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AQUEOUS SOLUTIONS CONTAINING AMINO ACIDS AND PEPTIDES. PART 25. THE ENTHALPY OF INTERACTION AT 298.15 K OF GLYCINE WITH POTASSIUM HALIDES

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ABSTRACT

A study of the enthalpy of interaction between the potassium halides (KF, KBr and KI) and glycine is described. The results obtained are compared with those reported earlier for glycine interacting with alkali metal chlorides. Qualitative differences are evident between the cationic and anionic series. Using the Kirkwood electrostatic model for the interaction between ions and dipoles, the electrostatic contributions to the enthalpic parameters have been estimated. The marked discrepancies between these and the experimental quantities are briefly discussed.

INTRODUCTION

It is well known that salts can have marked effects on the stability of protein structures, and that some electrolytes have a tendency to disrupt some, at least, of the structural features of proteins whereas other electrolytes show a propensity to buttress such structures [1]. If one considers the monoatomic cations then it is generally found that small, highly charged ions tend to denature (i.e. remove structural regularities) proteins, with consequent loss of biological activity [2]. In contrast to this it is found for monoatomic anions that it is the larger ions which are the most potent at inducing denaturation and the fluoride ion tends to stabilize tertiary and secondary structures in proteins. One other broad qualitative feature which is found is that the range of discrimination shown by the halide ions is much greater than that shown by the alkali metal cations. The molecular reasons for these observations are presently unknown although there is some evidence [3] which indicates that the major effects of salts on protein structures arise primarily through interactions occurring between the ions and the peptide groups in the backbone of the protein. It also seems reasonable that there should be interactions occurring between ions and other polar and ionic groups on the macromolecules. However in contrast, there is opposing evidence [4] which seems to indicate that the discriminatory effects of salts

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on proteins arise principally from the interaction of the ions with hydrophobic groups in the amino acid side chains.

In some of the previous work in this series on the interactions occurring between amino acids or peptides with salts, one objective was to obtain information on the ways in which such interactions varied with the nature of the cation. In the studies of glycine with alkali metal chlorides [5], we deliberately chose the amino acid which is generally recognised as being the most hydrophilic so that any interactions with ions would be maximised. It is fair to say that at the outset the expectation was that rather marked differences would be observed in the extent of interaction as the cationic size was increased, with the lithium salt interacting most effectively. The enthalpies and entropies of interaction did indicate that there is a marked difference at the molecular level between how the lithium ion interacts with glycine compared to the interactions exhibited by the other cations with glycine. It was experimentally observed, however, that the free energy of interaction of these salts with glycine changed little from one salt to another and that there was no indication of any monotonic variation with cationic size. One other feature which became apparent from this work was that the electrostatic approach developed by Kirkwood [6] was inadequate to explain the results obtained for the enthalpic components of the interactions. In view of the above comments and because of the resurgence of interest in the use of electrostatic models to explain some of the properties of protein structures [7,8], we felt it would be both useful and timely to investigate the enthalpic behaviour of aqueous solutions containing potassium halides and glycine.

EXPERIMENTAL

All solutions were made with deionised water. AR grade glycine was recrystallised from water and stored over P_2O_5 prior to use. The KBr and KI used were the purest materials available commercially and were used without further treatment after storage over P_2O_5. The KF was zone refined material.

The enthalpy of mixing measurements were obtained using a LKB batch microcalorimetric system [9], which was modified so that the output from the thermopiles, after amplification, was digitised to facilitate analysis using a microcomputer. In the experiments one cell was loaded with salt and glycine solutions whilst the other cell, which was in thermal opposition to the first, contained salt solution and water.

The excess enthalpy (H^{ex}) of a solution containing 1 kg of solvent, an electrolyte KX at molality m and a non-electrolyte A of molality m_A is given by [10]

$$H^{ex} = mH^{ex,0}_{m,KX}(m) + m_A H^{ex,0}_{m,A}(m_A) + 2mm_A(h_{KA} + h_{XA}) + 3m^2 m_A(h_{KKA} + 2h_{KXA} + h_{XXA}) + 3mm_A^2(h_{KAA} + h_{XAA}) + \ldots \quad (1)$$

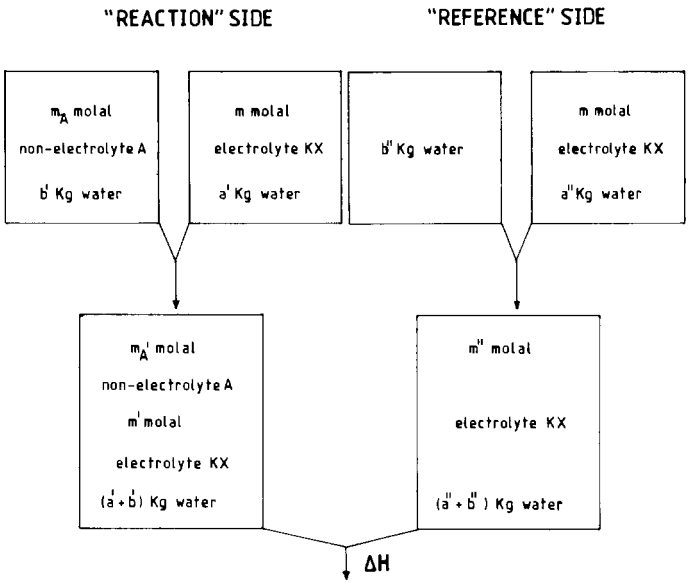

Fig. 1. A schematic illustration of a typical heat of mixing experiment.

In this equation $H^{ex,0}_{m,KX}(m)$ and $H^{ex,0}_{m,A}(m_A)$ are the molar excess enthalpies of single solute solutions containing the salt at molality m, and the non-electrolyte at molality m_A, respectively. The coefficients h_{ijk} are the enthalpic virial coefficients representing the heterotactic interactions [11] between the subscripted species. The experimental data were obtained from experiments in which approximately equimolar solutions of salt and glycine were mixed in the calorimeter and where most of the heat contribution arising from the dilution of the salt was annulled by simultaneously diluting in the calorimeter approximately the same amount of salt solution with a suitable amount of water (see Fig. 1). The experimental enthalpy change (ΔH) consequently consists of contributions from (a) the interaction of the electrolyte with glycine, (b) the dilution of the glycine, and (c) a residual contribution from the dilution of the electrolyte. Since it is the first of these with which we are concerned it is convenient to define a term (ΔH^{inter}) in which the superfluous terms are subtracted. The resulting expression is [5]

$$\begin{aligned}\Delta H^{inter} &= \Delta H - m\{a'[H^{ex,0}_{m,KX}(m') - H^{ex,0}_{m,KX}(m)] \\ &\quad - a''[H^{ex,0}_{m,KX}(m'') - H^{ex,0}_{m,KX}(m)]\} \\ &\quad - m_A b'[H^{ex,0}_{m,A}(m'_A) - H^{ex,0}_{m,A}(m_A)] \\ &= (a' + b')m'm'_A[2(h_{KA} + h_{XA}) + 3(h_{KKA} + 2h_{KXA} + h_{XXA})m' \\ &\quad + 3(h_{KAA} + h_{XXA})m'_A + \text{higher order terms}] \qquad (2)\end{aligned}$$

(See Fig. 1 for definitions of terms.)

TABLE 1

Experimental enthalpy of mixing results for potassium halide solutions with glycine solutions at 298.15 K

m (mol kg^{-1})	m' (mol kg^{-1})	m'' (mol kg^{-1})	m_A (mol kg^{-1})	m'_A (mol kg^{-1})	$10^3 a'$ [a] (kg)	$10^3 b''$ (kg)	$10^3 b'$ (kg)	$-10^3 \Delta H$ (J)	10^3 (salt correction) [a] (J)	10^3 (glycine correction) [b] (J)	$-10^3 \Delta H^{inter}$ (J)
KBr + glycine											
0.4999	0.3351	0.3401	0.4998	0.1648	3.9612	1.8589	1.9481	314.9	7.1	127.2	449.1
0.4999	0.2521	0.2504	0.4998	0.2477	1.9533	1.8810	1.9200	228.5	4.9	92.8	326.2
0.4999	0.1649	0.1639	0.4998	0.3350	1.9564	4.0473	3.9744	316.3	−2.3	123.5	437.5
0.3735	0.1882	0.1857	0.3736	0.1853	1.9801	2.0101	1.9494	139.7	−1.9	54.5	193.2
0.3735	0.2501	0.2486	0.3736	0.1234	3.9599	1.9676	1.9538	182.2	0.1	73.4	255.7
0.3735	0.1241	0.1235	0.3736	0.2494	1.9634	3.9885	3.9457	175.6	−0.3	71.9	247.2
0.2498	0.1670	0.1636	0.2488	0.0825	3.8954	2.1089	1.9314	72.6	−2.0	33.1	103.8
0.2498	0.1245	0.1238	0.2488	0.1249	1.9500	1.9774	1.9625	60.1	0.1	24.9	85.1
0.2498	0.0824	0.0816	0.2488	0.1668	1.9419	4.0438	3.9451	74.9	−0.1	32.9	107.7
KF + glycine											
0.5000	0.3363	0.3354	0.4977	0.1630	3.9960	1.9643	1.9451	−117.6	1.4	126.4	10.2
0.5000	0.2498	0.2484	0.4977	0.2490	1.9672	1.9954	1.9704	−79.9	1.2	93.6	14.9
0.5000	0.2538	0.2472	0.4977	0.2451	1.9728	1.9790	1.9137	−89.6	1.6	92.4	4.4
0.5000	0.1655	0.1639	0.4977	0.3330	1.9420	4.0219	3.9251	−126.2	3.8	121.5	−0.9
0.3750	0.2513	0.2501	0.3750	0.1237	4.0061	1.9853	1.9719	−75.9	0.4	74.7	−0.8

0.3750	0.1896	0.1875	0.3750	0.1854	1.9866	1.9994	1.9426	−53.0	2.0	54.9	3.9
0.3750	0.1252	0.1225	0.3750	0.2498	1.9520	4.0195	3.8947	−79.2	2.4	71.8	−5.0
0.2498	0.1274	0.1266	0.2498	0.1225	2.0569	2.0176	1.9762	−31.4	1.0	25.9	−4.5
0.2498	0.1678	0.1672	0.2498	0.0820	3.9939	1.9724	1.9518	−41.1	0.5	33.9	−6.6
0.2498	0.0823	0.0829	0.2498	0.1675	1.9508	3.9569	3.9703	−38.5	0.1	33.3	−5.1
KI + glycine											
0.4947	0.3260	0.3230	0.4977	0.1697	3.8439	2.0565	1.9891	505.8	−8.9	126.5	623.4
0.4947	0.3283	0.3278	0.4977	0.1675	3.9889	1.9898	1.9964	503.3	1.9	127.9	633.2
0.4947	0.2438	0.2448	0.4977	0.2524	1.9285	2.0223	1.9846	374.0	−6.4	92.9	460.5
0.4947	0.1621	0.1652	0.4977	0.3346	1.9432	3.9784	3.9871	502.3	−6.9	122.1	617.6
0.3745	0.1851	0.1858	0.3748	0.1895	1.8916	1.9564	1.9355	209.8	−2.2	53.4	261.0
0.3745	0.2483	0.2489	0.3748	0.1263	3.9236	1.9667	1.9942	288.0	2.3	74.7	364.9
0.3745	0.1243	0.1245	0.3748	0.2504	1.9427	3.9175	3.9104	270.2	−0.6	71.6	341.2
0.2488	0.0806	0.0801	0.2484	0.1680	1.8872	4.0355	3.9382	112.2	−0.9	32.1	143.4
0.2488	0.1236	0.1207	0.2484	0.1251	1.9460	2.0614	1.9712	88.2	−0.7	24.9	112.4
0.2488	0.1677	0.1658	0.2484	0.0810	4.0104	2.0023	1.9394	116.6	−1.3	33.5	148.8

[a] This corresponds to the second term on the right-hand side of eqn. (2).
[b] This corresponds to the third term on the right-hand side of eqn. (2).

TABLE 2

Heterotactic virial coefficients for potassium halides and glycine in water at 298.15 K

Salt	KF	KCl	KBr	KI
$(h_{KA} + h_{XA})$ (J kg mol^{-2})	−7 (6)	−490 (10) [a]	−680 (11)	−962 (10)

[a] Taken from ref. 5.

TABLE 3

A comparison of the experimental and electrostatic h_2 terms

Salt	h_2^{expt} (J kg mol^{-2})	h_2^{elec} (J kg mol^{-2})	$h_2^{specific}$ (J kg mol^{-2})
KF	−7	438	−445
KCl [a]	−490	418	−908
KBr	−680	412	−1092
KI	−962	404	−1366
LiCl	71	450	−379
NaCl	−504	438	−942
KCl	−490	418	−908
CsCl	−508	404	−912

[a] Data for the alkali metal chlorides taken from ref. 5.

The experimental results are given in Table 1 and we have included in this the various correction terms which allow ΔH^{inter} to be evaluated. The molar excess enthalpies of the solutions containing single solutes were obtained from previously published sources [12,13]. The corrected enthalpy data were fitted to eqn. (2) using a least squares routine and the coefficients obtained from these analyses are given in Table 2. For each of the three systems studied, only the coefficients representing pairwise interactions were required.

DISCUSSION

The results presented in Table 2 clearly show that as the halide ion radius increases, the enthalpic virial coefficients become increasingly negative, i.e. the larger the anion, the more thermochemically favourable is its interaction with glycine. This is in contrast to the results obtained for the alkali metal chlorides, where it was found that the values obtained for NaCl, KCl and CsCl were, within experimental uncertainty, the same [14]. The indications are, therefore, that the mode of interaction at the molecular level of the halide ions with glycine is quite different to that for cations interacting with glycine.

Before entering into a discussion of this it should be mentioned that although previously [5] we have performed transpositions of the experimen-

tal Lewis–Randall (LR) virial coefficients into the more meaningful McMillan–Mayer (MM) coefficients [15], for the present systems such transpositions are not possible because of the lack of free-energy information. This is probably relatively unimportant, because it appears that for glycine–salt systems there are close similarities both in sign and magnitude between the enthalpic virial (LR) coefficients and the corresponding internal energetic (MM) coefficients [5]. Consequently, we shall consider that the enthalpic virial coefficients consist of two components, one arising from electrostatic interactions and the other from specific (chemical) sources. We have estimated the electrostatic contributions using the Kirkwood [6,16] approach and from this we may write

$$(h_{KA} + h_{XA})^{elec} = RT^2\left[d\left\{-2A_1/(DT)^2 + A_2/(DT)\right\}\right.$$
$$\times\left\{(\partial \ln D/\partial T)_p + T^{-1}\right\} - \left\{-A_1/(DT)^2 + A_2/(DT)\right\}$$
$$\left.\times(\partial d/\partial T)_p\right] \quad (3)$$

where d and D are the density and relative permittivity of the pure solvent, respectively, and the terms A_1 and A_2 are given by

$$A_1 = 1.26 \times 10^{-2}\mu^2/a, \quad A_2 = 2.17 \times 10^{-2}V_A\alpha(\rho)/a$$

In these expressions the numerical factors pertain to the situation where μ (the dipole moment of the non-electrolyte) is in Debye units, the molar volume of the non-electrolyte (V_A) is expressed in $cm^3\ mol^{-1}$, and the distance a (the mean distance of closest approach of the ions and non-electrolyte) is in Ångstrom units. The function $\alpha(\rho)$ where $\rho = b/a$, b being the radius of the non-electrolyte, is given by

$$\alpha(\rho) = \left[(\rho^3 - 2)\ln(1+\rho) - (\rho^3 + 2)\ln(1-\rho) - 2\rho^2\right]/3\rho^4 \quad (4)$$

In calculating the electrostatic contributions to the enthalpic virial coefficients the following values were used: $\mu = 13.5$ D [17]; $V_A = 56.7\ cm^3\ mol^{-1}$ [18]; $D = 78.54$ [19]; $(\partial \ln D/\partial T)_p = -4.55 \times 10^{-3}\ K^{-1}$ [19]; $(\partial d/\partial T)_p = -2.54\ g\ cm^{-3}\ K^{-1}$ [20]; $b = 2.82$ Å [21]; along with Pauling ionic radii [22]. In Fig. 2 we have compared the calculated electrostatic contributions with the experimentally obtained quantities. It is apparent from this figure that the feature observed for the alkai metal chloride–glycine systems [5] is also present in the potassium halide–glycine systems, namely that the electrostatic predictions are of the opposite sign to the experimental values. Furthermore, the electrostatic contributions vary little with anionic size whereas experimentally a marked variation with size is observed. Included in Fig. 2a are the estimated specific contributions to the interactions. The specific contributions ($h_2^{specific}$) are defined by

$$h_2^{specific} = h_2^{expt} - h_2^{elec} \quad (5)$$

where

$$h_2^{expt} = h_{KA} + h_{XA}, \quad h_2^{elec} = (h_{KA} + h_{XA})^{elec}$$

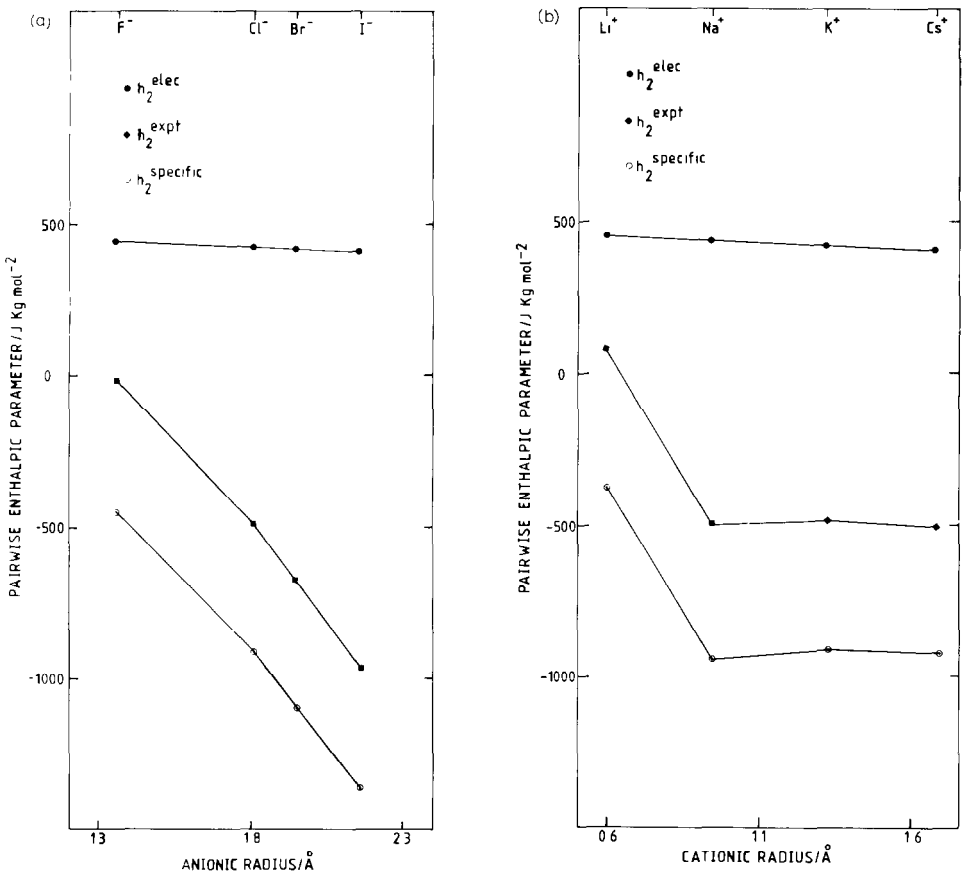

Fig. 2. The variation of h_2 coefficients for (a) potassium halide–glycine systems against anionic radius and (b) alkali metal chloride–glycine systems against cationic radius.

The results in Fig. 2a may be compared with the corresponding results for the alkali metal chloride–glycine systems (see Fig. 2b). Both parts of Fig. 2 indicate that there are considerable specific effects occurring in the interactions between glycine and the various ions.

When considering the variations evident in the potassium halide–glycine systems we assume that the potassium ion plays an invariant role in each system (see eqn. 1) and that it is the properties of the anions which are responsible for the observed trend. To rationalise this trend in the anionic series we further assume that the principal site for specific interactions of anions with glycine occurs at or near the protonated amino groups, and that the association of anions with the group may be envisaged as occurring in a two-stage process. These specific interactions can be represented pictorially (see Fig. 3) using a diagram similar to those which have been used earlier [10,23]. The initial process is partial desolvation of the $-NH_3^+$ moiety and the anion, and this is followed by short-range molecular interaction between

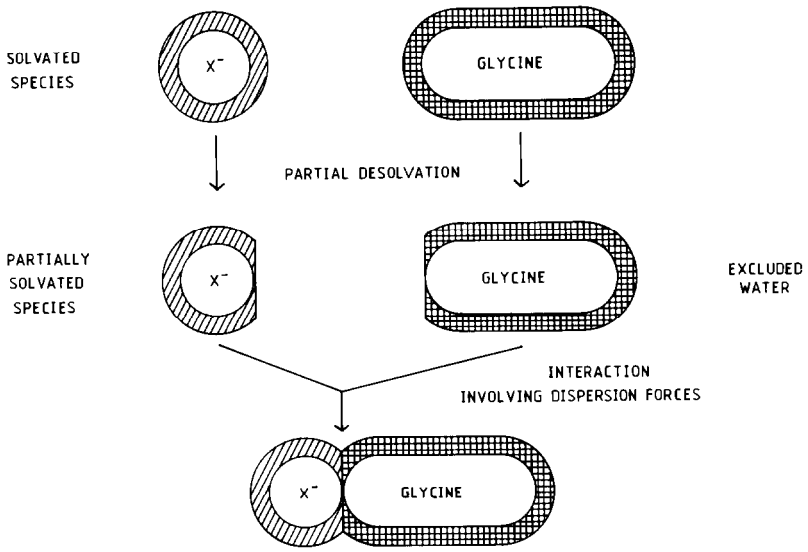

Fig. 3. Schematic representation of contributions to h_2^{specific}.

the two partially desolvated species. Qualitatively the desolvation contribution of the $-NH_3^+$ group will be approximately constant (a small variation can occur because co-sphere overlap will be greater with the larger anions) while the desolvation of the anions will become increasingly easier as the ionic size increases. Consequently one would expect that on descending the anionic series (F^- to I^-) the nett desolvation contribution would be enthalpically large and positive for F^- but smaller although still positive for I^-. Although anionic desolvation gives the correct trend in the specific contributions, it is always positive, and consequently a further favourable contribution (or contributions) must be operating. We envisage that such an effect can arise largely from a contribution containing a significant dispersion force component, since there is a correlation between h_2^{specific} and the polarisabilities of the anions but we also imagine that solvent reorganisation in the solvation shells of the interacting species also makes a contribution. It is not possible at the moment to analyse quantitatively each contribution to the overall interaction, but we are currently obtaining the Gibbs free energetic virial coefficients for the systems studied here in the hope that these, and the corresponding entropic coefficients, will throw some light on the problem. It is noteworthy, however, that, notwithstanding the above comments, there is an approximate parallel between the potency of the anions in inducing protein denaturation and the exothermicity of the anion–glycine interactions. This suggests that the enthalpic components associated with charged residues on proteins interacting with ions might well be implicated in protein denaturation but and importantly, the discrimination exhibited by ions arises from what may be termed non-electrostatic sources.

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