ON THE DIFFERENCE IN PHYSICO-CHEMICAL PROPERTIES OF VARIOUS PROTEINS IN PLANT SEEDS, (Second Report).

On the Differences in the Physico-chemical Properties of the Four Kinds of Rice Proteins which Vary in their Iso-electric Points.

By

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Introduction.

Rona and Michaelis[1] and Loeb[2] reported that the iso-electric point of edestin, a NaCl soluble protein, is pH 6.0–6.8 of acidic side, while that of gliadin, an alcohol soluble protein is pH 9.23 of basic side. Recently, Csonka, Murphy and Jones[3] have stated that the iso-electric point of albumin is pH 4.0–5.0, globulin, pH 5.0–5.5, and prolamin, pH 6.0–6.5. The present authors[4] stated in their first report that in the order water soluble-, NaCl soluble-, alkali soluble- to alcohol soluble-protein of rice, when the iso-electric point is decreased their acidity and sulphur contents also decrease. When the physico-chemical properties of NaCl soluble rice protein were compared to those of NaOH soluble the following differences were observed:

(1) In elementary composition, the carbon content of NaOH soluble protein is less than that of NaCl soluble. The nitrogen content of the former is higher than that of the latter.

(2) The specific rotatory power of NaOH soluble protein alkaline solution is higher than that of NaCl soluble.

(3) The combining power of NaCl soluble protein with HCl is less than that of NaOH soluble. The combining power of the former with silver is greater than that of the latter.

(4) In the determination of amino acid value by Van Slyke's ad-

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vanced method, NaCl soluble protein is found to be rich in monoamino-, lysin- and cystin-form nitrogen. NaOH soluble protein is rich in ammonia-, melanin-, diamino- and histidin-form nitrogen. The ammonia nitrogen and histidin nitrogen contents of NaCl soluble protein are about 59 % and 23 % respectively, of those of NaOH soluble protein. The melanin nitrogen of the NaCl soluble is about 82 % of that of the NaOH soluble.

(5) The free amino nitrogen content of NaOH soluble protein is greater than that of NaCl soluble, but its content of lysin is the reverse. The authors' results differ from Van Slyke's which showed that the free amino nitrogen is about half of the lysin nitrogen of protein.

(6) The histidin content of NaOH soluble protein is greater than that of NaCl soluble, while the tyrosin content shows no difference between the two kinds of protein.

(7) The NaCl soluble protein is digested by pancreatin more easily than the NaOH soluble which shows that the former has less complex radicals than the latter.

From the above results the difference of the physico-chemical properties of the two proteins which vary in their iso-electric point was observed. The authors intend in the following experiments to investigate further various proteins prepared by different methods and to discover how the difference of iso-electric point of proteins influences their physical and chemical properties.

I. Isolation and Purification of the Different Proteins.

(1) Preparation of Proteins Having Different Iso-electric Points.

In this experiment two different kinds of unhulled rice were used for preparation of the protein. One was a rice produced in the middle part of Japan (Shigaken) in 1924 and the other was produced in the northern part of Japan (Aomoriken) in 1925. Both kinds of rice were dried in the open air, powdered, extracted with ether in a Soxhlet's apparatus and dried again in the open air. A definite quantity of the defatted rice powder was mixed with 10 or 20 times its weight of 0.3 % NaOH solution, shaken for 30 minutes, and allowed to settle in a cold place. The upper liquid was decanted into a Buchner's funnel, the extraction repeated, and then filtered through a pulp layer. To the starch-freed filtrate were added various amounts of dilute HCl and the proteins were precipitated partially; these partial precipitates were named A1, A1′, B1, C1, D1 (Shiga) and

A2, B2, C2, D2, E2 (Aomori) from less to greater addition of HCl. Each precipitate was washed many times with 70 % alcohol, absolute alcohol, and ether, and dried in a H_2SO_4 desiccator.

(2) Preparation of NaCl- and Alcohol-soluble Proteins.

The following operations were performed, using unhulled rice produced in Korea in 1925. The defatted rice powder was extracted with 5–7 or 10 % solution of NaCl and all the extracts were filtered through pulp layer. From the starch-freed filtrates the protein was precipitated by addition of either acetic acid only or acetic acid and ammonium sulphate. Precipitates from the former were washed with water and those from the latter were dialysed in water for about one week in order to remove the reagent. Each precipitate was washed with 70 % alcohol to extract the alcohol soluble protein, then with absolute alcohol and ether, and dried in a H_2SO_4 desiccator (Samples 3, 4 and 5). The protein dissolved in the washings of 70 % alcohol was collected by evaporation and dried in the usual way (Sample 2).

The residue from NaCl extraction was washed with water until it showed no chlorine reaction. It was again extracted with 0.2 % NaOH solution and the extract was filtered to remove starch, after which the dissolved protein was precipitated by acetic acid (Sample 6). The resulting precipitate was washed with water, 70 % alcohol, absolute alcohol, and ether and dried in a H_2SO_4 desiccator. The protein dissolved in 70 % alcohol was collected by evaporation in vacuum (Sample 1).

The following six samples were prepared by the above procedures:

No. 1. 70 % alcohol soluble protein prepared from 0.2 % NaOH soluble.
No. 2. 70 % alcohol soluble protein prepared from NaCl soluble.
No. 3. The acid-precipitated protein from 10 % NaCl extracts.
No. 4. The acid-precipitated protein from 5–7 % NaCl extracts.
No. 5. The ammonium sulphate-precipitated protein from 10 % NaCl extracts.
No. 6. 0.2 % NaOH soluble protein (Oryzenin).

II. Water and Ash Contents of Samples.

The water and ash contents were determined by the ordinary method.

TABLE I

Water and Ash Contents of the Protein Samples.

Sample	H_2O (%)	Ash (Dry Matter %)	Sample	H_2O (%)	Ash (Dry Matter %)
A1	4.94	—	A2	7.56	1.48
A1′	4.13	—	B2	5.12	1.40
B1	7.10	—	C2	6.34	1.44
C1	2.83	—	D2	5.86	1.78
D1	1.03	—	E2	7.05	2.30
No. 1	2.35	0.326	No. 2	2.56	0.388
No. 3	8.22	0.613	No. 4	7.25	0.605
No. 5	7.27	0.702	No. 6	5.46	0.319

III. Preliminary Experiment on the Iso-electric Point Estimation of the Different Protein Solutions.

As described in the former report,[5] 0.1 g protein was dissolved in 10 cc of 1/50 n-NaOH solution and 1 cc of the protein solution was diluted with 9 cc of pure redistilled water. The mixture was titrated with 1/100 n-HCl in the earlier experiments and with 1/200 n-HCl in the present experiment. The point of maximum surface tension and turbidity was determined.

TABLE II

Surface Tension and Turbidity of the Protein Samples.

Surface Tension at 18°C.					
cc of HCl	A1	A1′	B1	C1	D1
1.0	69.7	69.9	69.7	69.0	69.0
1.1	69.0	70.4	69.9	69.7	66.0
1.2	69.7	71.0	69.7	70.4	66.7

cc of HCl	A1	A1′	B1	C1	D1
1.3	69.9	70.4	70.4	69.9	68.5
1.4	70.4	71.0	71.0	70.4	68.7
1.5	72.0	72.5	72.0	71.0	69.0
1.6	69.9	71.0	72.3	72.0	69.3
1.7	69.9	70.4	69.9	73.2	74.7
1.8	—	—	69.0	72.0	69.3
			Turbidity		
1.1	+	+	—	—	—
1.2	+	+	—	+	—
1.3	++	++	+	++	+
1,4	++	++	+	++	+
1.5	+++	+++	++	++	++
1.6	++	++	+++	++	++
1.7	++	++	++	+++	+++
1.8	++	++	++	++	++
Approximate Isoelectric Point	5.6	5.6	5.4	5.3	5.1

Surface Tension at 18° C.						
cc of HCl	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
4.70	59.3	59.3	59.3	59.8	59.3	60.7
4.75	61.4	60.7	—	—	—	—
4.80	60.7	61.4	59.3	59.3	59.3	60.7
4.85	—	60.7	—	—	—	—
4.90	59.3	59.3	60.7	60.7	60.7	61.4
4.95	—	—	60.7	—	—	61.4
5.00	58.7	59.3	61.4	61.4	61.4	62.2
5.05	—	—	60.7	62.2	—	61.4

cc of HCl	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
5.10	—	58.7	60.7	61.4	61.4	60.7
5.15	—	—	—	—	60.7	—
			Turbidity			
4.70	++	++	++	+	+	+
4.75	+++	++	++	+	+	+
4.80	++	+++	++	++	++	++
4.85	++	++	++	++	++	++
4.90	++	++	++	++	++	++
4.95	++	++	++	++	++	++
5.00	+	+	+++	++	++	+++
5.05	+	+	++	+++	++	++
5.10	—	+	++	++	+++	++
5.15	—	—	+	++	++	+

From the results of samples A1, A1′, B1, C1, D1, the partial precipitation of protein due to difference of acidity indicates different iso-electric points. Thus A or B corresponds to oryzenin, and C or D corresponds to NaCl or water soluble protein. From the results of samples No. 1 to No. 6, the alcohol soluble protein showed the least acidic nature, alkali soluble was slightly more acidic and NaCl soluble was most acidic, especially in the 10 % NaCl soluble.

IV. Nitrogen, Sulphur and Phosphorus Contents of the Different Kinds of Proteins.

Nitrogen, sulphur and phosphorus of protein being determined by the ordinary methods which are stated in the former report,[6] the following results were obtained.

TABLE III

Nitrogen, Sulphur and Phosphorus Contents of the Protein Samples.

Sample	Nitrogen (Dry Matter %)	Ratio	Sulphur (Dry Matter %)	Ratio	Phosphorus (Dry Matter %)	Ratio
A1	16.064	100	0.7108	100	—	—
A1′	—	—	0.7806	109	—	—
B1	15.877	98	0.8561	120	—	—
C1	15.570	90	0.8764	123	—	—
A2	15.793	100	—	—	0.1579	100
B2	15.617	98	—	—	0.1341	85
C2	14.915	94	—	—	0.0850	53
D2	14.214	90	—	—	0.0756	47
E2	13.336	84	—	—	—	—
No. 1	17.150	100	0.3867	100	—	—
No. 2	17.344	101	0.4072	105	0.1562	100
No. 3	16.348	95	1.0473	270	0.3575	228
No. 4	16.108	94	1.1104	287	0.3477	222
No. 5	16.093	94	1.2507	325	0.3759	240
No. 6	16.385	96	0.7758	200	0.1892	121

In the above table, the protein precipitated by much addition of HCl, showed lower contents of nitrogen, higher contents of sulphur, and lower contents of phosphorus. The same facts were observed in the order; water soluble-, alkali soluble-, and alcohol soluble-protein.

V. Carbon and Hydrogen Contents of Different Proteins.

The carbon and hydrogen contents of partially precipitated protein were determined by the ordinary method and the following results were obtained. In this experiment the quantity of sample was not sufficient to treat each separately, so a mixture of B2, C2 and D2 was made and compared to A2.

TABLE IV

Carbon and Hydrogen Contents of the Protein Samples.

	Sample g	CO_2 g	H_2O g	Carbon (%)	Hydrogen(%)
A2 (H_2O 7.560 %)	0.0541	0.0960	0.0442	52.3636	8.9111
	0.0541	0.0954	0.0472	52.0360	9.5556
	0.0540	0.0954	0.0473	51.9768	10.2223
Average	—	—	—	52.1221	9.5630
B2–C2–D2 (H_2O 7.058 %)	0.0572	0 0949	0.0423	50.6494	7.8712
	0.0593	0.1004	0.0437	50.7506	7.8965
	0.0602	0.0989	0.0452	50.1301	8.0112
Average	—	—	—	50.4767	7.9263

In the above results the carbon content of protein which needs a large quantity of HCl to precipitate it from its alkaline solution, is less than that of protein which needs a smaller quantity of HCl.

VI. Determination of Free Amino Nitrogen of Different Proteins.

The free amino nitrogen contents of the following proteins were determined by Sörensen's method which procedure has already been described in the first report. One-tenth g of sample was dissolved in 25 cc of 1/5 n-NaOH solution and the experiment was carried out after six hours.

TABLE V

Free Amino Nitrogen of the Protein Samples.

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Equivalent cc of 1/5 n-NaOH	0.55	0.60	0.55	0.43	0.40	0.50
Free Amino Nitrogen in mg	1.540	1.680	1.540	1.204	1.120	1.400
% of Free Amino N (Dry Matter)	1.925	2.100	1.925	1.550	1.400	1.750
% of Free Amino N (Total Nitrogen)	11.2241	12.1079	11.7746	9.6225	8.6990	10.6804

From the above results the free amino nitrogen content of alcohol soluble protein obtained from NaCl soluble is seen to be the greatest, that of NaCl soluble is next, and then the alcohol soluble and alkali soluble are smaller. There are two different samples which showed less content of free amino nitrogen than alkali soluble (No. 5 is the ammonium sulphate-precipitated protein from 10 % NaCl extracts and No. 4 is the 5–7 % NaCl soluble protein).

VII. Separation and Determination of Amino Acids.

Differences of the amino acids of different proteins both in quantity and kind were determined by Van Slyke's advanced method which was described in the first report. The following results were obtained.

TABLE VI

Kinds of Nitrogen Found in Various Protein Samples.

	Ammonia-N	Melanin-N	Mono-amino-N	Diamino-N	Arginin-N	Lysin-N	Histidin-N	Cystin-N
				(*Dry Matter %*)				
A2	0.7019	0.4387	9.2127	5.4399	1.8250	0.8880	2.5407	0.1860
B2	0.6668	0.3861	9.2653	5.2995	2.0274	1.5744	1.4752	0.2220
C2	0.4457	0.5264	8.6793	5.2644	2.3864	1.6662	0.9719	0.2400
D2	0.4387	0.4422	8.5980	4.7380	2.1058	2.0088	0.3654	0.2580
				(*Total Nitrogen %*)				
A2	4.445	2,778	58 352	34.450	11.557	5.624	16.094	1.178
B2	4.269	2.472	59.317	33.930	12.978	10.083	9.443	1.422
C2	2.987	3.528	58.170	35.280	15.962	11.169	6.514	1.609
D2	3.087	3.112	60.488	33.340	15.519	14.138	2.571	1.816

(*Dry Matter %*)

	Ammonia-N	Melanin-N	Mono-amino-	Diamino-N	Arginin-N	Lysin-N	Histidin-N	Cystin-N
No. 1	1.8836	0.2813	10.3253	4.6654	2.9613	0.6182	1.3818	0.1575

	Ammonia-N	Melanin-N	Monoamino-N	Diamino-N	Arginin-N	Lysin-N	Histidin-N	Cystin-N
No. 2	1.7673	0 2918	10.0205	5.2645	3.3117	0.5726	1.4163	0.1639
No. 3	1.1768	0.2637	9.1271	5.7812	3.9674	1.0788	0.4386	0.2964
No. 4	1.1707	0 3065	9.0251	5.6058	3.8989	1.0454	0.3595	0.3040
No. 5	1.1779	0.2609	9.9735	5 7416	4.4541	0.7407	0.2168	0.3300
No. 6	1.6726	0 2509	8.6586	5.8031	3.8233	0.6687	1.1144	0.1967
(*Total Nitrogen %*)								
No. 1	10.9827	1.6402	60.2037	27.2025	17.2665	3.6045	8.0567	0.9183
No. 2	10.1896	1.6824	57.7745	30.3533	19.0941	3.3014	8.1659	0.9450
No. 3	7.1981	1.6130	55.8273	35 3616	24.2672	6.5986	3.6828	1.8130
No. 4	7.2678	1.9028	56.0283	34.8011	24.2046	6 4600	2.2194	1.8872
No. 5	7.3190	1.6211	61.9707	35,6756	27.6757	4.6024	1.3471	2.0505
No. 6	10.2080	1.5313	52.8440	35.4167	23.3339	4.0811	6.8013	1.2005

From the above table it would be seen that the proteins requiring increasing amounts of HCl for precipitation include a decreasing amount of ammonia-, diamino- and histidin-nitrogen, but an increasing amount of monoamino-, arginin- and lysin-nitrogen. These differences are also observed between alcohol soluble, alkali soluble, and NaCl soluble protein i. e. arginin and lysin contents are increased while ammonia and histidin contents are decreased in the above order.

VIII. Specific Rotatory Power of Different Protein Alkaline Solutions.

The rotatory power of the alkaline solution of the following different proteins was examined by means of a Haensch-Schmidt's half-shadow polariscope with the same procedure as that reported in the former paper.

TABLE VII

Specific Rotatory Power of the Various Protein Alkaline Solutions.
(0.15 g of sample was dissolved in 15 cc of 1/5 n-NaOH solution.)

Sample	A1	A1′	B1	C1
Reading	−2.00	−1.90	−1.95	−1.85

(*0.1 g of sample was dissolved in 15 cc of 1/10 n-NaOH solution.*)

Time After Dissolution.		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
5 hrs.	Reading	−2.40	−2.50	−2.40	−2.35	−2.35	−2.60
	D	−62.28	−67.47	−62.28	−60.98	−60.98	−67.47
24 hrs.	Reading	−2.55	−2.60	−2.50	−2.45	−2.45	−2.70
	D	−66.17	−67.47	−66.47	−63.58	−63.58	−70.06
48 hrs.	Reading	−2.45	−2.50	−2.40	−2.35	−2.40	−2.55
	D	−63.58	−67.47	−62.28	−60.98	−62.28	−66.17
74 hrs.	Reading	−2.35	−2.40	−2.35	−2.30	−2.30	−2.50
	D	−60.98	−62.28	−60.98	−59.68	−59.68	−67.47
96 hrs.	Reading	−2.30	−2.35	−2.30	−2.25	−2.25	−2.45
	D	−59.68	−60.98	−59.68	−58 39	−58.39	−63.58
1 week	Reading	−2.15	−2.20	−2.10	−2.05	−2.10	−2.30
	D	−55.80	−57.09	−54.50	−53.19	−54.50	−59.68

The specific rotatory power of the protein, precipitated with a small quantity of HCl from alkaline solution, is greater than that of protein precipitated with a large quantity of HCl. The specific rotatory power of alcohol soluble and alkali soluble protein is greater than that of NaCl soluble and the smallest rotation was observed in 5–7 % NaCl soluble protein solution.

IX. SUMMARY.

From the results of the above experiments we could summarize the following differences in physico-chemical properties between the different kinds of rice proteins:—

(1) In the first report it was published that the pH value of the isoelectric point of the four kinds of rice protein is increased, while the sulphur content is decreased according to the following order i. e. water soluble, NaCl soluble, alkali- and alcohol-soluble protein. By comparing NaCl soluble protein to alkali soluble, we observe also that the former is inferior in its specific rotatory power, HCl combining power, free amino nitrogen, ammonia nitrogen and histidin nitrogen, while the latter is inferior in its Ag combining power, lysin nitrogen and cystin nitrogen content.

(2) The same differences were observed between the partially precipitated proteins from alkaline solution. The protein, precipitated with a small quantity of HCl, showed a high specific rotatory power, ammonia-,

arginin-, lysin-, and cystin-nitrogen contents.

(3) Thus the sulphur content of alkali soluble protein is about 2 times that of alcohol soluble, and that of NaCl soluble is about 2.5–3 times that of the latter. So the sulphur content is increased according to the increasing quantity of HCl needed to precipitate the protein from alkaline solution.

(4) The amino acid content of the alcohol, alkali, and NaCl soluble proteins varied in the following order:

Ammonia and Histidin	Alcohol soluble>Alkali soluble>NaCl soluble
Arginin, lysin and cystin	NaCl soluble>Alkali soluble>Alcohol soluble

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