Dissociation Constants of *p*-Amino- and *p*-Guanidino-Substituted Phenylalanines

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The concentration acid dissociation constants of lysine, *p*-aminophenylalanine, *p*-aminomethylphenylalanine, *p*-*N,N*-dimethylaminophenylalanine, *p*-guanidinophenylalanine, and *p*-guanidinomethylphenylalanine have been determined by electrometric titration in cells with transport using glass and saturated calomel electrodes (KCl bridge) at 37 °C and an ionic strength of 0.16. The constants have been calculated from titration data by a computer program based on the method of Schwarzenbach, Willi, and Bach with a modification for matching statistically the observed and theoretical curves as a test of the validity of the calculated constants. The constants together with their probable errors are presented.

Data obtained during experiments in rats (*8*) to determine whether chemically substituted phenylalanines could modify renal tubular resorption of endogenous phenylalanine revealed two *p*-guanidinophenylalanines that inhibited particularly lysine and to a small extent cystine resorption and three *p*-aminophenylalanines that did not. The former compounds therefore have considerable experimental importance and we sought to determine whether their effect was related to similarity of their dissociation constants to those of lysine and so provide additional information to help elucidate unknown details of diamino monocarboxylic amino acid transport. The analyses of titration data and p*K* calculation were carried out by a computer program following the approach described by Datta and Grzybowski (*3*) and based on the original method of Schwarzenbach et al. (*9*).

Experimental Section

Materials. All the phenylalanine derivatives were kindly donated by Dr. D. F. Elliott, Ciba Laboratories Ltd. *p*-Aminophenylalanine, *p*-aminomethylphenylalanine monohydrochloride, and *p*-*N,N*-dimethylaminophenylalanine were synthesized by Elliott, Fuller, and Harrington (*4*) and *p*-guanidinophenylalanine monohydrochloride and *p*-guanidinomethylphenylalanine monohydrochloride were synthesized by Elliott and Harrington (*5*). They were all recrystallized before use and their melting points checked.

The following general procedure was used. The compound was dissolved in the minimum amount of water, heating if necessary, ethanol was added, and the solution was filtered. Crystals were produced in the filtrate either by allowing it to stand or by freezing. These were washed with ethanol and dried by vacuum filtration or in a desiccator.

Lysine was obtained commercially from Sigma Ltd., St. Louis, Mo.

pH Measurement. All pH measurements were performed in a cell with transport comprising glass and calomel electrodes (Radiometer, Copenhagen) in conjunction with a Radiometer pH meter. A scale expander was used, which enabled the pH to be read to within 0.002 unit. The cell temperature was maintained at 37 ± 0.2 °C by water from a thermostat circulating through a glass jacket. During the titration, the solution was bubbled with CO_2-free nitrogen to prevent access of atmospheric carbon dioxide. The titrant was approximately 0.1 N CO_2-free sodium hydroxide solution. Before and after the titration the pH was read in standard 0.01 M borate and 0.05 M phthalate buffers (*2*). No attempt was made to adjust the pH meter, since the computer program is designed to calculate the true pH values from these readings. An ionic strength of 0.16 was maintained by adding an appropriate amount of KCl.

The concentration acid dissociation constants were calculated by a computer program (*6*) following the approach of Datta and Grzybowski (*3*) and based on the original method of Schwarzenbach et al. (*9*), the essential features of which are as follows. If

$$y = a_1 f_1(y) + a_2 f_2(y) + a_3 f_3(y) + \ldots a_m f_m(y) \qquad (1)$$

then

$$a_1 A_1 + a_2 A_2 + a_3 A_3 + \ldots a_m A_m = 1 \qquad (2)$$

where $A_1 = f_1(y)/y$, $A_2 = f_2(y)/y$, $A_3 = f_3(y)/y$, $\ldots A_m = f_m(y)/y$. By choosing any m experimental points, it is possible to write m equations of the type of eq 2 whose solution gives a_1, a_2, a_3, $\ldots$, a_m. If there are n experimental points, the number of sets of m equations is

$$T = \frac{n!}{(n-m)!m!} \qquad (3)$$

We thus obtain T sets of the constants a_1, a_2, a_3, $\ldots$, a_m, a statistical treatment of which yields the optimum values.

Schwarzenbach et al. considered the use of two constants only, for which a two-dimensional graphical solution is possible.

Here eq 2 becomes

$$a_1 A_1 + a_2 A_2 = 1$$

When $a_1 = 0$, we have $a_2 = 1/A_2$ and, when $a_2 = 0$, we have $a_1 = 1/A_1$. Consider plots of a_1 against a_2. If we join the ordinates $1/A_1$ with the abscissas $1/A_2$ by straight lines, the latter should intersect at the same point corresponding to the true values of a_1 and a_2. Owing to experimental errors, the intersections do not coincide exactly, but the point of the highest density of intersections should give the closest approximation to the true constants. If there are three, four, and more constants, we have intersections of planes, volumes, and hypervolumes. A graphical solution is then impracticable, but a computer solution is feasible.

For acid dissociations, we have a general equation of the type

$$K_1 \frac{(1-\bar{h})}{[H^+]\bar{h}} + \frac{K_1K_2(2-\bar{h})}{[H^+]^2\bar{h}} + \ldots \frac{K_1K_2 \ldots K_m(m-\bar{h})}{[H^+]^m\bar{h}} = 1 \qquad (4)$$

where $\bar{h}$ is the average number of protons dissociated per acid molecule. Thus $\underline{a}_1 = K_1$, $a_2 = \underline{K}_1K_2, \ldots, a_m = K_1K_2 \ldots K_m$, $\underline{A}_1 = (1-\bar{h})/[H^+]\bar{h}$, $A_2 = (2-\bar{h})/[H^+]^2\bar{h}$, $\ldots$, $A_m = (m-\bar{h})/[H^+]^m\bar{h}$. Our computer program is based on the above principle.

It has now been elaborated further to calculate automatically any number of overlapping acid dissociation constants, at any

Table I. pK Values of the Amino Acids

Amino acid	pK_2	a	pK_3	a	pH$_{RMS}$ [b]
p-Aminophenylalanine			8.93	0.05	0.05
p-Aminomethylphenylalanine–HCl	8.40	0.03	9.42	0.05	0.04
p-*N*,*N*-Dimethylaminophenylalanine			8.86	0.03	0.08
p-Guanidinophenylalanine–HCl	8.44	0.04	10.91	0.06	0.09
p-Guanidinomethylphenylalanine–HCl	8.61	0.03	*c*		0.05
Lysine–HCl	8.83	0.02	10.23	0.01	0.02

[a] The standard error of the pK. [b] pH$_{RMS}$ is the root mean square of the differences between the calculated and measured pH values. [c] pK_3 is apparently outside the range susceptible to measurement.

temperature or ionic strength, and for titration with acid or base, according to specification. Provision is also made to allow for possible inert impurities by adjusting the total amount of acid or base present to give the best agreement between the calculated and experimental pH curves, i.e., the minimum sum of squares of the differences between the calculated and experimental pH.

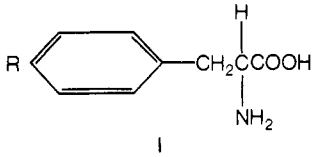
Results and Discussion

The second and third dissociation constants were measured and are displayed in Table I. The first dissociation (due to the carboxy group) was ignored because the carboxy group is always fully ionized in the physiological pH range.

$$K_2 = \frac{[H^+][HA]}{[H_2A^+]}$$

$$K_3 = \frac{[H^+][A^-]}{[HA]}$$

p-Aminomethylphenylalanine (I; R = $CH_2{\cdot}NH_2$), *p*-guanidinophenylalanine (I; R = $-NH{\cdot}C(NH_2){:}NH$, and *p*-guanidinomethylphenylalanine (I; R = $-CH_2{\cdot}NH{\cdot}C(NH_2){:}NH$) were titrated as the monohydrochlorides. The last compound, however, showed only one significant dissociation, probably because the pK of the guanidino group is outside the range susceptible to experimental determination by the technique employed. The pK value therefore approaches the value for aliphatic guanidine derivatives (13.5) (*1*) owing to the interposition of the methylene group between the guanidino and phenyl groups. This single pK value was found by allowing the program to seek one and two constants in turn, the former procedure giving a better curve fit.



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This is consistent with the view that the separation of the guanidino group from the aromatic ring by the methylene group facilitates the protonation of the former. If this assumption is correct, then at the ionic concentrations used the ionization of this group is unlikely to be of importance at physiological pH.

The compound with the guanidino group linked directly to the benzene ring shows clearly two deprotonations, the higher pK (10.91) being presumably that of the guanidino group. It is reduced relative to the pK's of aliphatic guanidines by approximately the same amount as the corresponding amino derivatives (2–3 pK units).

We were unable to find any pK values in the literature for any of the acids investigated except lysine. Klemperer, Hastings, and Van Slyke (*7*) measured the lysine pK's at 38 °C and an ionic strength of 0.1 (KCl). After reasonable adjustments to 37 °C and an ionic strength of 0.16 (pK_2 and pK_3 were increased by 0.02 units to allow for the temperature difference of 1 °C and reduced by 0.03 units to allow for the different ionic strength), their values were pK_2 = 8.92 and pK_3 = 10.30 compared with pK_2 = 8.83 and pK_3 = 10.23 obtained in the present study.

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