Sum of Unknown	•	•	1.30	•	•	4.37
Total	•	•	13.24	•	•	134.09
Total N (by Kjeldahl method)		13.3 mg.				134.1 mg.

alanine, glutamic acid and aspartic acid through paper chromatographic techniques after the hydrolysis. The amino acid sequence of the peptide has been under further investigation with the use of 1-fluoro 2,4-dinitrobenzen (FDNB) for the N-terminal assay.

The Rf values of the unidentified peak and the peptide at the chromatogram (Figure 1) were as follows:

Table 3. Rf values of the nihydrin Positive substances from unidentified Peaks

Solvent System	Rf Values			
	※1	※2	※3	※4
phenol-water (100:39 w/v)	—	0.18	0.68	0.32
butanol-aceticacid-water (4:1:1 v/v)	0.21	0.22	0.31	0.27
butanol-pyridine-water (7:7:4 v/v)	0.19	0.09	0.13	1.18

A Japanease worker[10] isolated 8.2 g. of alanine from hydrolyzate of 1 kilogram of the "*Undaria pinatifida*", however the result from this experiment showed, the alanine content was 9.5 g. in same amount of the alga, and one third of the amount occured in free state.

The red peak (Figure 2) that appeared prior to the elution of aspartic acid was considered to be due to the complex of ninhydrin with unknown substances resulting from hydrolysis of protein in the presences of carbohydrates. This fact was already described by Dustin and Moore et. al.[11] and also confirmed by theauthor in the hydrolysis of rice at this laboratory[12].

SUMMARY

The amino acid compositions of the protein and the nonprotein fractions obtained from a marine brown alga, "*Undaria pinnatifida*" were determined by use of Ion Exchange Column Chromatography.

The protein nitrogen in the alga was about ten times of the nonprotein nitrogen. Nonprotein fraction obtained from the extraction with 80 per cent ethanol contains considerable amount of free citrulline. Alanine content in the alga was the highest (about 1 per cent in dry weight) and one third of which was found in free state. The amino acid composition of the alga was well balanced and the content of the essential amino acids were relatively higher than soybean protein.

In addition, several peptide like substances were fractionated from nonproatein fraction, in which one was identified as a naturally occuring new tripeptide composed of alanine, glutamic acid and aspartic acid, and the remaining unknown substances are under investigation for the further information.

抄　　錄

Ion交換樹脂 cdumn chrcmatography法에 依해서 食用海藻인 "미역"의 蛋白性窒素劃分 및 非蛋白性窒素劃分의 amino酸含量을 定量하였다.

1. 蛋白性劃分의 窒素含量 (134.1mg/10g)은 非蛋白性劃分窒素(13i3mg/10g)의 約 10倍이다.

2. 兩者의 amino酸組成은 서로 對照的이고 그中 蛋白性劃分에는 必須 amino酸들이 고루 含有되어 있으며 그들 含有率은 大豆蛋白의 境遇보다 높았다.

3. 非蛋白性劃分 (80%ethanol可溶)에는 相當量의 Citrulline 이 含有되어 있는것 이는 Ion 交換樹脂 Column에서의 溶離順位, 一次元 및 二次元 paper chromatogram 上에서의 位置, 그리고 Ninhydrin 및 Ehrlich 試藥에 對한 陽性反應에 依해서 證明하였으나 그 正確한 含量은 150-cm column에서 Glycine과 重疊되어서 溶離되는 關係로 알수없고, 다만 이들 混合物의 窒素量이 이劃分全窒素中 約 30%에 相當함을 밝혔다.

4. 非蛋白性劃分에서 遊離狀態로 存在하는 새로운 peptide를 分離해 냈는데, 이는 鹽酸加水分解後 paper chromatography에 依해서 調査한바 Alanine, Gluatmic acid 및 Aspartic acid로 組成된 Tripeptide 임을 알았다.

5. "미역"(乾燥物)에는 約 1%에 該當하는 多量의 Alanine이 含有되어있는데 그中 約 1/3量이 遊離狀態로 存在함을 알았다.

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