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A Polarographic Study of Amino Acids in Aqueous Solution

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Only a few investigations have been made on the polarography of typical amino acids. Brdička[1] studied sulfur-containing amino acids and proteins using *Brdička's solutions*. Okazaki[2] reported a polarographic study of several α- and β-amino acids in a dioxane-water medium. He showed that there is a linear relation between the half-wave potentials and the pK_a for the amino acids, and suggested that the polarographic current observed in this system is due to the non-ionized form of the amino acids, *i.e.*, RNH_2COOH. In the present study a pH dependence of polarographic currents was analyzed in detail for several amino acids in water solutions with pH 2—4. A possible mechanism for reduction process is proposed, and a result which appears to be inherent in the polarography of amino acids is reported.

All chemicals are of reagent grade, and dissolved in water which has been deionized and distilled. Amino acids examined are L-alanine, β-alanine, γ-aminobutyric acid, L-arginine and L-lysine. Each sample solution contains 5 mM of amino acid and 1 M of LiCl. The pH values of the solutions were adjusted by adding HCl or LiOH. All measurements were performed at 23.0± 0.5°C using a Yanagimoto DC and AC polarograph Model PA-102. The flow rate of the dropping mercury electrode was 0.866 mg/sec, and the drop time was 8.16 sec in distilled water at a mercury column height of 55.0 cm with an open circuit.

All sample solutions give a polarographic wave at −1.55——1.65 V *vs.* mercury pool anode. This wave will be referred to hereafter as the main wave. A sub-wave accompanying a maximum of the first kind is observed over the shoulder of the main wave. This maximum increases in intensity with the lowering of the pH of the solution. AC polarograms for the present systems show two peaks A and B, which correspond to the main wave and the sub-wave in the DC polarograms, respectively. Peak B increases in intensity with the lowering of the pH of the solution (Figs. 1a and b). The limiting currents for the main wave (peak A) and the sub-wave (peak B) are proportional to the square root of the mercury column

1) R. Brdička, *Collection.*, **5**, 148, (1933); *Nature*, **139**, 330 (1937).
2) Y. Okazaki and T. Otsuki, *Rev. Polarog.* (Japan), **14**, 307 (1967).

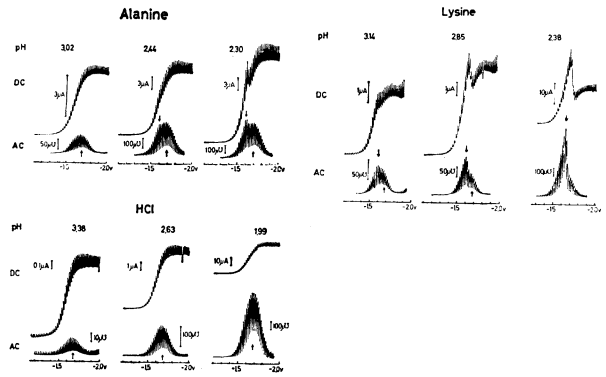

Fig. 1 a.

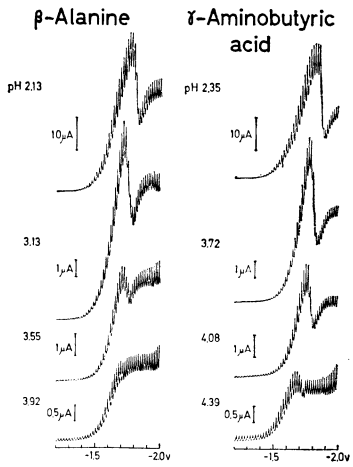

Fig. 1 b.

Fig. 1. DC and AC polarograms of typical amino acids.
a: DC and AC polarograms of some α-amino acids and HCl.
b: DC polarograms of β- and γ-amino acids.

height. This indicates that the main wave and sub-wave are both diffusion controlled.

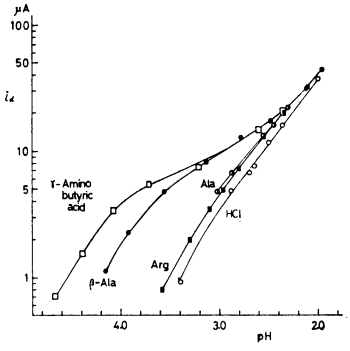

Fig. 2 a.

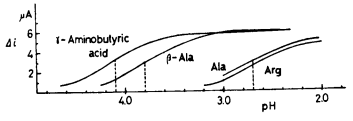

Fig. 2 b.

Fig. 2. pH dependence of the current intensities of some amino acids.
a: pH dependence of the sum of the current intensities of the main and the sub-wave.
b: pH dependence of the difference (Δi) in current intensity between HCl and the amino acids.

For the sake of comparison, the sum of the current intensities of the main and the sub-wave is plotted as a function of pH in Fig. 2a, where the values of the current intensity observed for HCl are also given. The difference Δi in current intensity between HCl and the amino acids in Fig. 2a is plotted in Fig. 2b. In the present systems it is sufficient to consider the equilibrium.

$$R(NH_3^+)COOH \rightleftharpoons R(NH_3^+)COO^- + H^+ \quad (1)$$

The following relation holds at a given pH value.

$$pH = pK_a + \log \frac{[R(NH_3^+)COO^-]}{[R(NH_3^+)COOH]} \quad (2)$$

Equation (2) is used along with the data given in Fig. 2b to estimate the pK_a values for the amino acids. The values are given in Table 1, and are in fairly good agreement with pK_a values determined by standard methods.[3]

Table 1. pK_a values for amino acids

	This work	Standard methods (A)[a]	Standard methods (B)[b]
L-alanine	2.7±0.2	2.34	2.348
L-arginine	2.7±0.2	2.18	—
β-alanine	3.8±0.1	3.60	3.551
γ-aminobutyric acid	4.1±0.1	—	4.031

a) determined on cells with liquid junction.
b) determined on cells without liquid junction.

From the results, it can be concluded that the main wave is due to the free H^+ ion, and that the sub-wave (peak B) accompanying the first kind of maxima corresponds to the electrode reaction.[4]

$$R(NH_3^+)COOH \xrightarrow{e^-} R(NH_3^+)COO^- + \frac{1}{2}H_2 \quad (3)$$

The maxima of the first kind are observed over the sub-waves (peak B). They increase in intensity with the lowering of the pH of the solution, and differ in

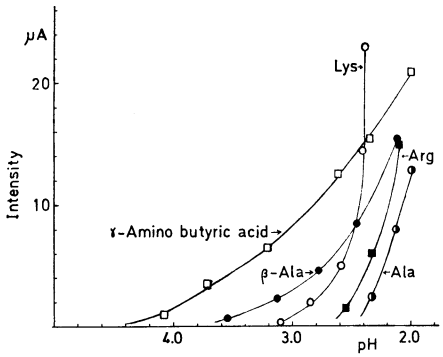

Fig. 3. pH dependence of the curreent intensities of the maxima.
Intensity: difference between the current intensity with the maximum and that without the maximum at the maximum potential.

3) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 1., John Wiley and Sons, New York (1961), p. 486.
4) Possibility of the following reaction cannot be excluded.

$$R(NH_3^+)COOH \xrightarrow[2H^+]{e^-} R(NH_3^+)CHO + H_2O$$

However, the reactant is definitely $R(NH_3^+)COOH$.

shape for different amino acids and pH values. As seen in Fig. 3, the maxima disappear at pH values nearly equal to the pK_a values for the amino acids, and the slopes for L-lysine and L-arginine are much greater than those for the remaining monoamino-monocarboxylic acids. It should be noted in Fig. 1b that the widths of the maxima increase in the order α-, β-, γ-amino acids, and that in the cases of β-alanine and γ-aminobutyric acid the widths of the maxima increase with decreasing pH value.

Further investigation of the maxima would be useful for the polarographic study of amino acids.