Thermodynamic Quantities for the Dissociation Equilibria of Biologically Important Compounds

8. THE FIRST AND SECOND ACID DISSOCIATIONS OF ARGININE*

By S. P. DATTA AND A. K. GRZYBOWSKI

Department of Biochemistry, University College London, Gower Street, London, W.C. 1

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Although the thermodynamics of the acid dissociations of a number of amino acids have been investigated (see, for example, Edsall & Wyman, 1958) no detailed study of arginine appears to have been made. Consequently we have determined the values of pK_{1a} (carboxyl) and pK_{2a} (ammonium) of arginine, on the molal scale, by e.m.f. measurements of cells without liquid junction from 0° to 55° at 5° intervals.

THEORY

Values of pK_{1a}

This dissociation constant refers to the equilibrium:

for the study of which the cell:

was used. In these solutions

$$\begin{aligned}
 m_{\text{R}^{2+}} &= m_2 - m_{\text{H}^+}, & m_{\text{R}^{2+-}} &= m_1 - m_2 + m_{\text{H}^+}, \\
 m_{\text{Cl}^-} &= m_1 + m_2 & \text{and } I &= m_1 + 2m_2 - m_{\text{H}^+}.
 \end{aligned}$$

From the equations for the e.m.f. of the cell and for the first dissociation of arginine, we get

$$pK_{1a} = \text{pWH} + \log (m_{\text{R}^{2+}}/m_{\text{R}^{2+-}}) + \log (\gamma_{\text{R}^{2+}} \cdot \gamma_{\text{Cl}^-} / \gamma_{\text{R}^{2+-}}) \quad (2)$$

where:

$$\text{pWH} = F(E - E_0)/(RT \ln 10) + \log m_{\text{Cl}^-}.$$

The individual activity coefficients in the last term

* Part 7: Datta & Grzybowski (1958c).

of eqn. (2) may each be represented, up to $I = 0.1$, by the equation

$$-\log \gamma_i = z_i^2 A I^{\frac{1}{2}} + B_i I + C_i I^{\frac{3}{2}} \quad (3)$$

where z_i is the charge on the i th ion, A is the Debye-Hückel constant and B_i and C_i are arbitrary constants (Datta & Grzybowski, 1958a).

Substituting for $m_{\text{R}^{2+}}$ and $m_{\text{R}^{2+-}}$, we may set

$$Y_1 = \text{pWH} + \log [(m_2 - m_{\text{H}^+})/(m_1 - m_2 + m_{\text{H}^+})] \quad (4)$$

then, combining eqns. (2), (3) and (4), we obtain

$$pK_{1a} = Y_1 - 4A I^{\frac{1}{2}} - B_1 I - C_1 I^{\frac{3}{2}}. \quad (5)$$

The values of pK_{1a} , B_1 and C_1 were determined by fitting values of $Y_1 = (Y_1 - 4A I^{\frac{1}{2}})$ to the equation $Y_1' = pK_{1a} + B_1 I + C_1 I^{\frac{3}{2}}$, by the method of least squares.

In eqn. (4), and for the evaluation of I , m_{H^+} was calculated from the relation

$$m_{\text{H}^+} = \text{antilog} [-(\text{pWH} + 2 \log \gamma_{\pm \text{HCl}})].$$

The values of $\log \gamma_{\pm \text{HCl}}$ being found from an equation, of the form of eqn. (3), fitted the data of Bates & Bower (1954). An approximate value of I was used to find a preliminary value of m_{H^+} , I was then corrected and final values of I and m_{H^+} were found by iteration. Although the use of $\log \gamma_{\pm \text{HCl}}$ is to some extent arbitrary, since the medium effect of arginine is neglected, it is nevertheless one of the most reasonable approximations available. Any error introduced by it should largely disappear on extrapolation to infinite dilution.

Values of pK_{2a}

This dissociation constant refers to the equilibrium:

for the study of which the cell:

was used.

In these solutions 'complexing' of Ag^+ ions by the species $\text{R}^\pm$ occurs; this increases the solubility of the AgCl of the Ag, AgCl electrode, and consequently corrections, Δm_{Cl^-} and $\Delta m_{\text{R}^\pm}$, have to be applied to m_{Cl^-} and $m_{\text{R}^\pm}$. These corrections are given by

$$\Delta m_{\text{Cl}^-} = \frac{K_s m_{\text{R}^\pm}}{K_{11} m_{\text{Cl}^-}} \left[1 + \frac{m_{\text{R}^\pm}}{K_{21}} \right]$$

$$\text{and} \quad \Delta m_{\text{R}^\pm} = \frac{K_s m_{\text{R}^\pm}}{K_{11} m_{\text{Cl}^-}} \left[1 + \frac{2m_{\text{R}^\pm}}{K_{21}} \right]$$

(Datta & Grzybowski, 1958a), where K_s is the solubility product of AgCl calculated from eqn. (10) of Owen & Brinkley (1938) and K_{11} and K_{21} are the first and second instability constants of the silver arginine complexes (Datta & Grzybowski, 1959).

Consequently we have the following relations:

$$m_{\text{R}^+} + - = m_1 - m_2 + m_{\text{OH}^-},$$

$$m_{\text{R}^\pm} = m_2 - m_{\text{OH}^-} - \Delta m_{\text{R}^\pm},$$

$$m_{\text{Cl}^-} = m_1 + m_3 + \Delta m_{\text{Cl}^-}$$

and

$$I = m_1 + m_3 + \Delta m_{\text{Cl}^-} + m_{\text{OH}^-}.$$

The hydroxide-ion concentration was calculated from: $m_{\text{OH}^-} = \text{antilog} [\text{p}w\text{H} - \text{p}K_w]$, where $\text{p}K_w$ is the negative logarithm of the ion product of water, calculated from the equation given by Robinson & Stokes (1955).

From the equations for the e.m.f. of the cell and for the second dissociation of arginine, we get

$$\text{p}K_{2a} = \text{p}w\text{H} + \log (m_{\text{R}^+} + - / m_{\text{R}^\pm}) \\ + \log (\gamma_{\text{R}^+} + - \cdot \gamma_{\text{Cl}^-} / \gamma_{\text{R}^\pm}). \quad (7)$$

Substituting for $m_{\text{R}^+} + -$ and $m_{\text{R}^\pm}$ we may set

$$Y_2 = \text{p}w\text{H} + \log [(m_1 - m_2 + m_{\text{OH}^-}) / \\ (m_2 - m_{\text{OH}^-} - \Delta m_{\text{R}^\pm})]. \quad (8)$$

Then combining eqns. (7) and (8) and representing the activity coefficients by eqn. (3) again, we have

$$\text{p}K_{2a} = Y_2 - 2AI^{\frac{1}{2}} - B_2I - C_2I^{\frac{3}{2}}. \quad (9)$$

The values of B_2 and C_2 and $\text{p}K_{2a}$ were determined, as for $\text{p}K_{1a}$, by fitting values of $Y'_2 = (Y_2 - 2AI^{\frac{1}{2}})$ to the equation $Y'_2 = \text{p}K_{2a} + B_2I + C_2I^{\frac{3}{2}}$, by the method of least squares.

The method of extrapolation to $I = 0$ used here (eqns. 5 and 9) was selected because it had previously been used successfully for $\text{p}K_{2a}$ of glycine (Datta & Grzybowski, 1958a) and also because the constants A , B and C can be used to compute the 'apparent' $\text{p}K$ values at given ionic strengths.

METHODS

The apparatus and technique for the measurements in cells without liquid junction were, in general, the same as have been described previously (Ashby, Crook & Datta, 1954; Ashby, Clarke, Crook & Datta, 1955). Stable e.m.f. values were not obtained with alkaline arginine solutions when the hydrogen electrodes consisted of Pt foil coated with Pt black. When, however, the Pt foil was coated with Pd black, by electrolysis, the e.m.f. values obtained were both stable and reproducible. Consequently in all the experiments with arginine the Pt foil of the hydrogen electrodes was palladized by electrolysis in a solution of 3 g. of $\text{PdCl}_2 \cdot 2\text{H}_2\text{O}$ in 100 ml. of 0.4N-HCl, with a Pd anode, at approx. 0.2A/cm.² for about 5 min. Electrolysis was continued until the plates were evenly covered with a thin black coating of Pd.

L-Arginine hydrochloride was purified by conversion into benzylidenearginine and subsequent hydrolysis with bromine-free HCl [Org. Synth. (1943), Coll. Vol. 2, 49]. The arginine hydrochloride was recrystallized and dried *in vacuo* over P_2O_5 . All solutions used in the cells without liquid junction were collected and the arginine was recovered as benzylidenearginine. This material was converted into arginine hydrochloride and used in subsequent sets of experiments.

The purity of the arginine hydrochloride was estimated by gravimetric chloride determination, and in some instances also by potentiometric titration. In all specimens the purity was found to be between 99.5 and 100%; the impurity was considered to be water, the necessary corrections being made to the weights.

Constant-boiling HCl was prepared from AnalaR-grade concentrated acid, its concentration being determined from the mean atmospheric pressure at the time of distillation and checked by gravimetric chloride determinations as AgCl .

Carbonate-free KOH was prepared electrolytically from either AnalaR KCl or AnalaR KOH in a laboratory-scale Castner-Kellner cell. Its concentration was determined by titration against the constant-boiling HCl.

Potassium chloride (AnalaR) was freed from bromine by chlorination of a concentrated solution. It was reprecipitated by HCl gas, dried, ignited at 350° and finally fused in an atmosphere of dry N_2 .

The values of E_0 for each of the Ag-AgCl electrodes used were determined by measurements of the e.m.f. of cells of the type:

at 25°. The HCl solutions were approx. 0.05 molal and their activity coefficients were calculated from the parameters of eqn. (3) fitted to the values given in Table 3 of Bates & Bower (1954). The E_0 values at other temperatures were calculated by using Bates & Bower's temperature coefficient.

RESULTS

The values of $(E - E_0)$ observed, the molalities of the solutions used and of the extrapolation functions Y_1 and Y_2 calculated for $\text{p}K_{1a}$ and $\text{p}K_{2a}$ respectively are given in Tables 1 and 2. The values of the constants B and C of eqns. (5) and (9) and the

Table 1. First dissociation of arginine (pK_{1a}): variations of molalities (m) and ionic strengths (I) of solutions, $e.m.f.$ values of cell 1 ($E - E_0$) and extrapolation functions (Y_1)

m_1 = molality of arginine hydrochloride; m_2 = molality of HCl; $I = m_1 + 2m_2 - m_{H^+}$; ($E - E_0$) is given in absolute volts corrected to 1 atm. hydrogen pressure and rounded temperatures. The values of E_0 used were determined for each Ag-AgCl electrode by measurements in cell 10; $Y_1 = p_w H + \log(m_{H^+} + m_{H_2S} + -)$.

Temp.		Set 1										Set 2																																																																																																																																																																																																																																																																																																																																																																																																																					
		$10^2 m_1$	$10^2 m_2$	0.8892	1.303	1.647	2.539	2.985	3.365	3.604	3.616	3.878	4.406	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$	Y_1	$10^5 (E - E_0)$	$10^2 I$

Table 2. *Second dissociation of arginine (pK_{2a}): variations of molalities (m) and ionic strengths (I) of solutions, e.m.f. values of cell 6 (E - E₀) and extrapolation functions (Y₂)*

m_1 = molality of arginine hydrochloride; m_2 = molality of KOH; m_3 = molality of KCl; $I = m_1 + m_2 + \Delta m_{Cl^-} + m_{OH^-}$; (E - E₀) is given in absolute volts corrected to 1 atm. hydrogen pressure and rounded temperatures; the values of E₀ used were determined for each Ag-AgCl electrode by measurements in cell 10; $Y_2 = pWH + \log(m_2^{1/2} + -/m_2 + -)$.

Set 1											
	$10^2 m_1$	0.5180	0.5948	0.8189	0.9076	1.280	1.383	1.701	1.797	2.032	2.064
	$10^2 m_2$	0.2554	0.2954	0.4066	0.4475	0.6310	0.6869	0.8446	0.8860	1.009	1.018
	$10^2 m_3$	0.3542	0.3703	0.5483	0.6206	0.8751	0.9391	1.159	1.229	1.329	1.411
Temp.	$10^5(E - E_0)$	64 242	64 057	63 327	63 073	62 399	62 264	61 862	61 721	61 534	61 461
	$10^2 I$	0.8730	0.9660	1.368	1.529	2.156	2.324	2.861	3.027	3.362	3.476
	Y_2	9.8090	9.8127	9.8288	9.8360	9.8606	9.8622	9.8781	9.8826	9.8875	9.8946
10°	$10^5(E - E_0)$	64 853	64 660	63 906	63 645	62 944	62 801	62 386	62 243	62 051	61 974
	$10^2 I$	0.8731	0.9662	1.368	1.529	2.156	2.324	2.861	3.027	3.362	3.477
	Y_2	9.4998	9.5032	9.5195	9.5271	9.5512	0.5522	9.5685	9.5735	9.5789	9.5856
20°	$10^5(E - E_0)$	65 485	65 292	64 515	64 238	63 512	63 371	62 938	62 791	62 596	62 510
	$10^2 I$	0.8733	0.9664	1.369	1.529	2.156	2.324	2.861	3.027	3.363	3.477
	Y_2	9.2154	9.2199	9.2367	9.2433	9.2671	9.2692	9.2850	9.2900	9.2961	9.3017
30°	$10^5(E - E_0)$	66 134	65 940	65 136	64 850	64 101	63 957	63 509	63 351	63 155	63 066
	$10^2 I$	0.8735	0.9666	1.369	1.530	2.157	2.324	2.862	3.027	3.363	3.477
	Y_2	8.9526	8.9578	8.9745	8.9810	9.0050	9.0075	9.0232	9.0273	9.0342	9.0398
40°	$10^5(E - E_0)$	66 800	66 602	65 774	65 477	64 709	64 559	64 096	63 941	63 734	63 640
	$10^2 I$	0.8737	0.9667	1.369	1.530	2.157	2.324	2.862	3.028	3.363	3.477
	Y_2	8.7094	8.7149	8.7319	8.7381	8.7630	8.7652	8.7807	8.7862	8.7924	8.7978
50°	$10^5(E - E_0)$	67 475	67 275	66 424	66 112	65 333	65 179	64 691	64 544	64 326	64 227
	$10^2 I$	0.8738	0.9669	1.369	1.530	2.157	2.325	2.862	3.028	3.363	3.478
	Y_2	8.4826	8.4890	8.5062	8.5116	8.5382	8.5408	8.5548	8.5622	8.5676	8.5726
Temp.	$10^2 m_1$	2.388	2.377	2.594	2.886	3.105	3.592	3.868	4.388	4.880	
	$10^2 m_2$	1.186	1.172	1.288	1.423	1.531	1.771	1.908	2.164	2.406	
	$10^2 m_3$	1.562	1.625	1.679	1.974	2.123	2.456	2.645	3.000	3.337	
Temp.	$10^5(E - E_0)$	61 232	61 171	61 069	60 810	60 655	60 379	60 252	60 008	59 814	
	$10^2 I$	3.951	4.003	4.274	4.861	5.229	6.049	6.515	7.390	8.218	
	Y_2	9.9020	9.9024	9.9061	9.9200	9.9231	9.9354	9.9442	9.9538	9.9641	
10°	$10^5(E - E_0)$	61 737	61 670	61 566	61 297	61 137	60 850	60 716	60 460	60 261	
	$10^2 I$	3.951	4.003	4.275	4.862	5.229	6.049	6.516	7.391	8.219	
	Y_2	9.5929	9.5928	9.5967	9.6106	9.6137	9.6258	9.6343	9.6434	9.6539	
20°	$10^5(E - E_0)$	62 270	62 199	62 092	61 812	61 647	61 351	61 212	60 950	60 743	
	$10^2 I$	3.952	4.003	4.275	4.862	5.229	6.050	6.516	7.391	8.219	
	Y_2	9.3100	9.3095	9.3135	9.3272	9.3304	9.3427	9.3510	9.3606	9.3712	
30°	$10^5(E - E_0)$	62 819	62 745	62 634	62 346	62 176	61 870	61 725	61 456	61 242	
	$10^2 I$	3.952	4.004	4.275	4.862	5.230	6.050	6.516	7.391	8.219	
	Y_2	9.0484	9.0477	9.0517	9.0655	0.0689	9.0811	9.0892	9.0992	9.1098	
40°	$10^5(E - E_0)$	63 390	63 315	63 199	62 902	62 725	62 402	62 258	61 982	61 763	
	$10^2 I$	3.952	4.004	4.275	4.862	5.230	6.050	6.516	7.391	8.219	
	Y_2	8.8069	8.8064	8.8102	8.8243	8.8273	8.8386	8.8476	8.8578	8.8685	
50°	$10^5(E - E_0)$	63 971	63 899	63 776	63 473	63 295	62 952	62 809	62 527	62 291	
	$10^2 I$	3.952	4.004	4.276	4.862	5.230	6.050	6.517	7.392	8.220	
	Y_2	8.5822	8.5826	8.5858	8.6003	8.6042	8.6139	8.6236	8.6343	8.6436	
Set 2 ($m_2 = 0$)											
	$10^2 m_1$	0.9503	1.887	3.011	4.060	4.445	5.775	7.046	8.203		
	$10^2 m_3$	0.3152	0.6258	0.9987	1.346	1.474	1.915	2.337	2.721		
5°	$10^5(E - E_0)$	62 762	61 342	60 401	59 778	59 617	59 089	58 732	58 412		
	$10^2 I$	0.9508	1.888	3.012	4.061	4.447	5.776	7.048	8.205		
	Y_2	9.6560	9.6962	9.7284	9.7452	9.7554	9.7734	9.7950	9.8030		
15°	$10^5(E - E_0)$	63 336	61 862	60 887	60 250	60 083	59 543	59 161	58 842		
	$10^2 I$	0.9509	1.888	3.012	4.061	4.447	5.776	7.048	8.205		
	Y_2	9.3619	9.4015	9.4338	9.4520	9.4621	9.4813	9.5009	9.5110		
25°	$10^5(E - E_0)$	63 924	62 399	61 396	60 740	60 567	60 014	59 613	59 294		
	$10^2 I$	0.9510	1.888	3.012	4.061	4.447	5.777	7.048	8.205		
	Y_2	9.0898	9.1295	9.1626	9.1815	9.1915	9.2116	9.2303	9.2423		
35°	$10^5(E - E_0)$	64 522	62 947	61 918	61 240	61 066	60 496	60 079	59 757		
	$10^2 I$	0.9511	1.888	3.012	4.061	4.447	5.777	7.048	8.205		
	Y_2	8.8374	8.8770	8.9114	8.9302	8.9411	8.9615	8.9796	8.9929		
45°	$10^5(E - E_0)$	65 139	63 502	62 455	61 741	61 579	60 985	60 560	60 230		
	$10^2 I$	0.9512	1.888	3.012	4.061	4.447	5.777	7.049	8.205		
	Y_2	8.6038	8.6415	8.6783	8.6949	8.7086	8.7281	8.7471	8.7608		
55°	$10^5(E - E_0)$	65 760	64 080	62 998	62 290	62 104	61 490	61 052	60 717		
	$10^2 I$	0.9513	1.888	3.013	4.061	4.447	5.777	7.049	8.205		
	Y_2	8.3850	8.4241	8.4606	8.4813	8.4922	8.5115	8.5306	8.5450		

Table 3. *First dissociation of arginine: values used for the constants B_1 and C_1 of eqn. (5) and the values of pK_{1a}*

pK_{1a} (obs.) was obtained from eqn. (5) and pK_{1a} (calc.) from eqn. (11). pK_{1a} (calc.) = $\frac{1087.6}{T} - 4.7526 + 0.009819T$;
 T (min.) = 59.7°; pK_{1a} (min.) = 1.783. σ is the standard error (Please, 1954).

Set	Temp.	B_1	C_1	pK_{1a} (obs.)	pK_{1a} (calc.)
1	0°	+3.851	-5.383	1.9140	1.911
2	5	+3.349	-3.814	1.8847	1.889
1	10	+3.635	-4.533	1.8697	1.869
2	15	+3.245	-3.311	1.8488	1.851
1	20	+3.522	-4.102	1.8374	1.836
2	25	+3.143	-2.851	1.8217	1.823
1	30	+3.408	-3.560	1.8138	1.812
2	35	+2.985	-2.266	1.8013	1.803
	37	—	—	—	1.799
1	40	+3.249	-2.879	1.7995	1.795
2	45	+2.690	-1.242	1.7852	1.790
1	50	+3.100	-2.325	1.7871	1.786
2	55	+2.276	+0.274	1.7731	$\sigma = 0.003$

Table 4. *Second dissociation of arginine: values used for the constants B_2 and C_2 of eqn. (9) and the values of pK_{2a}*

pK_{2a} (obs.) was obtained from eqn. (9) and pK_{2a} (calc.) from eqn. (11). pK_{2a} (calc.) = $\frac{2643.6}{T} - 0.8783 + 0.003363T$.
 σ is the standard error (Please, 1954).

Set	Temp.	B_2	C_2	pK_{2a} (obs.)	pK_{2a} (calc.)
1	0°	-0.115	+1.876	9.7178	9.718
2	5	-0.110	+2.122	9.5626	9.561
1	10	-0.080	+1.935	9.4073	9.410
2	15	-0.052	+1.974	9.2669	9.265
1	20	-0.001	+1.778	9.1226	9.125
2	25	-0.012	+1.876	8.9936	8.991
1	30	+0.057	+1.691	8.8589	8.861
2	35	-0.004	+1.848	8.7390	8.737
	37	—	—	—	8.688
1	40	+0.043	+1.825	8.614	8.616
2	45	+0.152	+1.401	8.5040	8.501
1	50	-0.044	+2.180	8.3852	8.389
2	55	+0.019	+1.892	8.2824	8.281
					$\sigma = 0.003$

Table 5. *First dissociation of arginine: thermodynamic quantities*

σ is the standard error (Please, 1954).

Temp.	ΔG^0 (kJ/mole)	ΔH^0 (kJ/mole)	$-\Delta S^0$ (J/mole deg.)	$-\Delta C_p^0$ (J/mole deg.)
0°	9.994	6.80	11.7	103
5	10.057	6.28	13.6	105
10	10.130	5.75	15.5	106
15	10.212	5.21	17.4	108
20	10.303	4.67	19.2	110
25	10.404	4.11	21.1	112
30	10.514	3.55	23.0	114
35	10.634	2.97	24.9	116
37	10.685	2.74	25.6	117
40	10.763	2.39	26.7	118
45	10.902	1.79	28.6	120
50	11.049	1.19	30.5	122
σ_0	0.014	0.36	1.3	14
σ_{25}	0.008	0.10	0.4	15
σ_{50}	0.014	0.40	1.3	16

Table 6. *Second dissociation of arginine: thermodynamic quantities* σ is the standard error (Please, 1954).

Temp.	ΔG° (kJ/mole)	ΔH° (kJ/mole)	$-\Delta S^\circ$ (J/mole deg.)	$-\Delta C_p^\circ$ (J/mole deg.)
0°	50.821	45.81	18.4	35
5	50.914	45.63	19.0	36
10	51.011	45.45	19.6	36
15	51.111	45.26	20.3	37
20	51.214	45.08	20.9	38
25	51.320	44.89	21.6	38
30	51.430	44.69	22.2	39
35	51.542	44.50	22.9	40
37	51.588	44.42	23.1	40
40	51.658	44.30	23.5	40
45	51.777	44.09	24.2	41
50	51.900	43.89	24.8	42
55	52.025	43.68	25.4	42
σ_0	0.013	0.29	1.0	10
σ_{25}	0.007	0.08	0.3	11
σ_{50}	0.013	0.33	1.0	12

values of pK_{1a} and pK_{2a} [pK_a (obs.)] calculated from these equations are given in Tables 3 and 4 respectively. In these tables are also given the values of pK_a calculated from Harned & Robinson's (1940) equation

$$pK = A/T - D + CT. \quad (11)$$

These are designated pK_a (calc.). The values of the constants of eqn. (11) were obtained by the method of least squares, the data from both sets of observations for each pK_a being combined.

The thermodynamic quantities for both dissociations of arginine have been calculated from the parameters of eqn. (11); they are given in Tables 5 and 6.

DISCUSSION

The values of both pK_{1a} and pK_{2a} for arginine are lower than those for the corresponding ionizations of simple monoamino monocarboxylic acids. Further, the differences between arginine and glycine are constant over a wide temperature range (Fig. 1); similar results are obtained when arginine is compared with α -amino-*n*-valeric acid (Smith, Taylor & Smith, 1937), the pK_a values of which are very close to those of glycine.

Since the magnitude of a pK_a value in aqueous solution is a direct indication of the free-energy change (ΔG°) on transferring a proton from an acid molecule to a water molecule, a discussion of the differences in pK_a values between acids resolves itself into a discussion of the corresponding thermodynamic quantities.

It is useful to consider the thermodynamic quantities associated with an ionization as arising from two sources: first, the environmental part due to the interaction of the reacting species and the products with the solvent water which we shall

Fig. 1. Temperature-dependence of the thermodynamic pK_a values of glycine (A) and arginine (B). Values of pK_{1a} for glycine are from King (1951) and of pK_{2a} from Datta & Grzybowski (1958a).

term ΔG° (env.), ΔH° (env.), ΔS° (env.) and ΔC_p° (env.); secondly, the non-environmental part due to the differences in the binding energy of the proton in the acid and in the hydronium ion (enthalpy) and to the intramolecular rearrangements occurring in the ionization of the acid molecule (entropy).

The latter part also includes a contribution arising from changes in the internal polarization of the molecule which is assumed to be invariant with temperature (cf. the general terms of eqn. 23). These non-environmental parts we shall term ΔG^0 (non.), ΔH^0 (non.) and ΔS^0 (non.). It is likely that ΔH^0 (non.) and ΔS^0 (non.) would remain invariant with temperature, while the environmental parts would reflect the changes in the properties of water with temperature. It follows, therefore, that ΔC_p^0 must arise entirely from environmental factors.

The environmental parts of the thermodynamic quantities are due principally to two effects (Datta & Grzybowski, 1958b): (a) the electrostatic effect of the reacting ions on the water molecules; (b) the stabilization of regions of ice-like structure in the water by large non-polar substituents in the reacting molecules (Frank & Evans, 1945). This latter effect is likely to be important only when the non-polar substituent is very close to the ionizing group, as in the methylammonium ions, sarcosine and *NN*-dimethylglycine; it is unlikely to be important in arginine. We may therefore assume that the electrostatic effect is largely, if not solely, responsible for the environmental parts of the thermodynamic quantities, which may therefore be evaluated from electrostatic theory as follows.

The electrostatic free-energy change on placing an ion, or ions, in solution is inversely proportional to the dielectric constant of the solvent (D); hence, for the ionization of arginine or glycine,

$$\Delta G^0 \text{ (env.)} = p/D, \quad (12)$$

where p is a constant. Consequently, we have the relations:

$$\Delta S^0 \text{ (env.)} = -p[d/dT(1/D)] \quad (13)$$

$$\Delta H^0 \text{ (env.)} = p[1/D - Td/dT(1/D)] \quad (14)$$

$$\text{and } \Delta C_p^0 \text{ (env.)} = \Delta C_p^0 = -p[Td^2/dT^2(1/D)]. \quad (15)$$

The variation with temperature of $1/D$ can be expressed by the relation:

$$1/D = a - bT - cT^2 \quad (16)$$

where, for the experimental values of Malmberg & Maryott (1956),

$$a = 8.5756 \times 10^{-3}, \quad b = 2.9915 \times 10^{-5}$$

$$\text{and} \quad c = 1.4741 \times 10^{-7}.$$

The first and second differentials with temperature of $1/D$ can easily be found from these parameters.

The value of p for any particular ion may be calculated by using an appropriate electrostatic model; the electrostatic contribution to the thermodynamic quantities for a given ionization may be found from a value of p which is the sum of values for the products less the sum of the values for the reactants. Thus for arginine we have

1st ionization:

$$p_{1 \text{ Arg}} = p_{\text{H}_3\text{O}^+} + p_{\text{Arg}^{2+}} - p_{\text{Arg}^{3+}} \quad (17)$$

and 2nd ionization:

$$p_{2 \text{ Arg}} = p_{\text{H}_3\text{O}^+} + p_{\text{Arg}^{2+}} - p_{\text{Arg}^{3+}}. \quad (18)$$

Similarly for glycine:

1st ionization:

$$p_{1 \text{ Gly}} = p_{\text{H}_3\text{O}^+} + p_{\text{Gly}^{2-}} - p_{\text{Gly}^{3-}} \quad (19)$$

and 2nd ionization:

$$p_{2 \text{ Gly}} = p_{\text{H}_3\text{O}^+} + p_{\text{Gly}^{2-}} - p_{\text{Gly}^{3-}}. \quad (20)$$

The value of $p_{\text{H}_3\text{O}^+}$ was obtained from the Born (1920) equation

$$p_{\text{H}_3\text{O}^+} = N\epsilon^2/2r_{\text{H}_3\text{O}^+} \quad (21)$$

where N is Avogadro's number, ϵ the electronic charge and $r_{\text{H}_3\text{O}^+}$ the ionic radius (cm.) of the hydronium ion, taken to be 0.92 Å (Datta & Grzybowski, 1958b). The values of $p_{\text{Gly}^{2-}}$ and $p_{\text{Gly}^{3-}}$ were also found from eqn. (21), assuming the ions to be spherical, the radius of the $\text{X} \cdot \text{CO} \cdot \text{O}^-$ ion being taken as 1.23 Å (Cohn, 1935) and that of the $\text{X} \cdot \text{NH}_3^+$ ion as 1.4 Å, the same as that of the ammonium ion (Kapustinsky, 1941). For the glycine dipolar ion, $p_{\text{Gly}^{2-}}$ was found from the equation of Scatchard & Kirkwood (1932), which assumes the molecule to be dumb-bell shaped, the term $1/2r$ in eqn. (21) being replaced by $(1/b - 1/R)$, where b is the radius of each charged group and R is the intercharge distance. The value of b was taken to be 1.315 Å, the mean of the radii of the carboxylate and the ammonium ions, and R was taken as 3.17 Å (Cohn, 1935).

The values of $p_{\text{Arg}^{2+}}$, $p_{\text{Arg}^{3+}}$ and $p_{\text{Arg}^{2-}}$ were obtained from a modification of Kirkwood's equation (1934), in which the multivalent ion is treated as a sphere of radius b and internal dielectric constant D_i , with point charges $e_1, e_2, \dots, e_m$ at distances $r_1, r_2, \dots, r_m$ from its centre; the angle between r_k and r_l is θ_{kl} . The work of charging such an ion at infinite dilution in water is given by:

$$W_0 = \frac{1}{2} \sum_{n=0}^{\infty} \frac{(n+1) Q_n (D_i - D)}{D_i \cdot b^{(2n+1)} [(n+1) D + n D_i]} \quad (22)$$

$$\text{where } Q_n = \sum_{k=1}^m \sum_{l=1}^m e_k e_l r_k^* r_l^* \text{Pn}(\cos \theta_{kl}),$$

in which $\text{Pn}(\cos \theta_{kl})$ etc. are Legendre functions. Eqn. (22) rearranges to

$$W_0 = \frac{1}{2} \sum_{n=0}^{\infty} \frac{(n+1) Q_n}{b^{(2n+1)} [(n+1) D + n D_i]} - \frac{(n+1) Q_n}{D_i \cdot b^{(2n+1)} (n+1 + n D_i/D)}. \quad (23)$$

In eqn. (23) the terms involving D_i may be ignored, since they arise from the effect of the electrostatic field on the internal environment of the ion, which should be largely temperature-independent. Thus we obtain the function W'_0 , which can be used to

find the temperature-dependence of the environmental part of the thermodynamic functions.

$$W'_0 = \frac{1}{2} \sum_{n=0}^{\infty} \frac{Q_n}{b^{(2n+1)}} \cdot \frac{1}{D}. \quad (24)$$

Hence p_i (eqn. 12) for any ion is given by

$$p_i = \frac{1}{2} \sum_{n=0}^{\infty} \frac{Q_n}{b^{(2n+1)}} = \frac{1}{2} z_i^2 \sum_{k=1}^m \sum_{l=1}^m \frac{mbe^2}{b^2 - r^2} + \frac{e_k e_l}{b} \frac{1}{\left(1 - 2 \cos \theta_{kl} \frac{r^2}{b^2} + \frac{r^4}{b^4}\right)^{\frac{1}{2}}} \quad (25)$$

the assumption being made that $r = r_k = r_l$.

The values of r , b and θ_{kl} for arginine were assigned from measurements of a molecular model (Catalin Ltd., Waltham Abbey, Essex). The distance between any two charges was taken as the mean of the maximum and minimum possible values, and from the three interchange distances in R^{2+-} the value of r was calculated. The radius of the sphere, b ($b - r$ = charge depth), was computed in such a way that the charge-solvent interaction

Fig. 2. Intercharge distances in arginine.

Table 7. Environmental and non-environmental parts of the thermodynamic quantities for the first and second dissociations of arginine and glycine, together with the observed values at 25°

p is the electrostatic constant for the various dissociations (eqns. 17-20). The observed values for the first dissociation of glycine are from King (1951) and for the second dissociation from Datta & Grzybowski (1958b).

	$10^{-5}p$	ΔG^0 (env.)	ΔG^0 (non.) (kJ/mole)	ΔG^0 (obs.)	ΔH^0 (env.)	ΔH^0 (non.) (kJ/mole)	ΔH^0 (obs.)	ΔS^0 (env.)	ΔS^0 (non.) (J/mole deg.)	ΔS^0 (obs.)	ΔC_p^0 (env.)	ΔC_p^0 (obs.)
Arginine	7.06	+9.01	+1.39	+10.40	-3.2	+7.3	+4.1	-40.9	+19.8	-21.1	-62	-112
Glycine	9.844	+12.56	+0.86	+13.42	-4.5	+8.5	+4.0	-57.1	+25.5	-31.6	-87	-144
Arginine-glycine	—	-3.55	+0.53	-3.02	+1.3	-1.2	+0.1	+16.2	-5.7	+10.5	+25	+32
Arginine	4.14	+5.30	+46.02	+51.32	-1.9	+46.8	+44.9	-24.0	+2.4	-21.6	-36	-38
Glycine	7.016	+8.95	+46.86	+55.81	-3.2	+47.4	+44.2	-40.7	+1.8	-38.9	-62	-46
Arginine-glycine	—	-3.65	-0.84	-4.49	+1.3	-0.6	+0.7	+16.7	+0.6	+17.3	+26	+8

energy, for each charge treated separately, should be the same as for a corresponding singly-charged spherical ion of approximate radius r_i . Thus:

$$\frac{1}{2} \frac{z^2 e^2 b}{b^2 - r^2} \cdot \frac{1}{D} = \frac{1}{2} \frac{z^2 e^2}{r_i} \cdot \frac{1}{D} \quad (26)$$

hence
$$\frac{b}{b^2 - r^2} = \frac{1}{r_i}.$$

The three values of b obtained were averaged. The value of r_i for $-\overset{+}{N}H_3$ and for $-\overset{+}{N}H-(NH_2)_2$ was taken as 1.40 Å, the same as for the ammonium ion (Kapustinsky, 1941), and for $-\overset{-}{C}O \cdot O^-$ it was taken as 1.23 Å (Cohn, 1935). From these parameters r for the arginine sphere was calculated to be 3.62 Å (Fig. 2) and b 4.35 Å. For the other species it was assumed that the disappearance of any charge would not alter the relative distance or position of the remaining charges.

The calculated values of p and both the environmental and non-environmental thermodynamic quantities are shown in Table 7. These values show that for the first dissociations of arginine and glycine almost the entire free-energy term arises from environmental factors. When, however, the individual enthalpy and entropy terms are examined, it is seen that there are substantial contributions from the non-environmental parts, but these almost entirely cancel out. The calculated and observed values of ΔC_p° do not agree very well but are nevertheless of the same sign. Further, almost the entire difference between the arginine and glycine pK_1 values resides in the entropy terms, the enthalpies being very similar.

For the second dissociations of these amino acids Table 7 shows a great similarity in the observed enthalpy terms, which arise almost entirely from the non-environmental parts. The agreement between the observed and calculated differences in ΔC_p° is not, however, quite so good as for the first dissociation. Again, for the second dissociation the major difference in the free-energy terms arises from the environmental parts of the entropy terms.

The implications of these results are: First, that the bond energies of a proton in an amino (or a carbonyl) group in glycine and arginine are virtually identical (non-environmental enthalpy changes). This may well be true for all α -amino acids (Edsall & Wyman, 1958). Secondly, it would appear that the major part of the differences between the dissociations of glycine and arginine is due to differences in the electrostatic interactions of the conjugate acid-base pairs (of different ionic structure) and the solvent water.

SUMMARY

1. The first and second dissociation constants of arginine have been determined over the range 0–55° at 5° intervals, from e.m.f. measurements of

cells without liquid junction containing hydrogen and silver-silver chloride electrodes.

2. The standard thermodynamic quantities associated with these dissociations have been calculated. These quantities have been divided into environmental and non-environmental parts.

3. The various factors which contribute to the differences between the first and second dissociation constants of arginine and glycine have been discussed. It is concluded that the pK_{1a} and pK_{2a} values of arginine are lower, by a constant amount, than those of glycine, mainly because of the differences in the electrostatic interactions of the various ionic conjugate acid-base pairs with the solvent water.

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