*The Separation of Amino Acids with an Ion-exchange Membrane**

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The group separation of a mixture of amino acids obtained from the hydrolysis of gluten has been carried out by electrodialysis, using the homogeneous ion-exchange membrane of the styrene-butadiene copolymer. It has been found that the five-compartment use of a cation-exchange membrane (C-2, with a water content of 50% and a specific resistance of 100 Ω-cm.) and an anion-exchange membrane (A-3, with a water content of 55% and a specific resistance of 120 Ω-cm.) effects a successful separation of amino acids into neutral, basic, and acidic groups. However, 10~20% of the neutral amino acids migrated to the basic and acidic amino acid groups.

The separation of amino acids by electrodialysis using such ordinary semi-permeable membranes as biological and collodion membranes has previously been attempted.[1] However, this method has not yet been carried out on an industrial scale because of the difficulty of manufacturing large-sized membranes with good electric properties and stability for long use. Moreover, ordinary semi-permeable membranes have a very low permselectivity; therefore, they cannot prevent the leakage of the hydrogen and hydroxyl ions formed in the electrode chambers. This makes it difficult to adjust the hydrogen ion concentration of amino acid solutions in the intermediate compartments. The author adopted ion-exchange membranes as diaphragms in the electrodialysis, and the experiments were carried out using homogeneous ion-exchange membranes of the styrene-butadiene copolymer produced by Kuwata and Yoshikawa.[2]

Experimental

The separation of amino acids by electrodialysis using ion-exchange membranes was limited to the separation of such groups as the acidic (AA), neutral (NA), and basic (BA) groups, all with similar isoelectric points. Various ion-exchange membranes have been made by polymerization or condensation.[3] The ion exchange membranes used in the present experiments were derived from the styrene-butadiene copolymer (commercial SBR) which has a styrene content of 50%.

The styrene-butadiene copolymer membrane has an advantage that the pore-size of the membrane can be changed by adjusting the butadiene content of the copolymer and/or by controlling the cross-linking reaction. Consequently, this kind of membrane is preferable over other ion exchange membranes for the separation of amino acids. The properties of these membranes are shown in Table I. The porosity of the membranes is compared in terms of the value of the water content (W), the water permeation ratio[4] (K), and the specific resistance (Ω-cm.) of the membranes.

An electrodialysis apparatus,* separated by two sets of anion- and cation-membranes (7.5×7.5 cm.), as illustrated in Fig. 1 (each intermediate compartment was 7.5×7.5×1.0 cm.), was used, with a nickel plate (7.0×7.0 cm.×0.3 mm.) as a cathode and a carbon electrode 7.5×7.5×0.8 cm.) as an anode. The experiment was carried out by changing the cation-exchange membrane, C_1, and the anion-exchange membrane, A_2, in various ways. The A_1 and C_2 membranes in Fig. 1 were fixed throughout the experiment by A-1 and C-1 respectively. A sodium chloride solution (0.1~0.2%) was placed in the intermediate compartments A and C, and 70 cc. of a solution (pH 5.6) containing 10% of mixed amino acids (Table II) obtained from the hydrolysis of gluten and by adjusting the pH in the vicinity of the isoelectric point of neutral

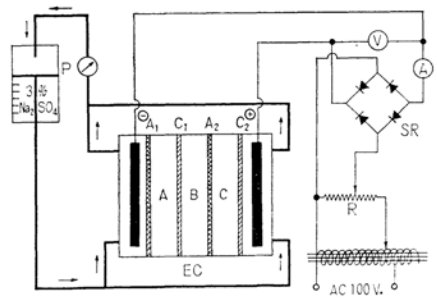

Fig. 1. Apparatus.

* This article reports on part of a long-range research program on ion-exchange membranes being led by Professor T. Kuwata and Assistant Professor S. Yoshikawa of The University of Tokyo.

1) G. L. Foster and C. L. Schmidt, *Biochem. J.*, **40**, 1709 (1926); K. Ikeda and S. Suzuki, U. S. Pat. 1015890 (1912).

2) T. Kuwata and S. Yoshikawa, Japanese Pat. 239596, 239724, 248669.

3) S. Yoshikawa, *J. Soc. Org. Chem. Japan*, **15**, 603 (1957).

4) "Kogyo Butsurikagaku," Ed. by Kogyo Butsurikagaku Kenkyu-kai, 2nd Ed., Korona-sha, Tokyo, p. 111.

* Electrodialysis cells made by assembling the various compartments in the form illustrated in Fig. 2 (for intermediate compartments) and in Fig. 4 (for electrode compartments) as indicated in a previous paper[5] were used.

5) Y. Hara, *J. Chem. Soc. Japan, Ind. Chem. Sec.* (*Kogyo Kagaku Zasshi*), **65**, 885 (1962).

Table I. Properties of the ion-exchange membranes used

Type	Notation	Ion-exchange capacity of membrane meq./g.	Fixed ion concentration in membrane meq./g.	Water content of membrane*1 W, %	Water permeation ratio*2 K, $\times 10^{-16}$ cm^3	Specific resistance Ω-cm.	Ion selective permeability n, 0.1 N NaCl	Thickness mm.
Cation-exchange membrane	C-1	1.05	2.57	33.4	0.24	272	0.90	0.24
	C-2	1.32	2.13	50.1	0.32	100	0.92	0.28
Anion-exchange membrane	A-1	1.68	3.91	31.2	0.18	300	0.90	0.23
	A-2	1.69	3.16	47.2	0.23	250	0.91	0.26
	A-3	1.78	1.84	54.8	0.45	120	0.85	0.45

*1 This value was calculated on the basis of the total weight of the resin comprising the membrane, excluding any supporting material.

*2 The degree of the penetration of a diaphragm is a value indicating the size and distribution of pores derived from Poiseville's law on the assumption that the cross-section of pores of a diaphragm is round. This is ordinarily indicated by the quantity (cc.) which transfers 1 cm^2 of diaphragm 1 cm. thick in one hour under a static pressure of 1 cm. of a water column when a 0.5 N sucrose solution and water are placed on opposite sides of the diaphragm.

Table III. Group separation of amino acids mixture

Runs No.	Electrodialysis cell: Ion-exchange membranes C_1	A_2	Catholyte	Anolyte	Initial current density amp./dm^2	Period of operation hr.	Experimental results: pH variation of the solution in each compartment A	B	C	Behavior of separating amino acids in each fraction A	B	C
1	C-1	A-1	3% Na_2SO_4	3% Na_2SO_4	0.3	5.0	10<	3.8	9.0	BA Gly Leu Ser Val Pro Ala Thr	NA His AA	AA Gly
2	C-1	A-1	3% Na_2SO_4	3% Na_2SO_4	0.1	24.0	8.8	5.6	8.6	Arg Lys Gly Pro Ser Thr Val Leu	NA His Arg AA	AA
3	C-1	A-1	N NaOH	N NaOH	0.1	24.0	10<	8.6	10<	Arg Lys Gly Leu Val Pro	BA NA AA	AA
4	C-1	A-1	N HCl	N NaOH	0.1	—	1	3.0	10<	—	— AA	AA Gly Ser
5	C-2	A-2	3% Na_2SO_4	3% Na_2SO_4	0.2	5.0	9.0	5.0	10.0	Arg Lys Gly Ser Ala Val	NA His Glu	AA
6	C-2	A-2	3% Na_2SO_4	3% Na_2SO_4	0.13	24.0	8.2	4.8	10	Arg Lys Gly Ser	NA His Glu	AA Gly Ser
7	C-2	A-3	3% Na_2SO_4	3% Na_2SO_4	0.15	22.0	8.0	5.6	8.2	Arg Lys Gly Ser Ala	NA His	AA Gly Ser Val Ala Leu
8	C-2	A-3	3% Na_2SO_4	3% Na_2SO_4	0.2	20.0	5.6	5.8	6.8	Arg Lys Gly Ser Ala Val Lew	NA His	AA Gly Ser Val Ala Leu
9	C-2	A-3	3% Na_2SO_4	3% Na_2SO_4	0.46	5.0	5.6	5.4	4.4	BA Gly Ser Ala Val Leu	NA	AA Gly Ser Val Ala Leu
10	C-2	A-3	6% Na_2SO_4	6% Na_2SO_4	0.5	3.5	10<	5.2	7.4	BA Gly Ser Ala Val Leu	NA	AA Gly Ser Val Ala Leu
11	C-2	A-3	6% Na_2SO_4	6% Na_2SO_4	0.8	2.0	10<	5.2	7.0	BA Gly Ser Ala Val Leu	NA	AA Gly Ser Val Ala Leu
12	C-1	A-3	6% Na_2SO_4	6% Na_2SO_4	0.5	2.5	—	5.2	—	BA Gly Ser Ala Val Leu	NA Arg His	AA
13	C-1	A-3	6% Na_2SO_4	6% Na_2SO_4	0.36	5.0	—	5.2	—	BA Gly Ser Ala Val Leu	BA NA AA	AA

TABLE II. COMPOSITION OF AMINO ACIDS MIXTURE USED

Kind of amino acids	Notation	Content %
Glutamic acid	Glu	12.7
Aspartic acid	Asp	
Acidic amino acids	AA	12.7
Glycine	Gly	5.3
Alanine	Ala	4.4
Leucine	Leu	18.5
Isoleucine	Ileu	
Valine	Val	7.2
Methionine	Met	
Proline	Pro	10.4
Serine	Ser	3.3
Threonine	Thr	2.0
Phenylalanine	Phe	8.5
Neutral amino acids	NA	71.5
Arginine	Arg	6.9
Histidine	His	5.4
Lysine	Lys	3.5
Basic amino acids	BA	15.7
		Sum 99.9

amino acids, were placed in the intermediate compartment B. Colloidal deposits on the C_1 cation-exchange membrane cause electric resistance when peptide and fumin are present in the feed amino acids, perticularly in the case of imperfect hydrolysis. Therefore, the muddy matter in the feed solution of amino acids was filtered off and purified through an ion-exchange resin bed. A 3~6% sodium sulfate solution was used as an electrolyte solution in the cathodic and anodic chamber and was circulated in the direction of the arrow at the rate of 1.0 l./hr. in order to cancel out the H ions and OH ions produced in the electrode solution at all times and thus to prevent any effect of pH fluctuation on the intermediate solution.

If the electrodialysis is carried out in such a way, sulfate ions will be furnished from the cathode to the A compartment and sodium ions from the anode to the C compartment, and, consequently, the pH of the solution of the B compartment will not be much changed during the electrodialysis.

The experiment was carried out using various kinds of anion- and cation-exchange membranes and under various operating conditions, such as current density, operating time, kind of electrolyte and pH of the solution of each compartment during electrodialysis. The degree of amino-acid separation was measured by two-dimensional paper partition chromatography and was determined quantitatively by the dinitrophenyl-paper chromatography method.[6]

Results and Discussion

To carry out a group separation of amino acids smoothly, it is necessary to keep the feed solution, viz., the solution in the intermediate compartment B at a constant pH during the electrodialysis. The pH fluctuation of the compartment B solution is due to the electrolysis of water within the membrane in addition to the effect of the H ions or OH ions formed on the electrodes. The effect of the H or OH ions can be minimized by mixing electrode solutions during circulation. When the sizes of the amino acid molecules are about as large as or larger than the pore-size of the ion-exchange membrane, the electrolysis of water occurs by forced electric current. Because of this phenomenon, the pH in each intermediate compartment varies markedly during electrodialysis.

The results are summarized in Table III. As No. 1 shows, when membranes having the smallest pore-size, C-1 and A-1, were adopted as membranes C_1 and A_2, respectively electrolysis of water took place apparently at an initial current density of 0.3 amp./dm^2 and the solution in compartment B gradually became acidic. In No. 2, if the initial current density was decreased at 0.1 amp./dm^2 and if the period of dialysis was extended, no electrolysis of water took place, but the results of paper partition chromatography showed that the separation of amino acids was imperfect. In Nos. 3 and 4, when sodium hydroxide or hydrochloric acid and sodium hydroxide were used as the electrolytes, the pH of the solution in the B compartment became unstable and the separation of amino acids was imperfect. In Nos. 5 and 6, when membranes wit ha little larger pore-sizes, C-2 and A-2, were adopted as membranes C_1 and A_2 respectively, the fluctuation of the pH of the solution in the intermediate compartment B was noticeable, even at a low current density, and it was found from the results of paper partition chromatography that a considerable amount of histidine and glutamic acid still remained in the B compartment. When the A-3 membrane, with the largest pore-size, was adopted as membrane A_2 almost no electrolysis of water took place, even at the high initial current density of 0.8 amp./dm^2, as Nos. 7—11 show, and the separation of amino acids took place within a short time. Further, the results of paper partition chromatography showed that no acidic and basic amino acids remained in the solution of the B compartment, although a small amount of remaining histidine was noticed in the feed solution at a current density below 0.2 amp./dm^2.

On the other hand, when the C-1 membrane, which has a smaller pore-size, was adopted as the C_1 membrane insteat of the C-2 membrane, both the basic and acidic groups remained in the solution of the B compartment,

6) S. Blackburm and A. G. Lowther, *Biochem. J.*, **48**, 126 (1951).

as shown in Nos. 12 and 13. Therefore, the separation of amino acids was unfavorably achieved. Further, it was found that when a set of C-2 and A-3 membranes, both of which have a larger pore-size, was applied and when electrodialysis was performed by applying a high initial current density (0.5~0.8 amp./dm^2), no electrodialysis of water took place and a favorable result was obtained.

Table IV. Quantitative analysis of amino acids separated by DNP-PPC method (Run No. 11)

Kind of amino acids	Each fraction of separated amino acids in the intermediate compartments, %		
	A	B	C
Glutamic acid	0	0	100
Aspartic acid	0	0	100
Glycine	10.0	81.7	8.2
Alanine	6.9	85.6	7.4
Leucine, Isoleucine	4.3	89.9	6.0
Valine, Methionine	6.7	86.3	7.0
Proline	5.7	89.0	5.1
Serine	7.0	81.5	12.1
Threonine	8.9	75.4	15.7
Phenylalanine	3.3	83.6	13.3
Neutral amino acids	7.2	84.3	9.4
Arginine	100	0	0
Histidine	100	0	0
Lysine	100	0	0

The results of the quantitative analysis on each fraction by means of the dinitrophenyl-paper partition chromatography method as an example (No. 11), are shown in Table IV, where the separation was almost ideal. These results show the complete absence of basic and acidic amino acids in the neutral amino acids remaining in the B compartment and a few per cent of neutral amino-acid transfer to both the basic and the acidic amino acid groups. This tendency is especially noted in neutral amino acids, which have a smaller molecular weight, and is probably due to the electro-osmosis accompanying the electrodialysis. In addition, it is interesting to note that some of the neutral amino acids, such as serine and threonine, which have an hydroxyl group, and phenylalanine, which has a phenyl ring, show a marked tendency to transfer to the acidic amino acid group. This seems to be because these amino acids have a particular affinity for the anion-exchange membrane.

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