Determination of the Dissociation Constants of α-Amino Acids by Ionophoretic Technique: II: Amino Acids with an Additional Functional Group

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The use of ionophoretic technique for the assesement of dissociation constant of some amino acid viz. Aspartic acid, Benzyl Aspartic acid, Asparagine, Glutamic acid and Glutamine is described.

Ionophoretic technique has been used to determine the dissociation constant of some amino acids. Here we have extended our technique to study the nature of dissociation as well as to assess the magnitude of dissociation constants of other amino acids. viz. aspartic acid, benzyl aspartic acid, glutamic acid, asparagine and glutamine.

EXPERIMENTAL

Instruments, chemicals and procedure are all as described in reference 1.

RESULTS AND DISCUSSION

In the present study two categories of amino acids have been taken. Category (A), consisting of asparagine and glutamine are H_2L variety whose dissociation in general is as follows[2]

$$NH_2CO - R - \underset{NH_3^+}{CH} - COOH \underset{K_1}{\overset{-H^+}{\rightleftharpoons}} NH_2CO - R - \underset{NH_3^+}{CH} - COO^-$$

I II

$$\underset{K_2}{\overset{H^+}{\rightleftharpoons}} \quad NH_2CO - R - \underset{NH_2}{CH} - COO^-$$

III

In category B includes aspartic acid, benzyl aspartic acid and glutamic acid which dissociate in 3 steps as

$$COOH - R - \underset{NH_3^+}{CH} - COOH \underset{K_1}{\overset{-H^+}{\rightleftharpoons}} COOH - R - \underset{NH_3^+}{CH} - COO^- \underset{K_2}{\overset{-H^+}{\rightleftharpoons}}$$

$$COO^- - R - \underset{NH_3^+}{CH} - COO^- \underset{K_3}{\overset{-H^+}{\rightleftharpoons}} COO^- - R - \underset{NH_2}{CH} - COO^-$$

The plot of overall electrophoretic mobility of amino acid spot against pH gives a curve with a number of plateaus as shown in Fig (1). Each plateau indicates the pH range where particular ionic species of amino acid is overwhelmingly formed. The first plateau occuring in the region of low pH values with positive mobility is due to the species with maximum protonation $NH_2CO - R - \underset{NH_3^+}{CH} - COOH$ of category A and $\underset{NH_3}{\overset{|}{}}$

COOH-R-CH-COOH of Category B.

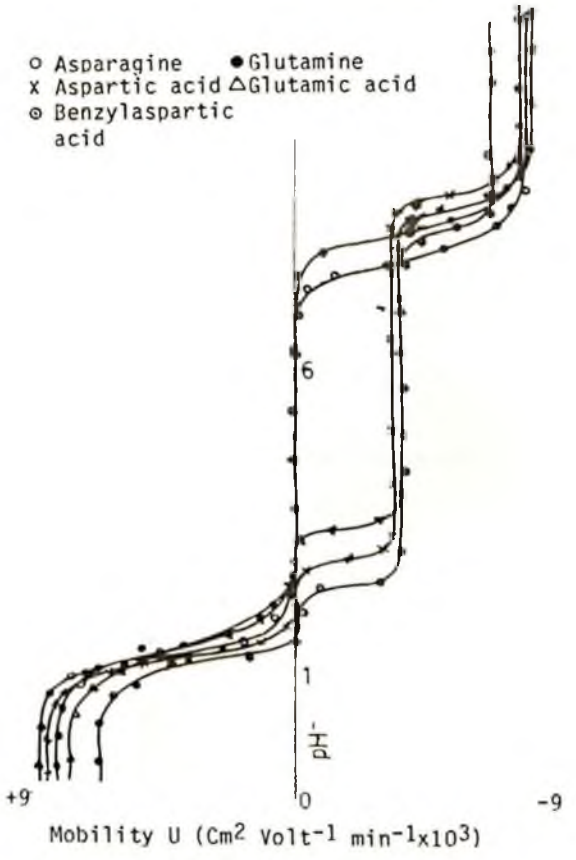


Fig. 1 Mobility Curves [H - Ligand] System

On increase of pH the speed of the spot decreases due to formation of increasing amount of less

TABLE 1

DISSOCIATION CONSTANTS OF AMINO ACIDS

(temp. 35°C, Ionic Strength 0.1)

Amino acid	Calculated value			Litrature value			Ref. No.
	log K_1	log K_2	log K_3	log K_1	log K_2	Log K_3	
Category A							
Aspartic acid	2.03	3.75	9.69	1.87	3.95	9.71	4
				--	3.75	9.48	4
				--	3.70	9.62	4
				1.93	3.70	9.63	5
Benzyl aspartic acid	1.90	3.26	9.05				
Glutamic acid	2.09	4.25	9.50	2.30	4.28	9.67	4
					4.23	9.45	4
				2.18	4.20	9.59	5
Category B							
Asparagine	2.10	8.73		2.16	8.70		4
				2.14	8.72		5
Glutamine	2.15	9.10		2.21	8.91		4
				2.39	8.97		4
				2.17	9.01		5

Protonated ionic variety. The decrease continues till the second plateau is reached. These ionic species in case of all amino acids are Zwitter ion with overall neutral charge which is confirmed because of zero speed in the region of second plateau. On further increase of pH, mobility further decreases and becomes constant in the range of third plateau in both types of amino acids, which is ascribed to the excessive formation of $NH_2CO - R - \underset{NH_2}{CH} - COO^-$ (category A) and $COO^- - R - \underset{NH_3^+}{CH} - COO^-$ (category B). On further increase in pH mobility does not obviously change in case of category A acids whereas a fourth plateau is evenced in case of category B acids which is ascribed to overwhelming formation of $COO^- - R - \underset{}{\overset{NH_2}{CH}} - COO^-$.

For category A acids, the behaviour is exactly similar to that found in reference 1. The dissociation constants are calculated by the same method and presented.

As for amino acids of category B, whose most protonated variety is H_3L, zwitter ion is H_2L and anionic varieties as HL and L, the following equation interrelating overall mobility with mobilities of individual ionic species can be anologously derived.

$$U = \frac{U_{H_3L} + U_{H_2L}\, K_1/[H] + U_{HL} K_1 K_2/[H]^2 + U_L K_1 K_2 K_3/[H]^3}{1 + K_1/[H] + K_1 K_2/[H]^2 + K_1 K_2 K_3/[H]^3} \quad ..(2B)$$

The region between first and second plaeau corresponds to deprotonation of most protonated variety of amino acid to zwitter ion in which only these two ionic species are over-whelmingly present whereas others are practically non-existent.

Hence for calculating first dissociation constant 'K_1' first and second plateaus have been considered. The average of the mobilities of the two plateaus corresponds to a point at which $K_i/[H^+] = 1$. The value of K_1 can thus be calculated. Similarly this principle of average mobility is used again for calculation of K_2 taking into consideration the region between second and third plateeaus and so on. Values of K's are reported in Table(1).

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