Thermodynamic Considerations in Co-ordination. Part XI.[1] Enthalpies and Entropies of protonating Asparaginyl, Aspartyl, Cysteinyl, and Phenylalanyl Anions

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Calorimetrically determined enthalpies and potentiometrically determined Gibbs free energies of protonation of asparaginyl, aspartyl, cysteinyl, and phenylalanyl anions at 25·0 °C and $I = 3{\cdot}00$M-(Na)ClO_4 are reported. The results are compared with those for similar studies (*a*) of these amino-acids at other ionic strengths and (*b*) of other amino-acids studied at $I = 3{\cdot}00$M.

We report the standard enthalpies and entropies for protonating the anions of four biologically important amino-acids, asparagine, aspartic acid, cysteine, and phenylalanine. Our results refer to adding protons to carboxylate, primary amine, and sulphide groups and were measured in 3M-(Na)ClO_4 at 25 °C. Protonation thermodynamics for these amino-acids have not been reported previously in this medium although some values are available for other ionic strengths.[2] Nevertheless, comparable results are available for related species since we have already reported protonation thermodynamics for hydroxymethylimidazoles, acetate, histidyl$^-$(hist), tryptophyl$^-$, Ln(hist)$^{2+}$, Cu(hist)$^+$, Cu(hist)$_2$, and Cu(hist)$_2$H$^+$ in perchlorate solutions at 25 °C.[1]

EXPERIMENTAL

Bases.—Commercial amino-acids were dried and used without further purification: L-(−)-Asparagine,H_2O (B.D.H., Biochemical grade; m.p. 233—235 °C; lit., 235 °C) (Found: C, 31·9; H, 7·0; N, 18·5. Calc. for $C_4H_{10}N_2O_4$: C, 32·0; H, 6·7; N, 18·7%); L-(+)-aspartic acid (B.D.H., Biochemical grade; m.p. 270 °C; lit., 270—271 °C) (Found: C, 35·6; H, 5·1; N, 10·2. Calc. for $C_4H_7NO_4$: C, 36·1; H, 5·3; N, 10·5%); L-(+)-cysteine (E. Merck, Biochemical grade; m.p. 220 °C; lit., 217—228 °C) (Found: C, 29·6; H, 5·9; N, 11·2. Calc. for $C_3H_7NO_2S$: C, 29·7; H, 5·8; N, 11·6%); L-(−)-phenylalanine (B.D.H., Biochemical grade; m.p. 284 °C; lit., 283—284 °C) (Found: C, 65·2; H, 6·9; N, 8·6. Calc. for $C_9H_{11}NO_2$: C, 65·4; H, 6·7; N, 8·5%). Other reagents, the potentiometric procedure, and computational analysis were as previously described.[3] The calorimetry was as described in ref. 3 with the following exceptions. (i) the phenylalanyl results were obtained with the thermistor detector calorimeter [4] rather than the quartz thermometer model; (ii) the heat of carboxylate protonation for asparagine was also measured with an LKB batch microcalorimeter; [5] and (iii) in addition to the more usual potentiometric and calorimetric approach, the pK and $\Delta H°$ of carboxylate protonation for cysteine were measured by the entropy titration approach,[6-8] a procedure that produces both $\Delta G°$ and $\Delta H°$ (and thus the entropy change $\Delta S°$) simultaneously from the same calorimetric titration results. Additional checks (ii) and (iii) were introduced because the carboxylate pK values occur at the end of the working range of glass electrodes.

The mathematics of our version of the entropy titration method are as follows: For the nth point in a titration of a monobasic ligand, if A and H represent the total, and a and h the free, concentrations of ligand and hydrogen ions respectively, [AH] the concentration of protonated ligand, V_n the total volume (in l) at point n, and Q_n the heat change in the system up to point n, the formation constant (β) and the enthalpy of formation ($\Delta H°$) are related to these terms through the relationships (1)—(7), leading to (8).

$$\beta = [AH]/ah \quad (1)$$

$$A = a + [AH] \quad (2)$$

$$H = h + [AH] \quad (3)$$

$$Q_n = \Delta H°[AH]V_n \quad (4)$$

$$\therefore\ Q_n/\beta = \Delta H°V_n(A - [AH])(H - [AH]) \quad (5)$$

$$= \Delta H°V_n\{AH - [AH](A + H) + [AH]^2\} \quad (6)$$

$$\therefore\ = \Delta H°V_n[AH - Q_n(A + H)/\Delta H°V_n + Q_n^2/(\Delta H°V_n)^2] \quad (7)$$

$$\therefore\ \Delta H°/\beta = \Delta H°^2V_nAH/Q_n - \Delta H°(A + H) + Q_n/V_n \quad (8)$$

The final expression (8) contains only two unknown parameters, $\Delta H°$ and β. These are, at first, estimated from pairs of titration-point equations of the form (8) and then these approximate values are refined by a least-squares method designed to minimize the error square sum (9) for s points of the titration. The final value is

$$U = \sum_{n=1}^{s} (Q_{\text{guessed}} - Q_n)^2 \quad (9)$$

approached by use of Newton's iterative procedure. Representative experimental points are plotted in Figure 1 and the enthalpic curves have been calculated by use of $\Delta H°$ values from the Table. The errors quoted are three times the computed standard deviations.

DISCUSSION

pK *Considerations.*—For the anions asparaginyl, aspartyl, and phenylalanyl, the first pK values quoted (9·30, 10·01, and 9·61) refer to protonating the primary amine site and the second pK values refer to protonating

[1] Previous parts of this series are IV = *Acta Chem. Scand.*, 1967, **21**, 341; VI, VII, VIII, IX, and X are *J. Chem. Soc.* (*A*), 1968, 2965; 1970, 1550; 1970, 3138; 1971, 3159; and *J.C.S. Dalton*, 1972, 790, respectively.

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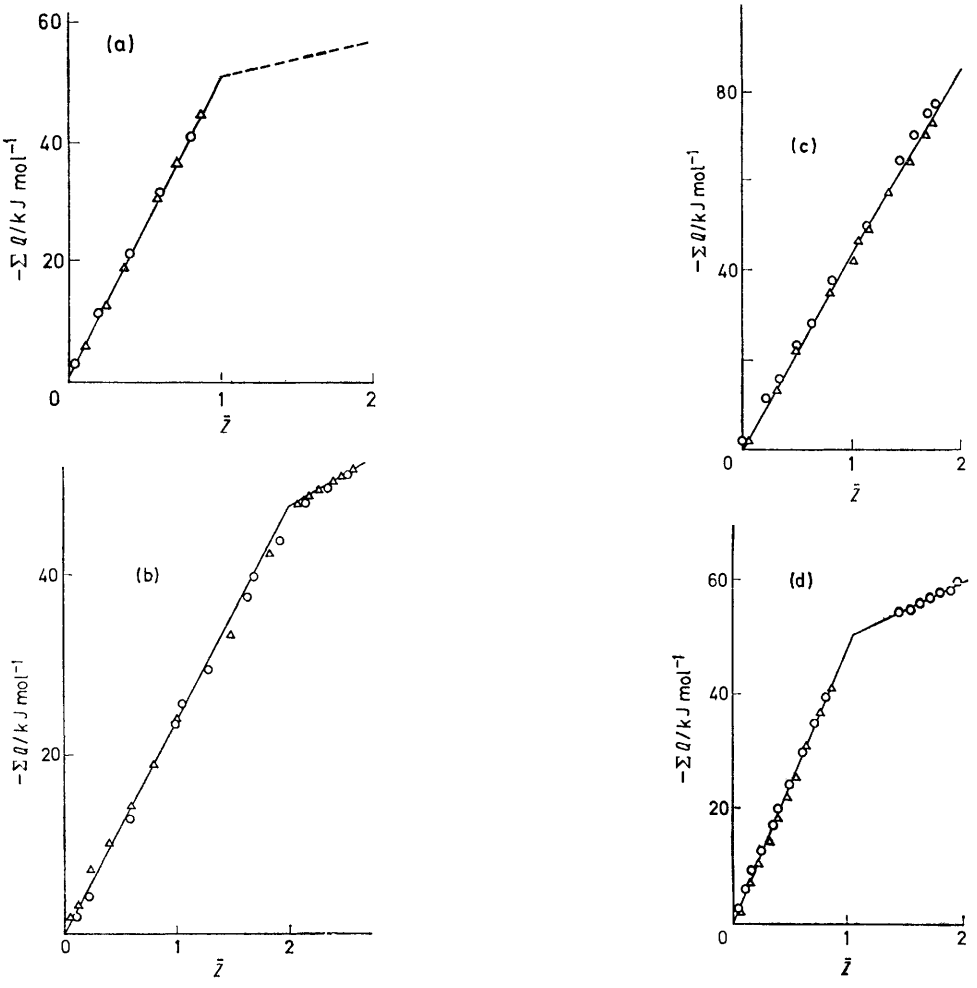

FIGURE 1 Enthalpic curves for the protonation of amino-acids at 25·0 °C, I = 3·00M-(Na)ClO_4. $\bar{Z}$ is the average number of protons per base. Different concentration conditions are represented by different symbols; (a) asparaginyl, (b) aspartyl, (c) cysteinyl, (d) phenylalanyl

Thermodynamic parameters for protonating amino-acids at 25 °C and I = 3·00M-(Na)ClO_4. n Denotes the number of experimental observations used to calculate each set of enthalpies

Base group protonated	pK	$-\Delta G°$ / kJ mol^{-1}	$-\Delta H°$ / kJ mol^{-1}	$\Delta S°$ / J mol^{-1} K^{-1}	n
Asparaginyl					
$-NH_2$	9·303 ± 0·018	53·10 ± 0·10	50·5 ± 0·4	8·9 ± 1·0	24
$-CO_2^-$	2·586 ± 0·022	14·76 ± 0·13	1·5 ± 3·5	*ca.* 44	
$-CO_2^-$ (microcal)			5·10 ± 0·05	32·4 ± 0·6	
Aspartyl					
$-NH_2$	10·007 ± 0·028	57·12 ± 0·16	23·6 ± 1·5	112·0 ± 5·3	57
$-CO_2^-$	4·067 ± 0·034	23·21 ± 0·19	23·9 ± 0·6	−2·3 ± 2·6	
$-CO_2^-$	2·345 ± 0·036	13·39 ± 0·21	7·3 ± 0·1	20·4 ± 1·0	
Cysteinyl					
$-S^-$	10·709 ± 0·030	61·13 ± 0·18	40·4 ± 1·0	69·5 ± 3·9	31
$-NH_2$	8·784 ± 0·040	50·12 ± 0·23	38·8 ± 1·5	38·0 ± 5·8	
$-CO_2^-$	2·4 ± 0·3	13·7 ± 1·7	−1·4 ± 1·5	50·6 ± 10·7	
$-CO_2^-$ (entropy titration)	2·44 ± 0·09	13·93 ± 0·6	0·3 ± 0·6	45·6 ± 4·0	12
Phenylalanyl					
$-NH_2$	9·610 ± 0·002	54·84 ± 0·01	50·40 ± 0·13	14·9 ± 1·0	49
$-CO_2^-$	2·754 ± 0·011	15·72 ± 0·06	9·74 ± 0·50	20·1 ± 1·8	

a carboxylate group. For cysteinyl, it is the third pK value that refers to the carboxylate protonation. However, for the other cysteinyl pK values (10·71 and 8·78) and for the aspartyl pK values (4·07 and 2·34) it is not

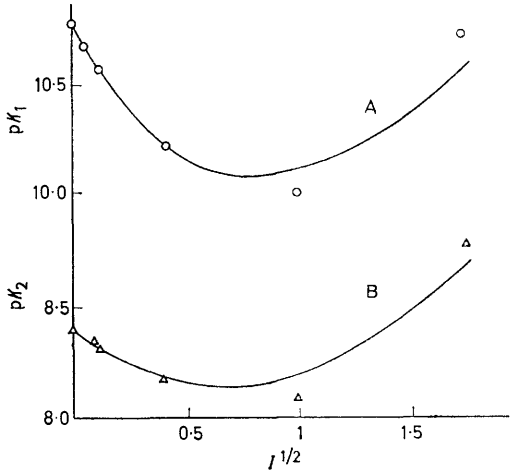

FIGURE 2 Plots of pK against $I^{\frac{1}{2}}$ for cysteine. Values of $I \neq 3{\cdot}00$M are from ref. 2. The curves shown are drawn for A, $\log K_1^\circ = \log K_1 - \log f_2$ ($b = -0{\cdot}0180$), and B, $\log K_2^\circ = \log K_2 - 2\log f_1$ ($b = -0{\cdot}0140$)

possible definitely to assign a value to that of protonating any one site since some concurrent occupation between $-S^-$ and $-NH_2$ and between carboxylate sites must occur. Nevertheless, aspartyl has donor oxygens separated by four other atoms and it appears that the 4·07 pK refers to adding protons predominantly to a carboxylate group such as occurs in acetate (4·52) [2] and the 2·34 pK to protonating a carboxylate group that is α to an amine group such as occurs in histidyl (2·28) [2] or phenylalanyl (2·75). On the other hand, when the first proton is added to cysteinyl it has an almost equal chance of occupying either site since comparable amino-acid amines and simple thiols have similar pK values (usually in the range 9·5—10·5), or possibly of being concurrently suspended between both sites since the cysteinyl donor atoms are closer than in aspartyl, being separated by only two other atoms.

As in previous papers our 3·00M-ClO_4^- constants are higher than those values reported in the literature for lower ionic strengths (I). Examples of plots of $\log K$ against $I^{\frac{1}{2}}$ are shown in Figure 2 and appear to follow the lines dictated by the Guggenheim extension to the Debye–Hückel equation (10) where z = charge and f = activity coefficient.

$$-\log f_z = 0{\cdot}5115 Z_+ Z_- I^{\frac{1}{2}}/(1 + I^{\frac{1}{2}}) - bI \quad (10)$$

Thermodynamic Parameter Considerations.—Øjelund and Wadsö have noted that thermodynamic data for aqueous solutions need to be interpreted in terms (*a*) of changes in solute–solvent interactions and (*b*) of changes in the parameters of the acid–base system currently under investigation.[9]

(*a*) In terms of simple electrostatics, solvation contributions will clearly be different for carboxylate or for sulphide protonation, $RCO_2^- + H^+ \rightleftharpoons RCO_2H$ or $RS^- + H^+ \rightleftharpoons RSH$, than for amine protonation, $RNH_2 + H^+ \rightleftharpoons RNH_3^+$, because for the latter the number of formal charges present does not change.

(*b*) Attempts have been made to relate ΔG°, ΔH°, and ΔS° for carboxylate protonation in terms of the extent of alkyl substitution,[10] and for aliphatic amino-protonation in terms of the numbers and positions of carbons and other amino-groups present.[11] However, the structures of the four amino-acids currently being reported are not related to each other in a stepwise manner and so correlations between ΔH° and ΔS° values and changes in acid–base parameters must be mainly qualitative. We shall consider the protonation of carboxylate and amine groups in turn.

Carboxylate. ΔG° (or pK) is essentially entropy-dependent.[12] Hansen *et al.* suggested that $RCO_2^-H^+$ exist in aqueous solution as an ion pair and so ΔH° approximates to zero and is not dependent upon the nature of R. Thus, protonation is an electrostatic phenomenon and this is reflected in ΔS° because the number of water molecules involved depend far more upon the charges on the ions concerned than upon the

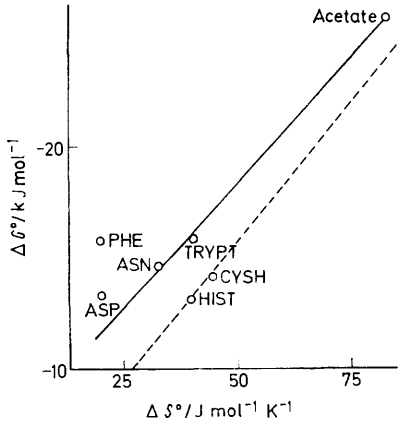

FIGURE 3 Plots of ΔG° against ΔS° for protonating carboxylate groups in perchlorate solutions. The full line has a slope of -218 K; the broken line is that reported in ref. 13 and has a slope of -243 K; ASN = asparaginyl, PHE = phenylalanyl; TRYPT = tryptophyl; CYSH = cysteinyl; ASP = aspartyl; and HIST = histidinyl

variety of groups being protonated. Christensen and Izatt [13] produced evidence for such concepts in the form of linear plots of ΔG° against ΔS° (I = 0M). The slope of their plots was significantly close to that predicted by Bjerrum's theory of electrostatic interactions (-243 *versus* -218). Although for 3M-(Na)ClO_4 we have far fewer data, Figure 3 (i) shows that our parameters also can be said to lie near the line of slope -218, and (ii)

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reinforces our pK assignment for the two aspartate carboxylates (pK = 4·07 is aliphatic and pK = 2·34 is amino-acid) because the plot of $\Delta G°$ against $\Delta S°$ for the lower pK of aspartate falls amidst those for other amino-acids.

Amino and Imino.—Protonating amines may be contrasted to protonating carboxylate groups because (i) amine protonation is an enthalpy-dependent process, (ii) substituents adjacent to the amines have more noticeable $\Delta H°$ effects, and (iii) whereas $-CO_2^-$ H^+ interactions are charge–charge, $-NH_2$ H^+ are mainly charge–dipole and so electrostatic contributions to the entropies of protonation are frequently masked by large substituent effects.

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