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Aqueous solutions containing amino acids and peptides. Part 29. The enthalpies of dilution of some amino acids at 25°C

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Abstract

The enthalpies of dilution of aqueous solutions at 25°C have been determined for the amino acids β-alanine, α-aminobutyric acid, γ-aminobutyric acid, ε-aminocaproic acid, α-aminovaleric acid (norvaline) and threonine. The results have been treated using the excess function concept and homotactic interaction coefficients have been obtained. These are briefly discussed in terms of intermolecular interactions between the hydrated solute species.

INTRODUCTION

In this continuing series of investigations [1], we are addressing the non-covalent bonding interactions which occur between amino acids and peptides, and their derivatives, when they are present in solutions. The principal reasons for studying such systems are (i) that some insights should be gained into the factors which affect the stability of globular proteins, and (ii) the information obtained contributes to the growing body of knowledge about solute interactions in aqueous media.

In most, if not all, of the previous works [2–27], we have investigated systems in which the side chains of the amino acid or peptide are apolar in nature. We continue this as part of the present paper but we have also turned our attention to an amino acid in which the side chain has a chemical functionality present. In particular, we consider the hydroxyamino acid threonine. The entire experimental study which is described, investigates the interactions using calorimetry.

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TABLE 1

Molar enthalpies of dilution of the indicated amino acids in aqueous solution at 25°C

m_{in}/ mol kg^{-1}	m_{fin}/ mol kg^{-1}	$\Delta_{dil}H_m$/ J mol^{-1}	m_{in}/ mol kg^{-1}	m_{fin}/ mol kg^{-1}	$\Delta_{dil}H_m$/ J mol^{-1}
β-Alanine					
0.4996	0.3304	24.8	0.9992	0.3217	114.0
0.4996	0.2884	31.3	0.9992	0.2402	114.0
0.4996	0.2468	37.5	1.4985	1.1013	63.9
0.4996	0.2053	42.9	1.4985	0.9724	85.6
0.4996	0.1639	48.5	1.4985	0.8450	106.5
0.9992	0.7396	38.0	1.4985	0.7197	127.5
0.9992	0.6545	52.2	1.4985	0.5960	145.7
0.9992	0.5701	65.2	1.4985	0.4736	165.2
0.9992	0.4867	77.8	1.4985	0.3529	184.1
0.9992	0.4039	89.2			
α-Aminobutyric acid					
0.2529	0.1889	44.5	0.5911	0.2567	171.3
0.2529	0.1254	76.0	0.5911	0.1914	205.6
0.3611	0.2690	38.3	0.5911	0.1432	227.9
0.3611	0.1982	75.0	0.6877	0.5094	86.2
0.3611	0.1781	86.6	0.6877	0.4512	116.6
0.3611	0.1581	87.7	0.6877	0.3738	158.6
0.3611	0.1181	103.8	0.6877	0.3355	176.3
0.3611	0.0885	118.5	0.6877	0.2974	200.9
0.4421	0.2423	99.7	0.6877	0.2217	236.5
0.4421	0.2177	111.3	0.6877	0.1657	258.4
0.4421	0.1931	127.1	0.8084	0.5290	140.7
0.4421	0.1443	147.5	0.8084	0.4379	186.4
0.4421	0.1080	175.7	0.8084	0.3929	207.8
0.5911	0.4387	77.6	0.8084	0.3481	236.8
0.5911	0.3887	100.7	0.8084	0.2593	277.5
0.5911	0.3223	138.1	0.8084	0.1937	311.9
0.5911	0.2894	155.8			
γ-Aminobutyric acid					
0.4997	0.3719	63.7	0.9993	0.4006	294.9
0.4997	0.3297	85.3	0.9993	0.3187	340.8
0.4997	0.2877	106.2	1.4992	1.0958	190.6
0.4997	0.2460	125.1	1.4992	0.9658	250.0
0.4997	0.2045	147.6	1.4992	0.8378	315.2
0.4997	0.1632	169.0	1.4992	0.7124	376.4
0.4997	0.1221	189.9	1.4992	0.5890	440.7
0.9993	0.5668	211.5	1.4992	0.4672	501.7
0.9993	0.4832	252.4	1.4992	0.3476	568.9

TABLE 1 (continued)

m_{in}/ mol kg^{-1}	m_{fin}/ mol kg^{-1}	$\Delta_{dil}H_m$/ J mol^{-1}	m_{in}/ mol kg^{-1}	m_{fin}/ mol kg^{-1}	$\Delta_{dil}H_m$/ J mol^{-1}
ε-Aminocaproic acid					
0.4999	0.3832	162.2	0.9996	0.3939	768.2
0.4999	0.3706	177.6	0.9996	0.3126	876.0
0.4999	0.3281	240.2	0.9996	0.2326	995.6
0.4999	0.2859	300.9	0.9996	0.2093	1023.2
0.4999	0.2442	363.1	1.5002	1.1238	411.9
0.4999	0.2028	423.2	1.5002	1.0842	459.0
0.4999	0.1616	483.5	1.5002	0.9522	608.1
0.4999	0.1207	557.7	1.5002	0.8231	769.0
0.4999	0.1087	569.8	1.5002	0.6974	923.1
0.9996	0.7574	287.5	1.5002	0.5747	1082.9
0.9996	0.7316	319.5	1.5002	0.4543	1254.3
0.9996	0.6451	429.8	1.5002	0.3969	1415.4
0.9996	0.5599	537.7	1.5002	0.3027	1487.2
0.9996	0.4762	648.4			
α-Aminovaleric acid					
0.3101	0.2301	68.5	0.5219	0.3430	171.4
0.3101	0.2039	95.8	0.5219	0.2843	213.5
0.3101	0.1690	115.8	0.5219	0.2553	244.7
0.3101	0.1518	134.1	0.5219	0.2264	269.8
0.3101	0.1346	151.0	0.5219	0.1688	309.8
0.3101	0.1004	171.9	0.5219	0.1263	346.7
0.3101	0.0751	192.9	0.5786	0.4287	146.1
0.4203	0.2767	130.6	0.5786	0.3797	186.4
0.4203	0.2295	168.3	0.5786	0.3146	239.3
0.4203	0.2062	190.6	0.5786	0.2824	277.2
0.4203	0.1829	217.3	0.5786	0.2503	306.7
0.4203	0.1021	267.3	0.5786	0.1866	359.7
Threonine					
0.4114	0.3063	10.8	0.7401	0.5473	13.4
0.4114	0.2717	12.9	0.7401	0.4845	17.1
0.4114	0.2256	15.5	0.7401	0.4011	24.1
0.4114	0.2027	21.2	0.7401	0.3599	28.6
0.4114	0.1799	27.2	0.7401	0.3189	29.6
0.4114	0.1344	33.7	0.7401	0.2375	33.8
0.4114	0.1006	37.6	0.7401	0.1775	75.6
0.5168	0.3839	8.4	0.8545	0.6301	11.6
0.5168	0.2535	22.0	0.8545	0.5572	15.1
0.5168	0.1678	41.4	0.8545	0.4607	18.6
0.5168	0.1255	38.1	0.8545	0.4131	21.2
0.6313	0.4143	13.1	0.8545	0.3658	25.6
0.6313	0.3081	23.8	0.8545	0.2721	36.6
0.6313	0.2731	34.4	0.8545	0.2032	46.5
0.6313	0.2036	34.0			

EXPERIMENTAL

All the amino acids were recrystallised at least twice from appropriate solvents. The microcalorimeter, its associated equipment and methodologies used have been described elsewhere [28].

RESULTS

The thermodynamic procedures used have been previously described [28–30] and only a summary will be given here.

The molar enthalpy change $\Delta_{dil}H_{m,A}$ on diluting a solution of non-electrolytic solute from an initial molality $m_{A,in}$ to a final molality $m_{A,fin}$ can be written

$$\Delta_{dil}H_{m,A} = H^{ex,o}_{m,A}(m_{A,fin}) - H^{ex,o}_{m,A}(m_{A,in})$$
$$= h_{AA}(m_{A,fin} - m_{A,in}) + h_{AAA}(m^2_{A,fin} - m^2_{A,in}) + \ldots \quad (1)$$

where $H^{ex,o}_{m,A}(m_{A,in})$ and $H^{ex,o}_{m,A}(m_{A,fin})$ are the molar excess (relative apparent molar) enthalpies of the solute in the solutions before and after dilution, and h_{AA}, h_{AAA}, etc., are the enthalpic coefficients representing pairwise and, at least notionally, triplet and higher order interactions between solvated solute species. Table 1 gives the experimental results obtained for the dilutions which were performed and Table 2 lists the coefficients of eqn. (1) which were obtained from least-squares analyses of these results.

DISCUSSION

The homotactic [14] interaction coefficients representing the pairwise interactions of the various solutes in water are collected in Table 3. We have included in this table results from some earlier studies in which enthalpic [31] and free energetic [32] information was obtained. In the discussion which follows, attention will be directed only to the pairwise

TABLE 2

Coefficients from the fitting of experimental dilution enthalpies to eqn. (1)

Amino acid	h_{AA}/(J kg mol^{-2})	h_{AAA}/(J kg^2 mol^{-3})	h_{AAAA}/(J kg^3 mol^{-4})
β-Ala	139(7)	10(3)	–
α-ABA	505(5)	–	–
γ-ABA	528(11)	−22(5)	–
ε-ACA	1662(79)	−370(92)	79(32)
α-AVA	767(50)	186(62)	–
Threonine	−139(18)	65(15)	–

Key: Ala is alanine; ABA is aminobutyric acid; ACA is aminocaproic acid; AVA is aminovaleric acid. Numbers in parentheses are 95% confidence limits.

TABLE 3

Homotactic pairwise enthalpic, free energetic and entropic interaction coefficients of some amino acids in water at 25°C

Amino acid	h_{AA}/(J kg mol^{-2})	g_{AA} [b]/(J kg mol^{-2})	s_{AA}/(J K^{-1} kg mol^{-2})
Glycine	−439(5) [a]	−238	−0.674
α-Ala	217(0) [a]	−61	0.932
α-ABA	505(5)	62	1.486
α-AVA	767(50)	85	2.287
β-Ala	139(7)	−136	0.922
γ-ABA	528(11)	−137	2.230
ε-ACA	1662(79)	−249	6.410
Serine	−720(5) [a]	−168	−1.851
Threonine	−139(18)	−115	−0.080

Key: see Table 2. Numbers in parentheses are 95% confidence limits. [a] See ref. 31. [b] See ref. 32.

coefficients, for reasons which have been given earlier [30]. The coefficients are displayed in Fig. 1 as a function of the number of atoms in the amino acid residues, for the α- and the α, ω-acids.

For the α-amino acids, as the apolar side chain is extended, the free energetic coefficients become increasingly positive, which reflects increased net repulsion between the acids. A similar but more pronounced effect is seen for the enthalpic coefficients. The molecular situation as we perceive it is illustrated in a pictorial way in Fig. 2. The hydration of the amino acids can be represented as consisting of three principal regions. One of these is from the solvent peripheral to the carboxylate group on the zwitterionic head group and there is a corresponding region about the amino group; both of these solvation regions will be under the relatively intense influence of the ionic charges. The third solvation region, which will be qualitatively

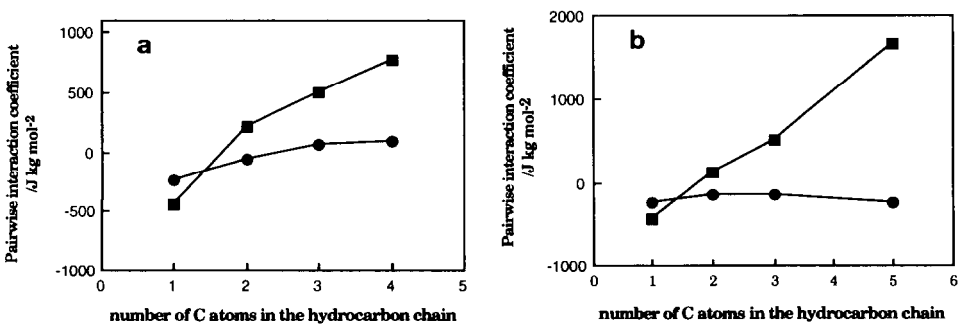

Fig. 1. Dependence of the pairwise homotactic free energetic (●) and enthalpic (■) coefficients on the number of carbon atoms in the hydrocarbon chain for: (a) α-amino acids and (b) α,ω-amino acids.

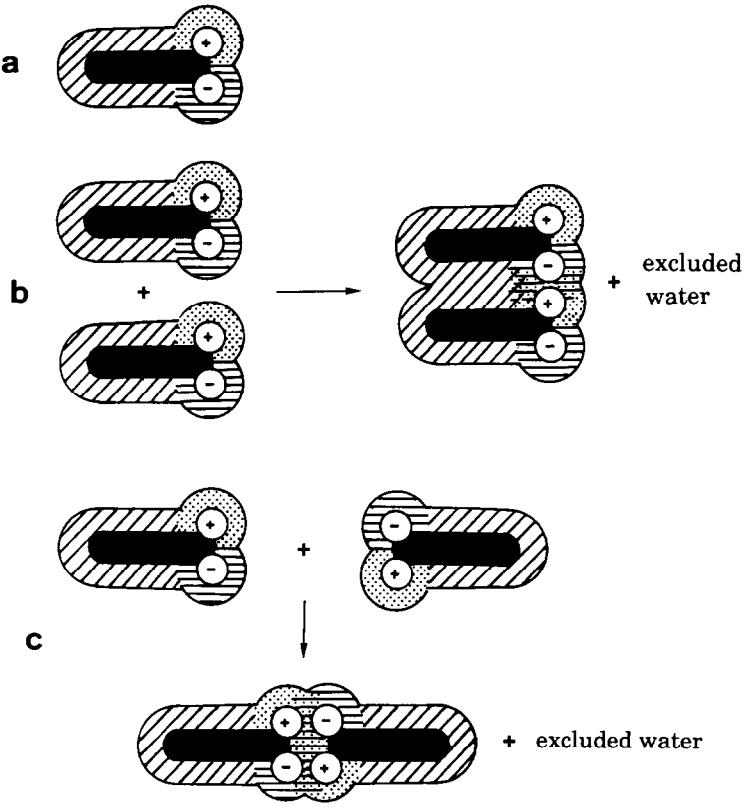

Fig. 2. Schematic representation of (a) the solvation regions of an α-amino acid, and the interaction of two α-amino acid molecules, (b) in a side-by-side manner, and (c) in a head-on way. The solvation regions about the apolar side chain and the positively and negatively charged groups in the amino acid are inducted as being distinct, although it is to be expected that at junctions some mutual perturbations will be present.

different to the other two, is that around the apolar side-chains. The marked increase towards more positive values for both types of coefficients almost certainly indicates that these solutes have a propensity to associate in a side-by-side manner: if the association was principally in a head-on manner, it would be expected that there would be little variation as the apolarity of the chain increases. The entropic coefficients calculated from

$$s_{AA} = (h_{AA} - g_{AA})/T \quad (2)$$

also increase towards more positive values and this certainly arises principally because of the release to the bulk solvent of the hydrophobic water of hydration peripheral to the apolar groups.

The α,ω-amino acids appear to show less regular trends than the α-acids in their homotactic interaction coefficients as they increase in size. This is particularly marked for the free energetic coefficients which exhibit a

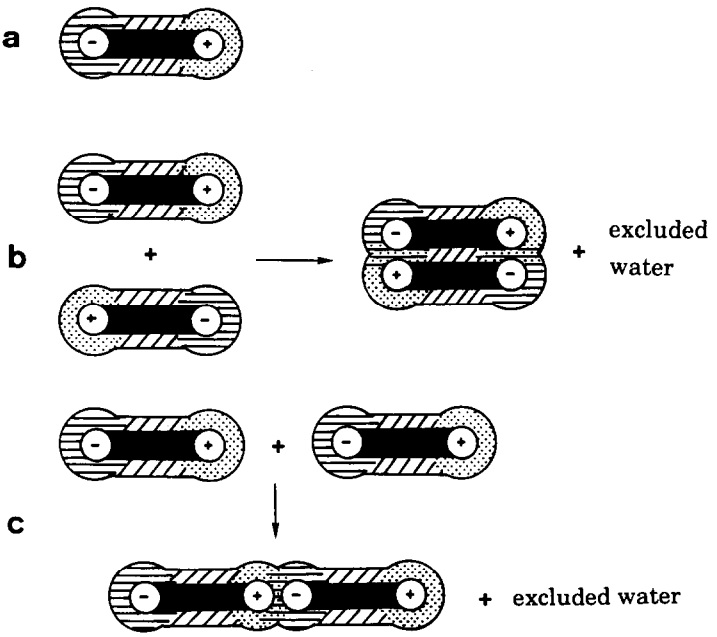

Fig. 3. Schematic representation of (a) the solvation regions of an α,ω-amino acid, and the interaction of two α,ω-amino acid molecules, (b) in a side-by-side manner, and (c) in a head-to-tail way. (See also Fig. 2.)

maximum at intermediate chain lengths. The overall trends in the enthalpic and entropic coefficients are similar to those shown for the α-acids and this again probably indicates that these acids also tend to associate in a side-by-side way and, in addition, in a head-to-tail configuration as shown in Fig. 3. The indications are that as well as seeing the consequences of hydrophobic solvation regions interacting, one is also seeing contributions arising from the interactions between hydrophilic solvation regions and hydrophobic solvation regions.

The addition of hydroxyl groups to amino acids has a marked effect on the homotactic enthalpic coefficients. The data available are somewhat limited but if we compare isoelectronic pairs, then the enthalpic coefficient for serine is more negative, by about 1200 J kg mol^{-2}, than that for α-aminobutyric acid. Moreover, the coefficient for threonine is about 900 J kg mol^{-2} more negative than that for α-aminovaleric acid (norvaline). Similarly, the free energetic coefficients for serine and threonine are some 230 and 200 J kg mol^{-2} more negative than those for α-aminobutyric acid and norvaline, respectively. It is apparent that the increased hydrophilicity induced by the presence of the hydroxyl groups has a marked effect on the interactions occurring between the solutes. The reason for these increased attractions must arise from contributions stemming from hydroxyl group–hydroxyl group, and hydroxyl group–zwitterionic head group interactions,

because it is known that interactions between hydroxyl groups and zwitterionic groups with hydrophobic groups are repulsive and consequently these last would lead to positive contributions to the homotactic interaction coefficients.

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REFERENCES

1 J. Fernández and T.H. Lilley, J. Chem. Soc. Faraday Trans., 88 (1992) 2503.
2 C.C. Briggs, T.H. Lilley, J. Rutherford and S. Woodhead, J. Solution Chem., 3 (1974) 649.
3 T.H. Lilley and R.P. Scott, J. Chem. Soc. Faraday Trans. 1, 72 (1976) 184.
4 T.H. Lilley and R.P. Scott, J. Chem. Soc. Faraday Trans. 1, 72 (1976) 197.
5 B.P. Kelley and T.H. Lilley, J. Chem. Thermodyn., 10 (1978) 703.
6 B.P. Kelley and T.H. Lilley, J. Chem. Soc. Faraday Trans. 1, 74 (1978) 2771.
7 B.P. Kelley and T.H. Lilley, J. Chem. Soc. Faraday Trans. 1, 74 (1978) 2779.
8 B.P. Kelley and T.H. Lilley, J. Chem. Thermodyn., 11 (1979) 513.
9 T.H. Lilley, E. Moses and I.R. Tasker, J. Chem. Soc. Faraday Trans. 1, 76 (1980) 906.
10 G.M. Blackburn, T.H. Lilley and E. Walmsley, J. Chem. Soc. Faraday Trans. 1, 76 (1980) 915.
11 G.M. Blackburn, T.H. Lilley and E. Walmsley, J. Chem. Soc. Chem. Commun., (1980) 1091.
12 T.H. Lilley and R. Tasker, J. Chem. Soc. Faraday Trans. 1, 78 (1982) 1.
13 G.M. Blackburn, T.H. Lilley and E. Walmsley, J. Chem. Soc. Faraday Trans. 1, 78 (1982) 1641.
14 K. Nelander, G. Oloffson, G.M. Blackburn, H.E. Kent and T.H. Lilley, Thermochim. Acta, 78 (1984) 303.
15 G.M. Blackburn, T.H. Lilley and P.J. Milburn, J. Solution Chem., 13 (1984) 789.
16 G.M. Blackburn, T.H. Lilley and P.J. Milburn, J. Chem. Soc. Chem. Commun., (1985) 299.
17 G.M. Blackburn, T.H. Lilley and P.J. Milburn, Thermochim. Acta, 83 (1985) 289.
18 T.E. Leslie and T.H. Lilley, Biopolymers, 24 (1985) 695.
19 G.M. Blackburn, T.H. Lilley and P.J. Milburn, J. Chem. Soc. Faraday Trans. 1, 81 (1985) 2191.
20 G.M. Blackburn, H.E. Kent and T.H. Lilley, J. Chem. Soc. Faraday Trans. 1, 81 (1985) 2207.
21 G.M. Blackburn, T.H. Lilley and P.J. Milburn, J. Solution Chem., 15 (1986) 99.
22 G.M. Blackburn, T.H. Lilley and P.J. Milburn, J. Chem. Soc. Faraday Trans. 1, 82 (1986) 2965.
23 K.G. Davis and T.H. Lilley, Thermochim. Acta, 107 (1986) 267.
24 A.H. Sijpkes, A.A.C.M. Oudhius, G. Somsen and T.H. Lilley, J. Chem. Thermodyn., 21 (1989) 343.
25 A.H. Sijpkes, G. Somsen and T.H. Lilley, J. Chem. Soc. Faraday Trans., 86 (1990) 2943.
26 M. Abbate, G. Barone, G. Castronuovo, P.J. Cheek, G. Giancola, T.E. Leslie and T.H. Lilley, Thermochim. Acta, 173 (1990) 261.

27 G.R. Hedwig, J.F. Reading and T.H. Lilley, J. Chem. Soc. Faraday Trans., 87 (1991) 1751.
28 P.J. Cheek and T.H. Lilley, J. Chem. Soc. Faraday Trans., 84 (1988) 1927.
29 T.H. Lilley, in G.W. Neilson and J.E. Enderby (Eds.), Water and Aqueous Solutions, Adam Hilger, Bristol, 1986.
30 T.H. Lilley, in M.N. Jones (Ed.), Biochemical Thermodynamics, Elsevier, Amsterdam, 2nd edn., 1988, Chapt. 1.
31 R.S. Humphrey, G.R. Hedwig, I.D. Watson and G.N. Malcolm, J. Chem. Thermodyn., 12 (1980) 595.
32 T.H. Lilley, in G.C. Barrett (Ed.), The Chemistry and Biochemistry of Amino Acids, Chapman Hall–Methuen, 1984, p. 591.