# *Ion-exchange Behavior of Acidic and Basic Amino Acids*

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In the preceding paper[1] the ion-exchange behavior of some neutral amino acids was described and it was pointed out that amino acids are absorbed most predominantly on ion-exchange resins in the pH range near the isoelectric points of the amino acids. In this paper, some experimental results concerning the ion-exchange behavior of the acidic and the basic amino acid are presented and discussed.

## Experimental

Glutamic acid was used as an acidic amino acid and lysine monohydrochloride as a basic amino acid. These dissociation constants p$K$ and isoelectric points pI are as follows.

| | p$K_1$ | p$K_1'$ | p$K_2$ | p$K_2'$ | pI |
|---|---|---|---|---|---|
| Glutamic acid | 2.19 | 4.25 | 9.67 | — | 3.22 |
| Lysine | 2.18 | — | 8.95 | 10.76 | 9.70 |

p$K$'s are defined by equations given later. The ion-exchange resins used were Amberlite resins, IR-120, IRC-50, IRA-400 and IR-4B, the exchange capacities of which are 4.24, 10.0, 2.15 and 8.0 meq./g. dry resin, respectively.

The experimental procedures were the same as those in the preceding paper[1]. All measurements were carried out batchwise; a constant volume (50 ml.) of a solution containing a constant amount (about 2 mmol.) of an 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 are as follows.

| Amino acid | Total amounts $a$ mmol. | H- or OH-form resins $w$ g. (dried) IR-120 | IRC-50 | IRA-400 | IR-4B |
|---|---|---|---|---|---|
| Glutamic acid | 2.09 | 0.180 | 0.091 | 0.344 | 0.110 |
| Lysine | 2.13 | 0.170 | 0.097 | 0.336 | 0.109 |

After being allowed to stand for a few days, which were long enough for the attaining of the 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. All the experiments were made at room temperature, 5~15°C.

## Results and Discussion

Experimental results were shown in Figs. 1 and 2, in which the adsorbed amounts of amino acids were plotted against the pH of the equilibrium external solution. Like the neutral amino acids, the acidic and the basic amino acid are adsorbed most intensively at pH values close to the isoelectric point and, in the lower and the higher pH range, the adsorbed amounts of the amino acids decrease sharply owing to the competitive adsorption of hydrogen or hydroxyl ions. The amounts of uptake of amino acids by sodium- or chloride-form strongly dissociated resins are less than those by hydrogen-form or hydroxyl-form resins, especially in the pH range near the isoelectric point.

The pH dependence of adsorption of amino acids is more remarkable and more characteristic, compared to the case of neutral amino acids. As shown already by several investigators[2,3], glutamic acid is adsorbed predominantly on the anion-exchange resins and lysine on the cation-exchange resins. Glutamic acid is adsorbed very intensively in the limited pH range, 3~5, on a weakly basic ion-exchanger, IR-4B, while it is well adsorbed in a wider, namely, higher pH range in the case of a strongly basic ion-exchanger, IRA-400. This is owing to the strong affinity of IR-4B to the hydroxyl ion. Lysine shows a peculiar behavior, probably because it was used in the monohydrochloride form. The resulting solution from it is acidic, and sodium hydroxide must be added to bring the solution pH to the isoelectric point of lysine. The maximum adsorption of lysine takes place in pH 2~3 on IR-120, and in pH 7~9 on IRC-50. In other words, the addition of sodium ions decreases the amount of uptake of lysine on IR-120 and increases the amount adsorbed on IRC-50. This behavior is owing to the strong affinity of IRC-50 to the hydrogen ion and that of IR-120 to the sodium ion.

The adsorption mechanism of amino acids on the ion-exchange resins was described in the preceding paper. That is to say, when the amino acid cations are adsorbed on the hydrogen-form cation-exchanger, the hydrogen ions

1) M. Senō and T. Yamabe, This Bulletin, 33, 1532 (1960).

2) P. B. Hamilton, "Ion Exchangers in Organic and Biochemistry", Ed. by C. Calmon and T. R. K. Kressman, Interscience Pub., New York (1957), p. 255.

3) O. Samuelson, "Ion Exchangers in Analytical Chemistry", John Wiley & Sons, Inc., New York (1953), p. 210.

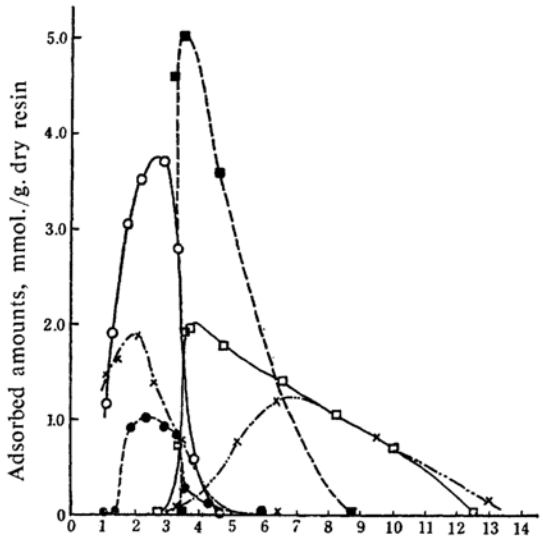



Fig. 1. Adsorption of glutamic acid on ion-exchange resins.

—○— IR-120, $H^+$-form
--●-- IRC-50, $H^+$-form
—□— IRA-400, $OH^-$-form
--■-- IR-4B, $OH^-$-form
-·×-· IR-120, $Na^+$-form
-··×-·· IRA-400, $Cl^-$-form

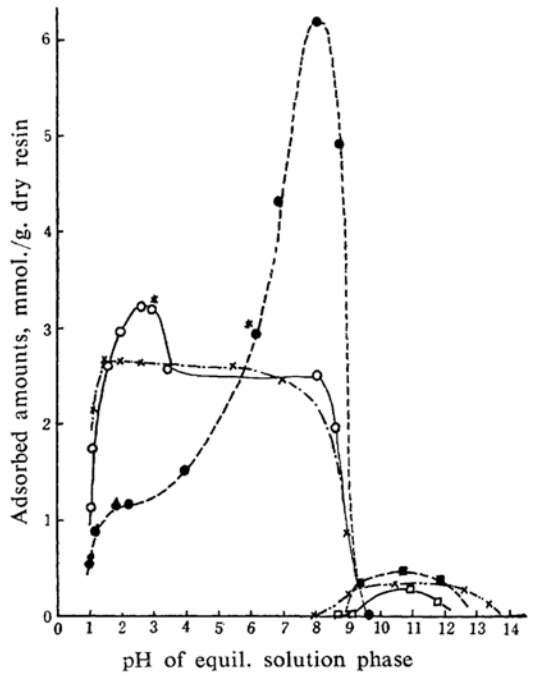



Fig. 2. Adsorption of lysine monohydrochloride on ion-exchange resins (Symbols are the same as those in Fig. 1).
A mark * represents for no addition of acid or base.

are liberated from resins and promote the association of the amino acid to produce the amino acid cations. These result in further exchange of the amino acid cations for the hydrogen ions. The exchange reaction attains equilibrium through such a repeating process. Similar reactions take place in other systems.

**Relations between the Adsorbed Amount of Amino Acids and the pH of the External Solution.**—The dissociation constants of the acidic amino acid are given as

$$\left.\begin{aligned} &AH^+ \rightleftarrows A^\pm + H^+ \quad K_1=\frac{[A^\pm]_s[H^+]_s}{[AH^+]_s} \\ &A^\pm \rightleftarrows A^- + H^+ \quad K_1'=\frac{[A^-]_s[H^+]_s}{[A^\pm]_s} \\ &A^- \rightleftarrows A^{2-} + H^+ \quad K_3=\frac{[A^{2-}]_s[H^+]_s}{2[A^-]_s} \end{aligned}\right\} \quad (1)$$

where the term arising from activity coefficients is neglected. In a case when the acidic amino acid is exchanged for hydrogen ions on the cation-exchanger, the selectivity coefficient is defined as

$$\left.\begin{aligned} &RH + AH^+ \rightleftarrows RAH + H^+ \\ &K_H=\frac{[AH^+]_r[H^+]_s}{[AH^+]_s[H^+]_r} \end{aligned}\right\} \quad (2)$$

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.

Under the present experimental condition, that is; (1) the total amount of the amino acid in the system is constant, $a$ mmol., (2) the amount of the exchangeable ions in the ion-exchanger is constant, $c$ meq., $c=Cw$, where $C$ is the exchange capacity (meq./g. dry resin) of the resin and $w$ is the weight of the resin used, and (3) the volume of the external solution is constant, $v$ ml., the following relationship holds between the adsorbed amount of the amino acid, $[AH^+]_r=y$, and the hydrogen ion concentration in the external solution, $[H^+]_s=x$,

$$\left.\begin{aligned} &\frac{(a-wy)(c-wy)}{y}=\frac{vw}{K_H}f(x) \\ &f(x)=\frac{x^3+K_1x^2+K_1K_1'x+K_1K_1'K_2}{x^2} \end{aligned}\right\} \quad (3)$$

This formula is similar to that derived on the system of neutral amino acids[1].

When the acidic amino acid is adsorbed on the hydroxyl-form anion-exchanger, two species of the amino acid anions participate in the exchange reaction as follows,

$$\left.\begin{aligned} &ROH + A^- \rightleftarrows RA + OH^- \\ &\qquad K_{OH} = \frac{[A^-]_r [OH^-]_s}{[A^-]_s [OH^-]_r} \\ &2ROH + A^{2-} \rightleftarrows R_2A + 2OH^- \\ &\qquad K'_{OH} = \frac{[A^{2-}]_r [OH^-]_s^2}{[A^{2-}]_s [OH^-]_r^2} \end{aligned}\right\} \quad (4)$$

and the following formulas are derived between quantities, $[A^-]_r = y_1$, $[A^{2-}]_r = 2y_2$ and $[OH^-]_s = x'$,

$$\left.\begin{aligned} &\frac{[a - w(y_1 + y_2)]\,[c - w(y_1 + 2y_2)]}{y_1} = \frac{vw}{K_{OH}} f(x') \\ &\frac{[a - w(y_1 + y_2)]\,[c - w(y_1 + 2y_2)]^2}{y_2} \\ &\qquad = \frac{vw^2}{K'_{OH}} \frac{K_w}{K_2} f(x') \\ &f(x') = \frac{K_1 K_1' K_2 x'^3 + K_1 K_1' K_w x'^2 + K_1 K_w^2 x' + K_w^3}{K_1 K_1' K_w x'} \end{aligned}\right\} \quad (5)$$

Similar formulas are obtained on the exchange reaction of a basic amino acid such as lysine, which dissociates as follows,

$$\left.\begin{aligned} &AH_2^{2+} \rightleftarrows AH^+ + H^+ \\ &\qquad K_1 = \frac{2[AH^+]_s [H^+]_s}{[AH_2^{2+}]_s} \\ &AH^+ \rightleftarrows A^\pm + H^+ \\ &\qquad K_2 = \frac{[A^\pm]_s [H^+]_s}{[AH^+]_s} \\ &A^\pm \rightleftarrows A^- + H^+ \\ &\qquad K_2' = \frac{[A^-]_s [H^+]_s}{[A^\pm]_s} \end{aligned}\right\} \quad (6)$$

where the selectivity coefficients are defined as

$$\left.\begin{aligned} &RH + AH^+ \rightleftarrows RAH + H^+ \\ &\qquad K_H = \frac{[AH^+]_r [H^+]_s}{[AH^+]_s [H^+]_r} \\ &2RH + AH_2^{2+} \rightleftarrows R_2AH_2 + 2H^+ \\ &\qquad K'_H = \frac{[AH_2^{2+}]_r [H^+]_s^2}{[AH_2^{2+}]_s [H^+]_r^2} \end{aligned}\right\} \quad (7)$$

The relationship between the quantities, $[AH^+]_r = y_1$, $[AH_2^{2+}]_r = 2y_2$ and $[H^+]_s = x$, is

$$\left.\begin{aligned} &\frac{[a - w(y_1 + y_2)]\,[c - w(y_1 + 2y_2)]}{y_1} = \frac{vw}{K_H} f(x) \\ &\frac{[a - w(y_1 + y_2)]\,[c - w(y_1 + 2y_2)]^2}{y_2} = \frac{vw^2}{K'_H} K_1 f(x) \\ &f(x) = \frac{x^3 + K_1 x^2 + K_1 K_2 x + K_1 K_2 K_2'}{K_1 x} \end{aligned}\right\} \quad (8)$$

While, in a case when the basic amino acid is adsorbed on the hydroxyl-form anion-exchanger, the simpler relation is derived between $[A^-]_r = y$ and $[OH^-]_s = x'$,

$$\left.\begin{aligned} &\frac{(a - wy)(c - wy)}{y} = \frac{vw}{K_{OH}} f(x') \\ &f(x') = \frac{K_1 K_2 K_2' x'^3 + K_1 K_2 K_w x'^2 + K_1 K_w^2 x' + K_w^3}{K_1 K_2 K_2' x'^2} \end{aligned}\right\} \quad (9)$$

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

**$f(x)$ or $f(x')$ Functions.**—As described in the preceding paper, functions $f(x)$ or $f(x')$, which appear to the right of the above equations,

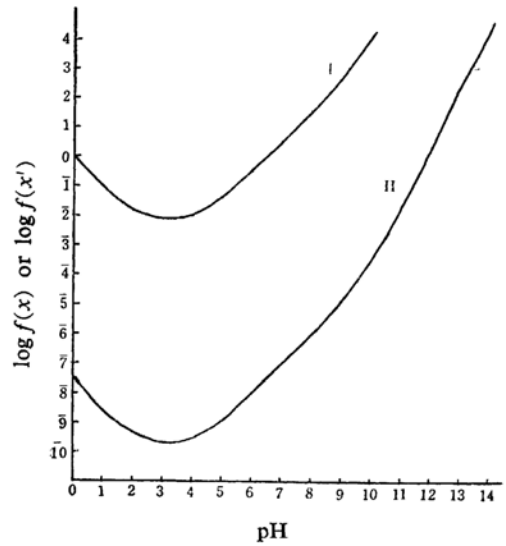



Fig. 3. $f(x)$ and $f(x')$ of glutamic acid.
I $f(x)$, II $f(x')$

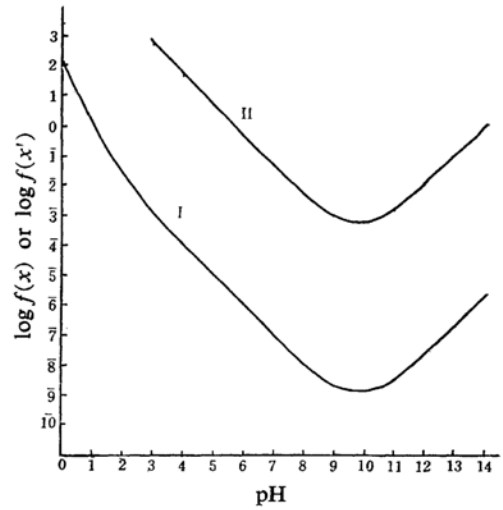



Fig. 4. $f(x)$ and $f(x')$ of lysine.
I $f(x)$, II $f(x')$

contain only a variable, the solution pH, and give the characteristic exchange behavior of amino acids. These functions have minimum values at the isoelectric points of amino acids as shown in Figs. 3 and 4, in which these are plotted against pH. At the minimum of these functions, the adsorbed amount of amino acids is the maximum. This can be shown directly by differentiating the above equations.

The amounts of adsorbed amino acids are related inversely to $f(x)$ or $f(x')$. For example, the relation derived from Eq. 3 is

$$y=\frac{ac}{w\left(a+c+\frac{v}{K_H}f(x)\right)}+\frac{a^2c^2}{w\left(a+c+\frac{v}{K_H}f(x)\right)^3}+\cdots \quad (10)$$

and the similar equation is obtained from Eq. 9. In the system where two ionic species of amino acids participate in the exchange reaction, such a simple relation can not be derived and, however, when $K'$ is not much larger than $K$ in Eqs. 7 or 8, $y_2$ is very small compared to $y_1$ in the pH range near the isoelectric point and the similar relations to Eq. 10 are derived in these cases.

This is closely related to the fact that $f(x)$ and $f(x')$ of the acidic and the basic amino acid are more strongly dependent on pH than those of neutral amino acids[1], that the ion-exchange behavior of the former has more remarkable pH effect than that of the latter. In passing, it is noticed that the exchange reaction takes place in a wider pH range in the cases where two ionic species praticipate.

**Ion Species Exchanged.**—In order to estimate the selectivity coefficients $K$ and $K'$, the ionic species exchanged for hydrogen or hydroxyl ions must be discriminated. This requires a complicated procedure experimentally[4]. Hence, it is assumed that the same dissociation equilibrium is attained in the resin phase as in the solution phase. Then, in the case of an acidic amino acid,

$$\frac{K_2}{K_w}=\frac{[A^{2-}]_r}{2[A^-]_r[OH^-]_r} \quad (11)$$

and under the present condition the following relation is obtained between $y_1$ and $y_2$,

$$y_2=\frac{K_2(c-wy_1)y_1}{w(2K_2y_1+K_w)} \quad (12)$$

Putting Ep. 12 into Eq. 5, $K_H$ and $K_H'$ are estimated at least in principle, if the amount (in mole) of adsorbed amino acids is known.

A similar relation is derived as that for a basic amino acid. The relation

$$K_1=\frac{2[AH^+]_r[H^+]_r}{[AH_2^{2+}]_r} \quad (13)$$

is assumed and the following formula is obtained.

$$y_2=\frac{(c-wy_1)y_1}{w(K_1+2y_1)} \quad (14)$$

It might be reasonably expected that these relations are valid for strongly dissociated ion-exchangers. In the case of weakly dissociated ion-exchangers, these relations are not always satisfied, because all the ions exchanged are not in the dissociated state[5].

From Eq. 12 or 14 and the known quantities, the numerical relation between $y_1$ and $y_2$ can estimated and the mole ratio of monovalent amino acid ion to all the exchanged amino acid ions in the resin phase, $y_1/(y_1+y_2)$, is shown against the total amino acid ions exchanges in mole in Fig. 5. Using this diagram

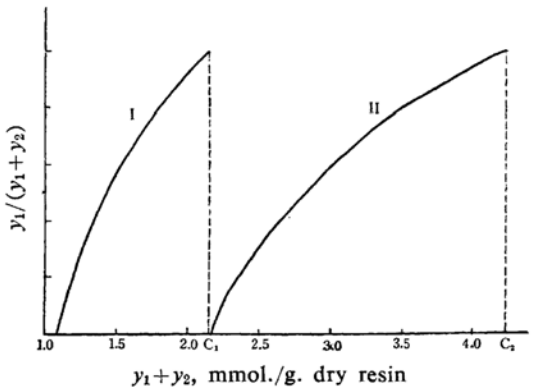



Fig. 5. Fraction of ionic species of amino acids in ion-exchange resins.
I Glutamic acid-IRA-400, II lysine-IR-120, $c_1$ and $c_2$ are the exchange capacities of IRA-400 and IR-120, respectively.

and the experimental data shown in Figs. 1 and 2, the adsorbed amino acids can be discriminated into two ionic species, mono- and di-valent ions. The result is given in Fig. 6.

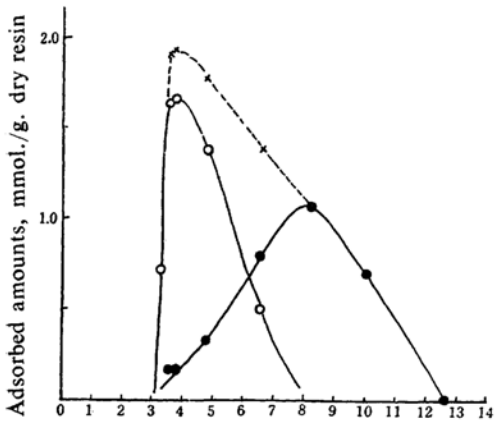
It is shown by differentiating Eq. 12 that, when $y_1$ has the following value

$$y_1=\frac{1}{2}\left(\sqrt{\left(\frac{K_w}{K_2}\right)^2+2C\frac{K_w}{K_2}}-\frac{K_w}{K_2}\right) \quad (15)$$

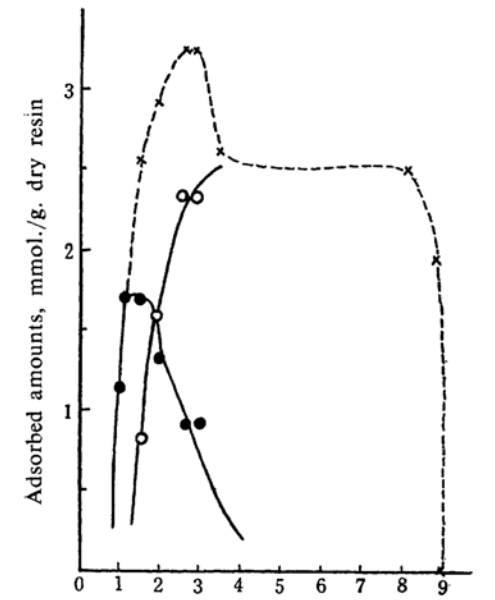
$y_2$ has a maximum value

4) In order to discriminate ionic species of adsorbed amino acids experimentally, the adsorbed amount must be measured in the two units, mole and equivalent number. The molar quantity was measured in this experiment and the equivalent quantity will be obtained by knowing the contents of hydrogen or hydroxyl ions in the ion-exchange resin in equilibrium in an appropriate way.

5) These relations can be extended to this case by assuming that the exchange capacity $C$ is a variable which depends on the dissociation constants of the exchange sites combined with various ions adsorbed on the resins.




Glutamic acid-IRA-400, —○— $A^-$, —●— $A^{2-}$, --×-- total ($A^-+A^{2-}$)




Lysine-IR-120, —○— $AH^+$, —●— $AH_2^{2+}$, --×-- total ($AH^++AH_2^{2+}$)

Fig. 6. Ionic species of amino acids adsorbed on ion-exchange resins.

$$y_{2,\max}=\frac{1}{2}\left(C+\frac{K_w}{K_2}-\sqrt{\left(\frac{K_w}{K_2}\right)^2+2C\frac{K_w}{K_2}}\right) \tag{16}$$

Putting these values into the second equation of Eq. 5, it is shown that $y_2$ has a maximum value when

$$f(x')=K'_{OH}\frac{\left(a-\frac{1}{2}c\right)}{v} \tag{17}$$

In the case of glutamic acid-Amberlite IRA-400, using the known values and the value of $K'_{OH}$ obtained in the next section, Eq. 17 becomes

$$f(x')=2.27\times10^{-7}$$

This value corresponds to pH=7.4.

A similar relation is derived in the case of lysine-Amberlite IR-120. The value of $y_2$ is maximum when

$$f(x)=K_H'\frac{\left(a-\frac{1}{2}c\right)}{v} \tag{18}$$

$$=1.48\times10^{-2}$$

This corresponds to pH 2.0. All the values explain well the experimental results presented in this paper.

**Selectivity Coefficient.**—The selectivity coefficients were estimated from the above-mentioned experimental data, using Eqs. 3, 5, 8 and 9, with Eqs. 12 and 14[6]. The latter equations can not be successfully applied to the cases of weakly dissociated resins. On this account, the selectivity coefficients in the systems, glutamic acid-IR-4B and lysine-IRC-50, can be obtained immediately. However, as revealed in the systems, glutamic acid-IRA-400 and lysine-IR-120, monovalent amino acid ions play the predominant role in the pH range close to the isoelectric point and divalent amino acid ions contribute predominantly to the ion-exchange in the pH-range for from the isoelectric point[7]. It may be safely assumed that similar reactions take place also in systems of weakly dissociated ion exchangers. The selectivity coefficients thus obtained are given in Table I.

Table I. Selectivity coefficients

| | Glutamic acid | | Lysine | |
|---|---|---|---|---|
| | $\log K_H$ | $\log K'_H$ | $\log K_{OH}$ | $\log K'_{OH}$ |
| IR-120 | 0.23 | — | 1.81 | $\bar{1}.62$ |
| IRC-50 | $\bar{2}.43$ | — | $\bar{5}.83$ | $\bar{3}.14$ |
| | $\log K_{OH}$ | $\log K'_{OH}$ | $\log K_{OH}$ | $\log K'_{OH}$ |
| IRA-400 | $\bar{3}.32$ | $\bar{6}.82$ | $\bar{3}.75$ | — |
| IR-4B | $\bar{8}.08$ | — | $\bar{3}.38$ | — |

6) In this calculation, it happens that $c-w(y_1+2y_2)$ equals nearly zero. Then, the following formulas derived from Eqs. 12 and 14 are used.

$$c-w(y_1+2y_2)=\frac{K_w(c-wy_1)}{2K_2y_1+K_w}$$ (glutamic acid-IRA-400)

$$=\frac{K_1(c-wy_1)}{K_1+2y_1}$$ (lysine-IR-120)

7) This situation is verified by the variation of the ionic species of amino acids due to the pH change. The predominant ionic species in solutions with various pH's are as follows.

| pH | | 2.2 | | 4.3 | | 9.7 | |
|---|---|---|---|---|---|---|---|
| Glutamic acid | $AH^+$ | | $A^\pm$ | | $A^-$ | | $A^{2-}$ |
| pH | | 2.2 | | 9.0 | | 10.8 | |
| Lysine | $AH_2^{2+}$ | | $AH^+$ | | $A^\pm$ | | $A^-$ |

In general, $K_H$'s of the amino acids are larger than $K_{OH}$'s. $K_H$ or $K_{OH}$ in the case of the weakly dissociated resins is rather small. This is owing to their strong affinity to hydrogen or hydroxyl ions and it is clear from Figs. 1 and 2 that IRC-50 of sodium-form or IR-4B of chloride-form has a strong affinity to amino acids.

## Summary

The ion-exchange behavior of the acidic and the basic amino acids was described and discussed. The maximum uptake of amino acids takes place in the pH range close to the isoelectric points and the pH effect is more remarkable and more characteristic, in comparison with the case of neutral amino acids. Theoretical treatment on ion-exchange equilibria, which takes account of the dissociation equilibria of amino acids, explains well the experimental results and the selectivity coefficients were estimated herefrom.

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