The Ion-Exchange Behavior of Some Neutral Amino Acids

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In many practical works on the separation of amino acids by means of ion-exchangers, some successful procedures using the ion-exchange chromatography have been established. Above all, the displacement development method has been applied with success to the separation by Partridge et al., and Moore and Stein pointed out that the elution method possessed higher resolving power for analytical purposes[1]. However, few fundamental researches have been carried out on the exchange behavior of amino acids on ion-exchange resins[2]. This problem is interesting, since it is expected that weak amphoteric electrolytes such as amino acids exhibit some characteristic behavior concerning the ion-exchange reaction.

In the present paper, the experimental resuls are described on the ion-exchange behavior of some neutral amino acids, particularly the influence of pH on the ion-exchange reaction and some general remarks are presented in this respect. The behavior of the acidic and the basic amino acids toword the ion-exchange resins will be given in the succeeding paper.

Experimental

Through the present investigation the following reagents were used; amino acids are glycine, DL-alanine and L-leucine, which are commercial special-grade and were used without further purification. The dissociation constants and the isoelectric points of these amino acids are as follows.

	pK_1	pK_2	pI
Glycine	2.34	9.60	5.97
Alanine	2.34	9.69	6.00
Leucine	2.36	9.60	5.98

The ion-exchange resins used were Amberlite resins, IR-120 (4.24) IRC-50 (10.0), IRA-400 (2.15) IR-4B (8.0), the exchange capacities of these resins being given in parentheses in the unit, meq./g. dry resin.

All the experiments were carried out batch-wise. Through a run of measurements, a constant volume, 50 ml., of a solution containing a constant amount, about 2 meq., of amino acid and being adjusted to a desired pH by adding hydrochloric acid or sodium hydroxide, was added onto a constant quantity, about 1 meq., of the ion-exchange resin. The details for each system are given in the following table.

Amino acids	Total amounts[a]	H- or OH-form resins, *w* g. dry resin			
	mmol.	IR-120	IRC-50	IRA-400	IR-4B
Glycine	1.95	0.169	0.094	0.330	0.101
Alanine	1.98	0.166	0.111	0.331	0.180
Leucine	2.02	0.175	0.103	0.343	0.134

After being allowed to stand for a few days, which were long enough for the attaining of the ion-exchange equilibrium, the pH and the remaining amount of the amino acid in the solution phase were measured and the adsorbed amount of the amino acid was estimated herefrom.

Amino acids were measured colorimetrically with ninhydrin[3] and pH of the solutions was measured with a glass-electrode pH-meter, model HM-5, Toa Dempa Kogyo Co., Ltd. All the measurements were made at room temperature, 5~15°C.

1) P. B. Hamilton, "Ion Exchangers in Organic and Biochemistry" Ed. by C. Calmon and T. R. E. Kressman, Interscience Publishers, Inc., New York (1957), p. 255.

2) D. T. Englis and H. A. Fiess, *Ind. Eng. Chem.*, **36**, 604 (1944); C. S. Cleaver, R. A. Hardy, Jr. and H. G. Cassidy, *J. Am. Chem. Soc.*, **67**, 1343 (1945); M. E. Carsten and K. Cannan, ibid., **74**, 5950 (1952).

3) K. Satake et al., "Biochemistry I (Seibutu Kagaku I)", Zikken Kagaku Koza, Vol. 23. Maruzen Co., Tokyo (1957), p. 124.

Results and Discussion

Experimental results were shown in Figs. 1–3, in which the adsorbed amounts of amino acids were plotted against the pH of the equilibrium external solution. As anticipated from the amphoteric nature of amino acids, these are adsorbed by the cation-exchange resins in the pH range lower than its isoelectric point and by the anion-exchange resins in the higher pH range.

As seen in these figures, it was revealed that the maximum adsorption of the neutral amino acids by hydrogen- or hydroxyl-form ion-exchange resins takes place in a rather broad pH range near the isoelectric points, while the acidic and the basic amino acid are extensively adsorbed in a relatively narrow pH range near the isoelectric points, as will be presented in the succeeding paper. Furthermore, it was noticed that, in the case of the sodium or chloride form resins, the amounts of amino acids are remarkably diminished in the pH range near the isoelectric point, compared to the case of the hydrogen- or hydroxyl-form resins. In the lower and the higher pH ranges, the adsorbed amounts of all the amino acids decrease sharply owing to the competitive adsorption of hydrogen or hydroxyl ions.

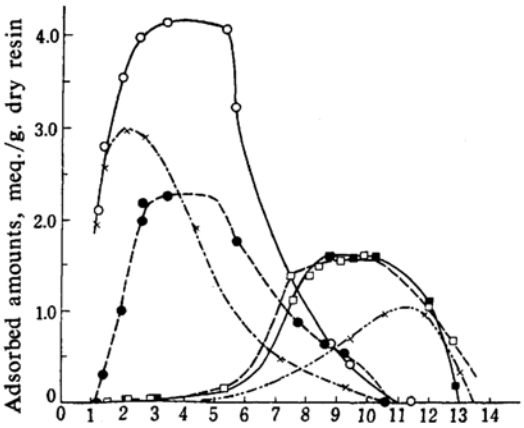

Fig. 1. Adsorption of glycine on ion-exchange resins.
—○— IR-120, H-form
--●-- IRC-50, H-form
--□-- IRA-400, OH-form
—■— IR-4B, OH-form
—·×—· IR-120, Na-form
—··×—·· IRA-400, Cl-form

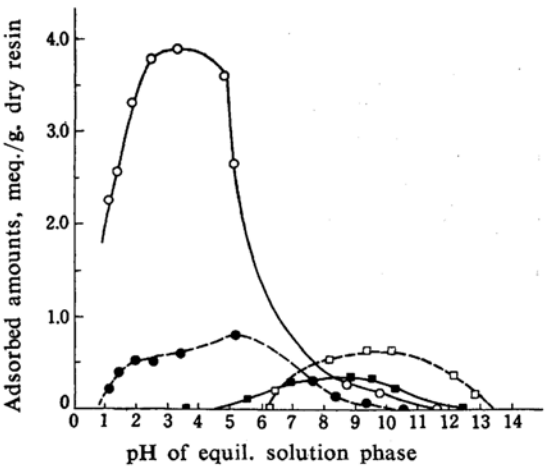

Fig. 2. Adsorption of DL-alanine (symbols are the same as those in Fig. 1).

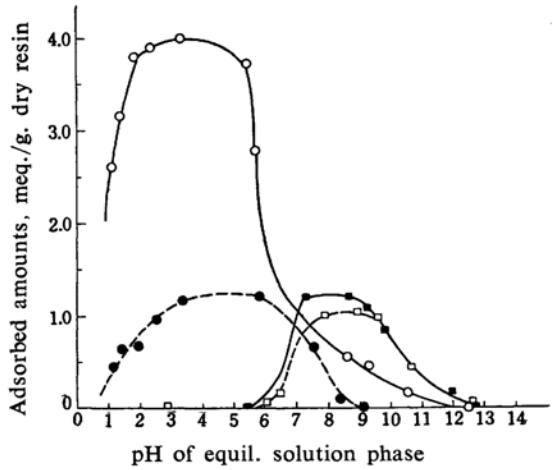

Fig. 3. Adsorption of L-leucine (symbols are the same as those in Fig. 1).

Adsorption Mechanism of Amino Acids on Ion-Exchange Resins.—The apparent equilibrium constants defined by

$$\left.\begin{aligned} K_{H}' &= \frac{[A]_r[H^+]_s}{[A]_s[H^+]_r} \\ K_{OH}' &= \frac{[A]_r[OH^-]_s}{[A]_s[OH^-]_r} \end{aligned}\right\} \quad (1)$$

are not constant but depend on pH in the solution phase; K_H' decreases and K_{OH}' increases, with increase of pH, where $[A]_s$, $[H^+]_s$ or $[OH^-]_s$ is concentration in meq./ml. of amino acid, hydrogen or hydroxyl ions in the equilibrium external solution, respectively, and $[A]_r$, $[H^+]_r$ or $[OH^-]_r$ is concentration in meq./(g. dry resin) of amino acid, hydrogen or hydroxyl ions in the resin phase at equilibrium, respectively. It seems that this constancy does not hold, because the dissociation equilibrium of amino acids in the solution is not taken into consideration.

When the neutral amino acid is adsorbed on cation-exchange resin of hydrogen-form,

the amino acid cations that exist in the following equilibrium state,

$$AH^+ \rightleftharpoons A^{\pm} + H^+ \quad \text{(i)}$$

$$A^{\pm} \rightleftharpoons A^- + H^+ \quad \text{(ii)}$$

exchange for hydrogen ions in the ion-exchange resin,

$$RH + AH^+ \rightleftharpoons RAH + H^+ \quad \text{(iii)}$$

and the hydrogen ions are liberated from the cation-exchange resin, resulting in production of more amino acid cations and further exchange of the amino acid cations for the hydrogen ions. The exchange reaction attains the equilibrium through such a repeating process[4].

This adsorption mechanism would be supported by the following empirical facts.

(1) The pH of the external solution remains nearly constant through the adsorption process of amino acids as shown in Table I, in which pH's of the solution phase before and after equilibration of ion-exchange were given in the case of glycine as an example.

TABLE I. pH OF THE SOLUTION PHASE BEFORE AND AFTER EQUILIBRATION OF ION-EXCHANGE OF GLYCINE

pH of orig. soln.	pH of equil. soln.	pH of orig. soln.	pH of eqil. soln.
Amberlite IR-120, H-form.		Amberlite IRC-50, H-form.	
1.20	1.19	1.20	1.20
1.35	1.38	1.35	1.40
1.90	1.90	1.98	1.98
2.50	2.42	2.50	2.58
3.37	3.32	3.37	3.40
6.60	5.30	6.60	5.60
8.72	5.60	8.72	7.65
9.72	8.70	9.72	8.55
10.80	9.39	10.80	9.20
12.55	12.50	12.55	12.45
Amberlite IRA-400, OH-form.		Amberlite IR-4B, OH-form.	
1.35	1.48	1.35	1.52
2.50	2.93	2.50	3.02
3.37	7.40	3.37	5.24
6.60	8.00	6.60	7.45
8.72	8.30	8.72	8.60
9.72	9.05	9.72	9.40
10.80	9.80	10.80	10.10
12.55	11.89	12.55	11.90
12.89	12.75	12.89	12.80
Amberlite IR-120, Na-form.		Amberlite IRA-400, Cl-form.	
1.10	1.12	1.92	1.94
1.38	1.42	3.37	3.60
1.92	2.09	6.60	5.25
2.48	2.70	9.80	9.40
3.37	4.37	10.72	10.51
6.60	7.16	11.98	11.81
9.80	9.33	13.19	13.02
13.49	13.42	13.49	13.41

(2) The amino acids are adsorbed remarkably on the hydrogen- or hydroxyl-form ion-exchanger in the pH range near the isoelectric points, while the amino acid adsorbed on the salt-form ion-exchanger is small in quantity in this pH range.

It should be noticed that the completion of the above-mentioned two processes, i and iii, lead to the same result as the following mechanism[2],

$$RH + A^{\pm} \rightleftharpoons RAH \quad \text{(iv)}$$

However, there is not any evidence that such a process takes place actually.

Relations between the Adsorbed Amount of Amino Acid and the pH of the External Solution.—In a case when the neutral amino acid is adsorbed on the hydrogen-form cation-exchanger, the selectivity coefficient is defined as follows:

$$K_H = \frac{[AH^+]_r [H^+]_s}{[AH^+]_s [H^+]_r} \quad (2)$$

where $[A^+]_s$ is the concentration of ion A in the equilibrium external solution in the unit, meq./ml., and $[A^+]_r$ is that in the resin phase in the unit, meq./g. dry resin. Strictly, the activity should be used instead of the concentration in these equations but was not used in the present paper to avoid the troublesome procedure.

The dissociation constants of the neutral amino acids are given as

$$\left.\begin{aligned} K_1 &= \frac{[A^{\pm}]_s [H^+]_s}{[AH^+]_s} \\ K_2 &= \frac{[A^-]_s [H^+]_s}{[A^{\pm}]_s} \end{aligned}\right\} \quad (3)$$

where the term arising from activity coefficient is also neglected.

Under the present experimental condition, that is;

(1) the total amount of amino acid in the system is constant, a meq.,

(2) the amount of the exchangeable ions in the ion-exchange resin is constant, c meq.,

4) Amino acids are taken up from the solution phase into the resin phase by the other processes, such as Donnan adsorption and physical or chemical adsorption, besides the ion-exchange process. Although it is difficult to estimate the contributions of these processes experimentally, it would be safely said on the basis of the preceding reports listed in Ref. 1, that the ion-exchange is the primary process and the other ones have a small contribution to be neglected to a first approximation, excepting for the case of amino acids with benzene rings.

$c=Cw$, where C is the exchange capacity (meq./g. dry resin) of the resin and w is the weight of the resin used,

(3) the volume of the external solution is constant, v ml., the following relationship holds between the exchanged amount of the amino acid, $[AH^+]_r=y$, and the hydrogen ion concentration in the external solution, $[H^+]_s=x$,

$$\frac{(a-wy)(c-wy)}{y}=\frac{vw}{K_H}\frac{x^2+K_1x+K_1K_2}{x} \quad (4)$$

The similar relation is gained in a case when the neutral amino acid is adsorbed on the hydroxyl-form anion-exchanger, where the selectivity coefficient K_{OH} is defined by

$$K_{OH}\frac{[A^-]_r[OH^-]_s}{[A^-]_s[OH^-]_r} \quad (5)$$

The exchanged amount of the amino acid anion, $[A^-]_r=y$, is related to the hydroxyl ion concentration in the equilibrium external solution, $[OH^-]_s=x'$, by the formula

$$\frac{(a-wy)(c-wy)}{y}=\frac{vw}{K_{OH}}\frac{K_1K_2x'^2+K_1K_wx'+K_w^2}{K_1K_2x'} \quad (6)$$

where K_w is the dissociation constant of water.

It must be noted that these relationships are valid for the two-component systems that contain only hydrogen or hydroxyl ions besides amino acid ions.

$f(x)$ or $f(x')$ Functions. — It is found that, in the ion-exchange behavior of the weak amphoteric electrolytes such as amino acids, a function of only the concentration of the hydrogen or the hydroxyl ion in the equilibrium solution phase, $f(x)$ or $f(x')$, which appears in the right hands of the Eqs. 4 and 6, gives the characteristic behavior. This function is, for cation exchange,

$$f(x)=\frac{x^2+K_1x+K_1K_2}{x} \quad (7)$$

and for anion exchange

$$f(x')=\frac{K_1K_2x'^2+K_1K_wx'K_w^2}{K_1K_2x'} \quad (8)$$

The numerical values of these functions for amino acids under investigation are given in Table II and shown against pH of the solution phase in Fig. 4[5,6].

The $f(x)$ or $f(x')$ of each amino acid has a minimum at its isoelectric point. At the minimum of $f(x)$ or $f(x')$, the adsorbed amount of the amino acid is the maximum. This can be shown directly by differentiating the Eqs. 4 and 6. It is supported by the present experiment that the adsorbed amount of the amino acid is the maximum at the isoelectric point.

The adsorbed amount of the amino acid is related inversely to the $f(x)$ or $f(x')$. For

TABLE II. $f(x)$ AND $f(x')$ FUNCTIONS

pH	log $f(x)$		
$(-\log x)$	Glycine	Alanine	Leucine
0	0.002	0.002	0.002
1	$\bar{1}$.021	$\bar{1}$.021	$\bar{1}$.017
2	$\bar{2}$.164	$\bar{2}$.164	$\bar{2}$.155
3	$\bar{3}$.746	$\bar{3}$.746	$\bar{3}$.729
4	$\bar{3}$.669	$\bar{3}$.669	$\bar{3}$.649
5	$\bar{3}$.661	$\bar{3}$.661	$\bar{3}$.641
6	$\bar{3}$.660	$\bar{3}$.660	$\bar{3}$.640
7	$\bar{3}$.661	$\bar{3}$.661	$\bar{3}$.641
pH	log $f(x')$		
$(-\log K_w/x')$	Glycine	Alanine	Leucine
6	$\bar{5}$.600	$\bar{5}$.691	$\bar{5}$.600
7	$\bar{5}$.601	$\bar{5}$.692	$\bar{5}$.601
8	$\bar{5}$.611	$\bar{5}$.700	$\bar{5}$.611
9	$\bar{5}$.697	$\bar{5}$.772	$\bar{5}$.697
10	$\bar{4}$.146	$\bar{4}$.173	$\bar{4}$.146
11	$\bar{3}$.017	$\bar{3}$.020	$\bar{3}$.017
12	$\bar{2}$.002	$\bar{2}$.002	$\bar{2}$.002
13	$\bar{1}$.000	$\bar{1}$.000	$\bar{1}$.000
14	0.000	0.000	0.000

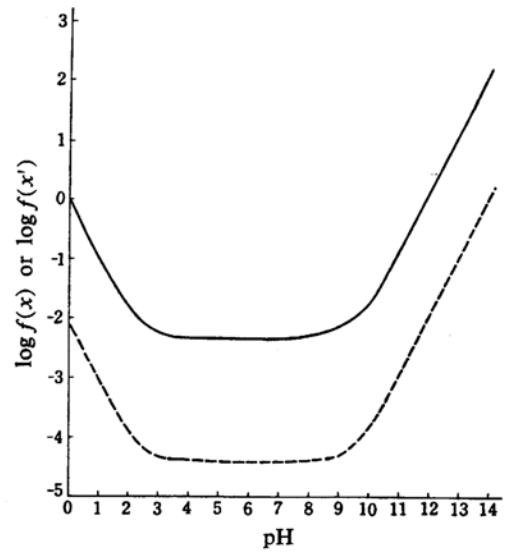

Fig. 4. $f(x)$ and $f(x')$ for glycine, which are shown by a full line and a dotted line, respectively.

5) $f(x)$ is related to $f(x')$ by the formula,
$$K_Wf(x)=K_1K_2\,f(x')$$
Then, for amino acid of which isoelectric point is 7, in this case $K_1K_2=K_W$, $f(x)$ is identical with $f(x')$.

6) $f(x)$ has a practical meaning in the pH range lower than the isoelectric point and $f(x')$ in the higher pH range.

example, the following formula is derived from Eq. 4,

$$y=\frac{ac}{w\left(a+c+\frac{v}{K_H}f(x)\right)}+\frac{a^2c^2}{w\left(a+c+\frac{v}{K_H}f(x)\right)^3}+\cdots\cdots \quad (9)$$

and the similar relation is obtained in another system. From these relations and the dependence of $f(x)$ or $f(x')$ on pH, it is concluded that the maximum adsorption of the neutral amino acids on the hydrogen- or hydroxyl-form exchanger takes place in the broad pH range near the isoelectric point, and this conclusion is also supported experimentally.

Selectivity Coefficients.—The selectivity coefficients K_H and K_{OH} were estimated using the above-mentioned experimental data and the formulas and are given in Table III. As for three kinds of neutral amino acids, glycine, alanine and leucine, K_H is very large in comparison with K_{OH}. This is partly because the carboxyl group of these amino acids is dissociated more strongly than the amino group and, on this account, the ratio of the concentration of the hydrogen ion to that of the amino acid cation is larger than the ratio of the concentration of the hydroxyl ion to that of the amino acid anion in the equilibrium solution phase[7].

Table III. Selectivity coefficients for ion-exchange of amino acids for hydrogen or hydroxyl ions

	log K_H		log K_{OH}	
	IR-120	IRC-50	IRA-400	IR-4B
Glycine	0.56	$\bar{2}.52$	$\bar{2}.00$	$\bar{3}.03$
Alanine	0.41	$\bar{2}.29$	$\bar{4}.73$	$\bar{5}.52$
Leucine	0.53	$\bar{2}.43$	$\bar{3}.24$	$\bar{4}.38$

The order of the selectivity coefficient does not agree with that of the chain length of the amino acid molecule and alanine has smaller K value than that of leucine. This was already reported[8] and it might be considered that a simple adsorption makes a contribution to the uptake of amino acids by ion-exchange resins to some extent in the case of amino acid as glycine which is small in size, and as leucine, which has a long nonpolar chain.

Summary

The ion-exchange behavior of three kinds of neutral amino acids was examined and the following results were obtained.

1) The ion-exchange behavior of amino acids can be explained when the dissociation equilibrium of amino acids in the solution was taken into account.

2) The maximum adsorption of neutral amino acids on the ion-exchange resins takes place in a rather broad pH range at the isoelectric point.

3) The neutral amino acids are adsorbed more extensively on cation-exchangers than on anion-exchangers. Glycine is adsorbed most extensively and leucine is more adsorbed than alanine.

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7) These ratios are given by
$[H^+]_s/[AH^+]_s=f(x)/[A]_s$
$[OH^-]_s/[A^-]_s=f(x')/[A]_s$
where $[A]_s$ is the concentration of amino acid remaining in the equilibrium solution phase, and, for example, $f(x')=0.087\times10^{-2}\,f(x)$ for glycine.

8) K. Narita, S. Fujiwara and S. Murasawa, This Bulletin, 31, 381 (1958).