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A NEW METHOD FOR THE STUDY OF DISSOCIATION CONSTANT

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DISSOCIATION of amino acids in general has been studied mainly with electrometric technique using glass electrode which gives reliable results. The ionophoretic technique has been recently introduced to investigate the formation of complexes and determination of stability constants of mononuclear complexes[1, 2]. The usual procedure is to study the mobility of metal cation spot on a paper strip soaked with background electrolyte buffered at a fixed pH containing progressively increasing concentration of the ligand. This procedure has been drastically modified[3-7]. Here the concentration of liganding sample is kept constant but the hydrogen ion concentration of the background electrolyte is progressively decreased by the addition of an alkali solution. Thus the previous technique failed to elucidate the effect of change of relative concentration of the different ionic species of a liganding polybasic acid. Our modified technique has also been used for the study of mixed complexes for the first time[8-13].

The electrophoretic technique usually suffers from a number of defects. Temperature during electrophoretic capillary flow on the paper, electroosmosis, adsorption and molecular sieving, affect the mobility of charged moieties. The technique described here is almost free from these vitiating factors. We have extended our modified method to study the nature of dissociation as well as to assess the magnitude of dissociation constants of some amino acids. The technique is very handy and simple. It gives results fairly in agreement with the literature values.

Instrument

Electrophoresis equipment (Systronics model 604 India) has been used. The apparatus consists of a PVC moulded double tank vessel. In order to avoid error due to heat generated during electrophoresis, two hollow rectangular plates each of one kg weight covered with thin plastic paper have been used through which thermostated water is circulated. The tank is closed with a transparent PVC moulded lid to prevent moisture changes that may upset the equilibrium in paper strip. Each electrolyte tank contains a separate platinum wire electrode. Voltage variation is eliminated with an electrical stabilizer. pH measurements were made with Elico model L_{1-10} pH meter using glass electrode.

Chemicals

A.R. grade perchloric acid, sodium hydroxide and β alanine, 3-amino butanoic acid, 4-amino butanoic acid, 4-amino butanoic acid, 5-amino pentanoic acid and 6-amino hexanoic acid (all BDH) are used. A 0.28% (w/v) solution of ninhydrin in acetone was used for detecting amino acid spots. A saturated solution of silver nitrate (A.R.) in acetone (pure) was sprayed on paper and subsequently fumed with ammonia to detect glucose spot.

Procedure

The level of hollow base plate in the instrument was made horizontal with a spirit level. Perchloric acid (150 ml of 0.1 N) was taken in each tank of the electrophoretic apparatus. The levels of the two tank solutions were equalised. These precautions were taken to check any gravitational and hydrodynamic flow; paper strips (Whatman No. 1) of (30×1) cm^2 size were socked in the background electrolyte and then excess of electrolyte solution was blotted. The strips in duplicate were then spotted with an aqueous solution (0.01 M) of amino acid and glucose solution (0.01 M) in the centre with a micropipette and were subsequently placed on the base plate and sandwiched under the upper hollow metallic plate and the ends of the strip were allowed 15 min. Then a potential difference of 200V is applied between the tank solutions to initiate electrophoresis. The electrolysis is carried for 60 min. The strips are then taken out and dried horizontally and the spots are detected. This observation was repeated at different pH values of the background electrolyte. The distance recorded in duplicates differed within $\pm 5\%$ and the average distance of the duplicates were noted for calculation. The actual distance of sample spot takes into account the distance travelled by the reference glucose spot. The potential gradient through the strip was found to be 7.57 V/cm.

The plot of overall electrophoretic speed of amino

acid spot against pH gives a curve with a number of plateaus as shown in figure 1. A plateau is obviously indicative of a pH range where speed is practically constant. This is possible only when a particular ionic species of amino acid is overwhelming. Thus every plateau indicates the formation of a certain ionic species of amino acid. The first pleateau occurring in the region of low pH values with positive mobility must be due to most protonated ionic variety of β-amino acids, *viz* NH_3^+–$(CH_2)_n$–CH_2–COOH.

With an increase in the pH, the speed of the spot decreases due to the formation of increasing amount of less protonated ionic variety. The decrease continued till the second plateau is reached. These ionic species in the case of all amino acids are zwitter ions with overall neutral charge which is confirmed because the speed is zero in the region of second plateau. On further increase of pH the mobility further decreases and becomes constant in the range of the third plateau. Since the last plateau lies in the negative region of curve which definitely implies abundant formation of anionic species *viz* NH_2–$(CH_2)_n$–CH_2COO^-. Mobility remains unchanged on further increase of pH.

In view of the above observation the dissociation of a β amino acid can be depicted as:

$$NH_3^+-(CH_2)_n-CH_2-COOH \underset{k_1}{\overset{-H^+}{\rightleftharpoons}} NH_3^+$$

$$-(CH_2)_n-CH_2-CO\bar{O} \underset{k_2}{\overset{-H^+}{\rightleftharpoons}} NH_2$$

$$-(CH_2)_n-CH_2-CO\bar{O}.$$

Considering most protonated variety of amino acids as H_2L, zwitter ion as HL and anionic variety as L (charges on the ions are being ignored). The dissociation can be expressed as:

$$H_2L \overset{k_1}{\rightleftharpoons} HL + H,$$

$$HL \overset{k_2}{\rightleftharpoons} H + L$$

The concentration of a zwitter ion and anionic species can be expressed as:

$$[HL] = \frac{k_1}{[H]}[H_2L],$$

$$[L] = \frac{k_1 k_2}{[H]^2}[H_2L].$$

Total dibasic acid distributes itself in the form of different-ionic species. The following expression holds

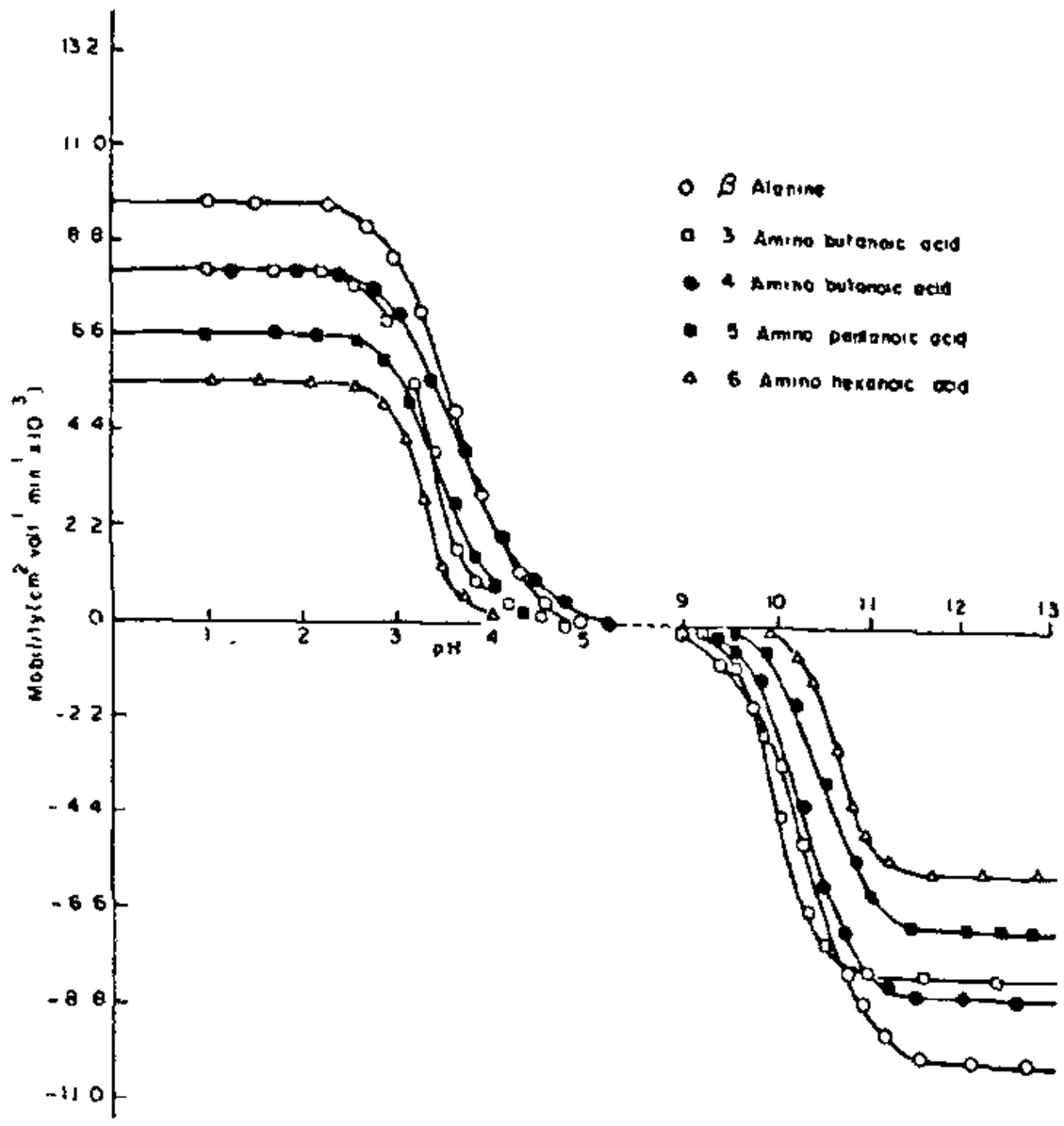

Figure 1. Mobility curves (temp. 35°C, ionic strength 0.1)

for total concentration:

$$L_T = \left\{1 + \frac{k_1}{[H]} + \frac{k_1 k_2}{[H]^2}\right\}[H_2L]$$

The relative abundance of a particular anionic species of the dibasic acid depends upon the pH of the background electrolyte in which it is present. Under the influence of electric field imposed on the strip, the amino acid spot which is essentially a conglomeration of number of species in equilibrium will move as single entity whose speed in an electric field can be given by the well-known equation[1]:

$$U = u_n f_n,$$

Where f_n and u_n are molefraction and mobility of a particular species of the amino acid under investigation respectively. Using this equation in the present case the overall mobility is given by the expression.

$$U = U_{H_2L} \cdot f_{H_2L} + u_{HL} \cdot f_{HL} + u_1 \cdot f_L \qquad (1)$$

where u_{H_2L}, u_{HL} and u_L are mobilities of H_2L, HL and L species respectively and f_{H_2L}, f_{HL} and f_L are their mole fractions. Substituting the values of mole fraction f_{H_2L} by $(1 + k_1/[H] + k_1k_2/[H]^2)^{-1}$; f_{HL} by $k_1[H](1 + k_1[H] + k_1k_2/[H]^2)^{-1}$ etc. in (1) and simplifying we obtain,

$$U = \frac{u_{H_2L} + u_{HL} \cdot k_1/[H] + u_L \cdot k_1k_2/[H]^2}{1 + k_1/[H] + k_1k_2/[H]^2}.$$

Table 1 *Dissociation constants of β amino acids at μ = 0.1, and temp. 35°C.*

	Inophoretic method		Literature value		Ref.
Amino acid	log k_1	log k_2	log k_1	log k_2	No.
β alanine	3.63	10.22	3.53	10.10	13
			–	10.55	15
			–	10.09	15
			3.68	10.16	15
			3.66	10.80	15
			3.65	10.21	15
			3.62	10.36	15
			3.60	10.26	15
			3.52	10.39	15
3-aminobutanoic acid	3.45	10.05	3.42	10.02	13
			–	10.14	14
4-aminobutanoic acid	3.70	1.25	–	10.31	13
			–	10.66	15
			–	10.46	15
5-amino-pentanoic acid	3.50	10.50	–	10.51	13
			–	10.94	14
			–	10.38	14
			–	9.64	14
			–	11.33	14
			–	10.75	14
			–	10.00	14
6-aminohexanoic acid	3.30	10.65	–	10.52	13

This expression is a general expression correlating overall mobility U with mobility of individual species present in spot and as well as with their relative abundance.

For calculating first dissociation cosntant k_1 first and second plateaus have been considered. The average mobility corresponds to a point at which $k_1/[H] = 1$. The value of k_1 can thus be calculated. Similarly this principle of average mobility is used again, for calculation of k_2 where second and third plateaus are kept in mind. Values of k's are reported in table 1.

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