Determination of the Dissociation Constants of α-Amino Acids by Ionophoretic Technique: I Simple Amino Acids.

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A novel method involving the use of the ionophoretic technique is described for the assessment of dissociation constants of some α-amino acids viz., serine, homoserine, threonine, isoleucine, α-amino - butyric acid and norvaline. The method is based on the migration of a spot of the acids on paper strips with variation of pH of background electrolyte containing 0.1 M perchloric acid. A plot of pH against mobility gives information about nature of dissociation and helps in calculating them. The calculated values are fairly in agreement with literature Values.

Dissociation of an acid, in general, has been studied mainly by the electrometric technique using the glass electrode. This technique is universal and gives reliable results. The ionophoretic technique has been recently introduced for investigating the formation of complexes[1,2] and determination of stability constants of mononuclear complexes[1,2] This procedure has been refined for the study of metal complexes[3] and the study of mixed complexes[4].

The technique has now been extended to the study of the dissociation, and to determine the dissociation constants of several α- amino acids. Perhaps the precision and universality of the ionophoretic technique is not equal to those of the electrometric technique, nevertheless this new approach gives results which are fairly in agreement with those in literature.

EXPERIMENTAL

Instrument

Electrophoresis equipment (Systonics model 604) has been used. It has a built-in power supply (A.C. -D.C.) which is directly fed to electrophoretic tanks. The electric current passing through paper strips generates heat which causes evaporation of back ground electrolyte leading to serious errors arising due to diffusion. A temperature control device has been designed by the two hollow metallic plates covered from outside with thin plastic paper in which water circulates at constant temperature (35°C). Thus errors due to heating and evaporation effects are eliminated. The pH measurements were made using Elico model 1-10 pH meter using glass electrodes.

Chemicals

All chemicals were of reagent grade. 0.28% (W/V) solution of ninhydrin in acetone was used for detecting amino acid spots. A saturated solution of silver nitrate in acetone was sprayed on paper and subsequently fumed with ammonia to detect the spot of glucose.

Procedure

Paper strips (Whatman No.1) 30x1 cm are spotted in the centre with aqueous amino acid solution (0.05M) with a micropipette. An extra strip was spotted with aqueous glucose,(5%). The strips are sandwiched between the two insulated hollow metal plates. The plates are then mounted in the electrophoresis equipment with each end of the paper strips dipping in one of the two tanks of the instrument. Each tank is filled with 150 ml of the background elevctrolyte containing 0.1 M perchloric acid. An hour is allowed for the strips to get moistened. Subsequently, electrophoresis is carried out for 60 minutes applying a potential of 200 volts between the two tank solutions. The strips are then removed from the tanks and dried. The migrated spots of amino acid are made visible by spraying ninhydrin solution. The distances travelled by the spots are measured, and correction for electro-osmosis is applied from the migration of glucose in the blank strip. Subsequently the pH of the background electrolyte solution is adjusted to different values by the addition of sodium hydroxide solution and the exeperiment repeated. The distances travelled towards anode are assumed to be negative and those towards cathode to be positive. The length of unimmeresed strips above the tank containing background electrolyte is 26.5 cm. Hence the potential gradient in each experiment is 7.5 V/cm. Dividing movement by potential gradient yields mobility.

RESULTS AND DISCUSSION

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. A plateau is indicative of pH range where the mobility is practically constant. The dissociation of an amino acid can be depicted as -

$$\underset{(I)}{R\text{-}CH(NH_3^+)\text{-}COOH} \xrightleftharpoons[K_1]{-H} \underset{(II)}{R\text{-}CH(NH_3^+)\text{-}COO^-} \xrightleftharpoons[K_2]{-H^+} \underset{(III)}{R\text{-}CH(NH_2)\text{-}COO^-}$$

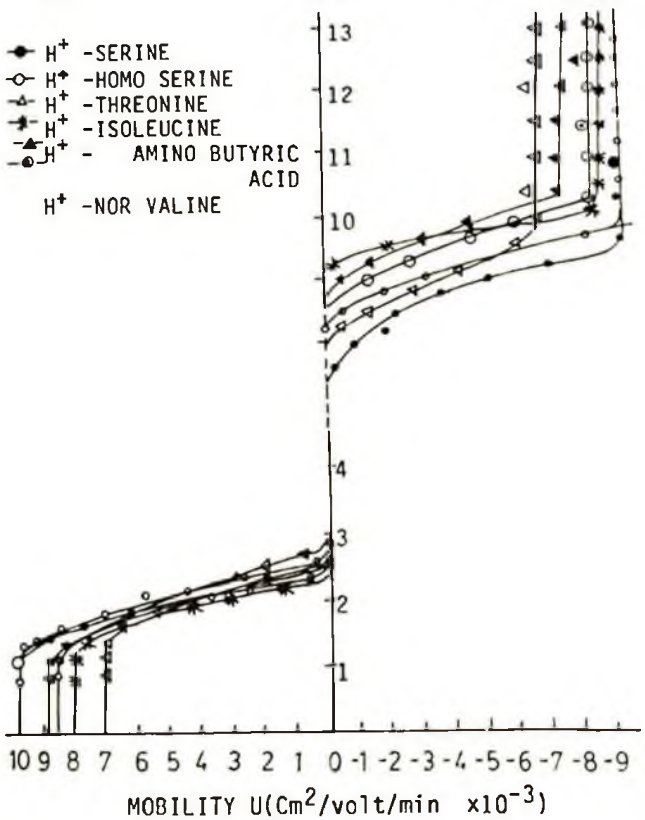


Fig. 1 Mobility Curves (Temp. 35°C Ionic Strength 0.1)

Thus every plateau indicates the formation of a specific ionic species resulting from the amino acid. The first plateau occurring in the region of low pH values with positive mobility must be due to the protonation of α-amino acids(I). On increase of pH, the speed of the spot decreases due to deprotonation. At the pH where species (II) is predominent,the second plateau occurs. These ionic species in case of all amino acids are zwitter ions (II) with overall neutral charge, This is confirmed by zero mobility in the region of the second plateau. On further increase of pH, mobility further decreases and becomes constant in the range of third plateau. The last plateau in the negative region of curve is due to the formation of the anionic species (III). The mobilitiy remains unchanged on further increase of pH.

Considering the 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 -

$$H_2L \xrightleftharpoons{K_1} HL + H \qquad (i)$$

$$HL \xrightleftharpoons{K_2} H + L \qquad (ii)$$

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

$$[HL] = \frac{K_1}{[H]}[H_2L] \qquad [iii]$$

$$[L] = \frac{K_1K_2}{[H^2]}[H_2L] \qquad [iv]$$

Total disbasic acid distributes itself in the from of different ionic species. The following expression holds for total concentration:

$$L_T = \left\{1 + \frac{K_1}{[H]} + \frac{K_1K_2}{[H]^2}\right\}[H_2L] \qquad [v]$$

The relative abundance of a particular 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 a number of species in equilibrium will move as single entity whose speed in an electric field can be given by the well known equation

$$U = \sum U_n X_n \qquad (vi)$$

Where X_n and U_n are mole fraction and mobility of a particular species of the amino acid under investigation. Using this equation in the present case, the overall mobility is given by the expression:

$$U = U_{H_2L} X_{H_2L} + U_{HL} X_{HL} + U_L X_L$$

Where U_{H_2L}, U_{HL} and U_L are mobilities of H_2L HL and L respectively and X_{H_2L}, X_{HL} and X_L ar their mole fractions. Substituting the values c mole fraction X_{H_2L} by $(1+K_1/[H] + K_1K_2/[H]^2)^{-1}$ X_{HL} by $K_1[H]^{-1}(1+K_1[H]+K_1K_2/[H]^2)^{-1}$ in equati (vii) and simplifying we obtain.

$$U = \frac{U_{H_2L} + U_{HL}(K_1/[H]) + U_L(K_1K_2/[H]^2}{1 + K_1/[H] + K_1K_2/[H^2]} \qquad (viii$$

This expression is a general expression corre ting overall mobility U with mobility of indi dual species present in the spot and their re tive abundance.

For calculating the first dissociation cons K_1, first and second plateaus have been con dered. The average mobility corresponds t point at which $K_1/[H] = 1$. The value of K_1

thus be calculated. Similalrly this principle of average mobility is used again, for calculation of K_2 using second and third plateau. Values of K's are reported in Table 1.

TABLE 1

DISSOCIATION CONSTANTS OF α - AMINO ACIDS
(Temperature 35°C, Ionic strength 0.1)

Amino Acid	Experimental value Log K_1	Log K_2	Literature values Log K_1	Log K_2	Ref.No.
Serine	2.1	9.0	2.18	8.84	5
			2.14	9.23	5
			2.56	9.57	5
Home Serine	2.2	9.2	2.26	9.84	5
			2.27	9.28	6
Threonine	2.2	8.9	2.10	9.07	5
			2.17	8.98	5
			2.21	8.92	5
			2.26	8.98	5
Isoleu-cine	2.2	9.6	2.25	9.62	6
			2.38	9.76	7
			2.66	9.70	7
α-amino butyric acid	2.3	9.6	2.11	9.62	5
			2.30	9.66	5
			2.34	9.61	5
nor Valine	2.3	9.6	2.31	9.65	6
			-	9.87	7
			2.31	9.68	5

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