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ENTHALPIES OF INTERACTION OF SOME *N*-ACETYL AMIDES OF L-SERINE, L-THREONINE AND L-HYDROXYPROLINE DISSOLVED IN *N*,*N*-DIMETHYLFORMAMIDE AT 298.15 K

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ABSTRACT

The enthalpies of dilution of five dipeptides, namely, *N*-acetyl-*N'*-methyl amides of L-serine, L-threonine and L-hydroxyproline, and *N*-acetyl amides of L-threonine and L-hydroxyproline dissolved in *N*,*N*-dimethylformamide were measured calorimetrically at 298.15 K. From these, the enthalpic interaction coefficients were calculated. The pair-wise interaction coefficients of these dipeptides are compared with related amino acid derivatives with non-hydroxylated side chains. The influence of the hydroxyl group is discussed in terms of solute–solute–solvent interactions. The relevance of the results for biochemical systems is indicated.

INTRODUCTION

In nature there are several amino acids which have an aliphatic side chain with a hydroxyl group. Two of these, L-serine and L-threonine, are standard amino acids, while L-hydroxyproline occurs almost exclusively in collagen proteins. It is manufactured in vivo via oxidation of proline residues in protocollagen [1]. The importance of the presence of a hydroxyl group lies in its ability to form hydrogen bonds. Serine is often found in β-turn regions of proteins where its hydroxyl group is involved in side chain–backbone interactions [2–4]. Recent studies of mutants of lysozyme of bacteriophage T4 have highlighted the role of a threonine residue in stabilizing the native state of a protein [5–7]. Hydroxyproline is one of the main contributors to the stability and rigidity of collagen proteins due to its potential to interconnect the polypeptide chains via hydrogen bonds [1,8].

In order to estimate the contribution of the OH groups to the interaction enthalpies of these amino acid residues, we determined the enthalpic interaction coefficients of some model dipeptides, namely, *N*-acetyl-*N'*-methyl amides of L-serine (SerMe), L-threonine (ThrMe) and L-hydroxyproline (HypMe), and *N*-acetyl amides of L-threonine ($ThrNH_2$) and L-hydroxypro-

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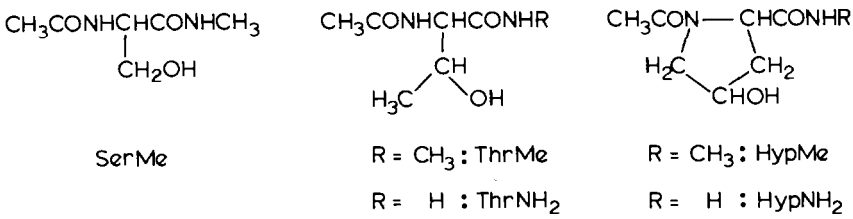

Fig. 1. Structural formulae of the compounds.

line ($HypNH_2$). Structural formulae and abbreviations are shown in Fig. 1.

The pair-wise interaction coefficients of the dipeptides of Ser, Thr and Hyp can be compared to analogous dipeptides with non-hydroxylated side chains [9,10].

EXPERIMENTAL

Apparatus

Enthalpies of dilution were measured with an LKB 10700-2 batch microcalorimeter system operating at 298.15 K. The experimental procedures have been previously described [10].

Materials

N,N-Dimethylformamide (DMF, Baker Analysed Reagent) was dried with 0.4 nm molecular sieves and used without further purification.

The dipeptides of Ser and Thr were synthesized from their parent amino acids (Janssen Chimica, Belgium) following the procedures described by Kent et al. [9] and Blackburn et al. [11]. The overall yields after several recrystallization steps of the final products were poor (below 5% relative to starting material). Therefore a different method was chosen for the syntheses of the dipeptides of Hyp. The amino acid (75 mmol) was acetylated at the imino group, which proceeds quantitatively [12]. The product, *N*-acetyl-L-hydroxyproline (75 mmol), was dissolved in 75 cm^3 DMF. *N*-Ethylmorpholine (1.1 equivalent) was added and, after cooling the solution to below $-15\,°C$, 1.1 equivalents isobutylchloroformate was added. After stirring for 15 min, the reaction mixture was poured into 250 cm^3 THF to precipitate the *N*-ethylmorpholinium chloride. After removing the precipitate by filtration, 2 equivalents of amine (33% NH_2CH_3 in EtOH or 25% NH_4OH in H_2O) were added. After evaporation of the solvents a yellow/brown oil remains from which the final products were crystallized. Yields after several recrystallization steps (EtOH) are 31% (HypMe) and 61% ($HypNH_2$) relative to starting material.

TABLE 1

Melting points, m.p., and optical rotations, $[\alpha]_D^{20}$, of some dipeptides

Dipeptide	m.p. (°C)	Literature value	$[\alpha]_D^{20}$ [a] (deg cm^3 dm^{-1} g^{-1})	Literature value
SerMe	117–118	117.5[13]	−15.4 (EtOH)	−50.6 (H_2O) [13]
				−33.6 (H_2O) [14]
				−29.5 (H_2O) [15]
ThrMe	159–161	161[13]	−9.2 (EtOH)	
			−31.1 (H_2O)	−57.5 (H_2O) [13]
HypMe	167–169	167–168.5[12]	−61.9 (EtOH)	−60.9 (EtOH) [12]
		167[14]	−90.0 (H_2O)	−180.0 (H_2O) [13]
$ThrNH_2$	173–174		+8.0 (H_2O)	
$HypNH_2$	163–164		−93.6 (H_2O)	

[a] 1–2% solutions.

The dipeptides were characterized by their melting points and degrees of optical rotation, which are given in Table 1. We cannot account for the fact that the optical rotatory powers given by Zahn and Reinert [13] differ by a factor of around two from our data and from those of others [14,15]. NMR spectra were recorded with a Bruker WH 90 using $CDCl_3$ ($CHCl_3$ as internal reference, 7.27 ppm) or D_2O (H_2O as internal reference, 5.56 ppm) as solvents. Chemical shifts (ppm) are listed below.

δ (SerMe [$CHCl_3$]): 6.8 (2H, s, NH + N′H); 4.40 (1H, m, αCH); 4.15 (1H, dd, J 3.0 Hz, J 11.9 Hz, βCH); 3.60 (1H, dd, J 4.7 Hz, J 11.9 Hz, βCH′); 2.83 (3H, d, J 4.8 Hz, N′CH_3); 2.4 (1H, s, OH); 2.08 (3H, s, CH_3CO).

δ(ThrMe [$CHCl_3$]): 6.7 (2H, s, NH + N′H); 4.32 (2H, m, αCH + βCH); 2.82 (3H, d, J 5.3 Hz, N′CH_3); 2.3 (1H, s, γ_1OH); 2.05 (3H, s, CH_3CO); 1.13 (3H, d, J 6.3 Hz, γ_2CH_3).

δ (HypMe [D_2O]): 5.25 (2H, m, αCH + γCH); 4.44 (2H, m, δCH_2); 3.55 (0.6H, s, N′$CH_{3,cis}$); 3.48 (2.4H, s, N′$CH_{3,trans}$); 2.94 (2H, m, βCH_2); 2.87 (2.4H, s, CH_3CO_{trans}); 2.74 (0.6H, s, CH_3CO_{cis}).

δ ($ThrNH_2$ [D_2O]): 5.09 (1H, d, J 6.0 Hz, αCH); 4.97 (1H, dqa, J 6.0 Hz, J 6.4 Hz, βCH); 2.82 (3H, s, CH_3CO); 1.98 (3H, d, J 6.4 Hz, γ_1CH_3).

δ ($HypNH_2$ [D_2O]): 5.22 (2H, m, αCH + γCH); 4.49 (2H, m, δCH_2); 3.0 (2H, m, βCH_2); 2.86 (2.4H, s, CH_3CO_{trans}); 2.77 (0.6H, s, CH_3CO_{cis}).

RESULTS

Enthalpies of dilution are measured by mixing a known amount of solvent with a known amount of solution containing N moles of solute with an initial molality m_i, resulting in a solution with final molality m_f. The

enthalpy change upon dilution, ΔH, can be written as [16]

$$\Delta H = N\left[H_m^E(m_f) - H_m^E(m_i)\right] \quad (1)$$

$H_m^E(m_f)$ and $H_m^E(m_i)$ are the molar excess enthalpies of the solution at final and initial molality, respectively. The molar excess enthalpies can be written as

$$H_m^E(m) = B_2^h\left(m/m^\ominus\right) + B_3^h\left(m/m^\ominus\right)^2 + \ldots \quad (2)$$

TABLE 2

Enthalpy change upon dilution, ΔH, of N moles of solute from initial molality, m_i, to final molality, m_f, of some N-acetyl (-N'-methyl) amides of L-serine, L-threonine and L-hydroxyproline

m_i (mol kg^{-1})	N (mmol)	m_f (mol kg^{-1})	ΔH (mJ)	Δ (%) [a]	m_i (mol kg^{-1})	N (mmol)	m_f (mol kg^{-1})	ΔH (mJ)	Δ (%)
SerMe									
0.0877	0.3362	0.0584	3.77	−3.6	0.1944	0.8004	0.1325	17.30	3.0
0.1515	0.3285	0.0696	9.33	−7.3	0.1944	0.3929	0.0625	18.94	1.4
0.1515	0.5614	0.0986	0.97	−8.1	0.1944	0.4610	0.0775	19.93	3.8
0.1515	0.2763	0.0480	10.23	−5.9	0.2877	1.0476	0.1875	30.65	0.1
0.1944	0.3088	0.0545	16.08	2.4	0.2877	0.5643	0.1002	33.38	−1.1
ThrMe									
0.0925	0.3721	0.0587	7.37	1.2	0.1219	0.2718	0.0419	12.29	−0.1
0.0925	0.2106	0.0341	7.57	1.5	0.1556	0.6369	0.1002	16.56	0.7
0.1219	0.5013	0.0818	10.83	2.9	0.1556	0.3459	0.0537	17.83	−2.0
HypMe									
0.1728	0.2885	0.0477	15.39	0.4	0.3572	0.8545	0.1393	74.46	0.6
0.1728	0.4444	0.0727	18.51	−4.4	0.3990	1.6195	0.2703	76.77	−0.9
0.2080	0.8110	0.1393	22.74	−2.8	0.5185	1.1096	0.2676	98.36	−0.3
0.2080	0.3764	0.0629	23.87	1.4	0.5185	2.0235	0.3494	117.0	−0.0
0.3572	1.1458	0.2012	69.21	−0.2	0.5185	1.0801	0.1778	135.9	0.4
$ThrNH_2$									
0.1419	0.1793	0.0329	28.63	1.8	0.1758	0.7389	0.1190	55.71	−3.0
0.1321	0.5111	0.0890	31.18	0.2	0.1758	0.6190	0.1043	61.78	1.5
0.1321	0.2827	0.0470	34.13	−1.3	0.2685	0.4277	0.0771	110.3	0.7
0.1321	0.5452	0.0840	36.13	−2.6	0.2685	0.5048	0.0873	123.5	1.5
0.1419	0.5189	0.0900	36.78	−2.9	0.2685	1.1279	0.1772	130.3	−0.9
0.1758	0.2189	0.0385	41.67	−2.1					
$HypNH_2$									
0.1019	0.3982	0.0627	10.09	2.4	0.3573	1.3369	0.2374	83.83	−0.0
0.1181	0.5081	0.0808	11.83	0.5	0.3573	0.7680	0.1254	97.34	−0.8
0.2212	0.9153	0.1895	16.03	−3.1	0.3573	1.4707	0.2234	103.6	−0.1
0.2212	0.4341	0.0697	39.07	−0.8	0.5001	0.7993	0.1388	149.7	1.2
0.2212	0.8321	0.1348	40.96	−2.3	0.5001	1.9817	0.3225	163.5	−0.5

[a] Δ (%) = 100[(ΔH(exp.) − ΔH(calc.))/ΔH(exp.)]; ΔH(calc.) is calculated from eqn. (3).

TABLE 3

Enthalpic interaction coefficients of some dipeptides dissolved in DMF at 298.15 K

Dipeptide	B_2^h (J mol^{-1})	B_3^h (J mol^{-1})	σ [a] (mJ)
SerMe	−442 (13) [b]	317 (35)	0.6
ThrMe	−745 (24)	1095 (113)	0.3
HypMe	−470 (5)	148 (6)	1.2
$ThrNH_2$	−1544 (31)	596 (85)	0.5
$HypNH_2$	−671 (8)	248 (11)	0.9

[a] Standard error of fit.

[b] Values in parentheses are standard deviations of the coefficients.

where $m^{\ominus} \equiv 1$ mol kg^{-1} and B_2^h, B_3^h, etc., are the pair, triplet, etc., enthalpic interaction coefficients. These enthalpic interaction coefficients are related to McMillan–Mayer cluster integrals [17,18] and are a measure of the interaction between pairs, triplets and higher order multiplets of solutes in the solution. They should not be confused with virial coefficients. Combining eqns. (1) and (2) yields

$$\Delta H = \sum_{n>1} N\left[\left(m_f/m^{\ominus}\right)^{n-1} - \left(m_i/m^{\ominus}\right)^{n-1}\right] B_n^h \qquad (3)$$

Experimental values of ΔH, m_i, m_f and N are given in Table 2. A least-squares analysis of these values in terms of eqn. (3) gives the desired B_n^h coefficients. Coefficients were only considered to be physically meaningful if the Student t-test indicated that they exceeded their 95% confidence level. In all cases, only the first two coefficients were necessary to represent the experimental data. The resulting enthalpic interaction coefficients are collected in Table 3.

TABLE 4

Pair-wise enthalpic interaction coefficients of dipeptides with and without a hydroxyl group

Dipeptide with OH	B_2^h (J mol^{-1})	Dipeptide without OH	B_2^h (J mol^{-1})
SerMe	−442	AlaMe	−348 [a]
ThrMe	−745	αiBMe	−480 [b]
HypMe	−471	ProMe	−158 [a]
$ThrNH_2$	−1544	$\alpha iBNH_2$	−1159 [b]
$HypNH_2$	−671	$ProNH_2$	−468 [a]

[a] Sijpkes and Somsen [10].

[b] Hypothetical values of α-isobutyric acid (αiB) are obtained by taking the average values of Ala and Val from refs. 9 and 10.

DISCUSSION

When the dipeptides of this investigation are compared with their non-hydroxylated analogs (see Table 4 and Fig. 2) several features are apparent. Introduction of an OH group shifts the B_2^h coefficients to a more negative value by an amount of 110–300 J mol^{-1}. An obvious explanation for this effect would be that an OH group increases the possibilities of the dipeptide forming intermolecular hydrogen bonds. When interacting with another solute molecule this will give a more negative B_2^h coefficient. On the other hand, a solute molecule can also interact with a solvent DMF molecule. Because the B_2^h coefficient is a measure of the solvent mediated solute–solute interaction, this implies that by hindering the solute–solute interaction, solute–solvent interactions give rise to a more positive B_2^h coefficient. In view of this, the net effect of the introduction of an OH group is the sum of two contributions, namely an enhanced solute–solute interaction which contributes via an exothermic shift of the B_2^h, and an enhanced solute–solvent interaction contributing via an endothermic shift of the B_2^h. The experimentally observed exothermic shift of the B_2^h coefficient indicates that solute–solute interactions are predominant over solute–solvent interactions upon introducing an OH group. When we apply the results of these model compounds to estimate the stabilisation energy of native protein structures due to OH groups, we must therefore take into account the fact that the

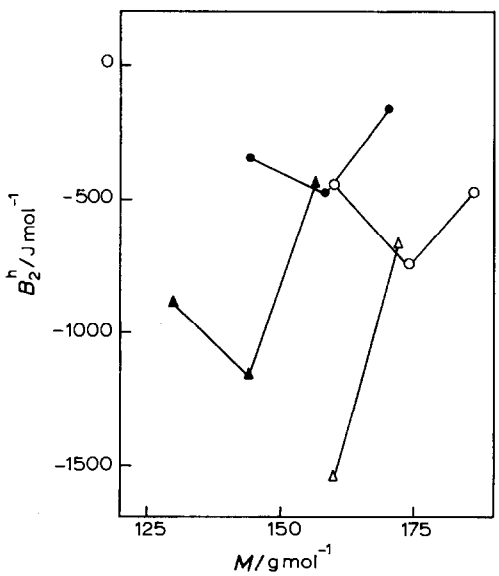

Fig. 2. Enthalpic pair-wise interaction coefficients of some dipeptides as a function of their molar masses: •, AlaMe, αiBMe and ProMe; ○, SerMe, ThrMe and HypMe; ▲, AlaNH$_2$, αiBNH$_2$ and ProNH$_2$; △, ThrNH$_2$ and HypNH$_2$.

experimental value of B_2^h underestimates the solute–solute interaction. The real interaction can be much more exothermic.

The positive B_3^h coefficients presented in Table 3 are much larger than those of related amino acid derivatives with non-hydroxylated side chains [9,10]. However, the interpretation of the triplet interaction coefficients is obscured by the fact that they also contain pair-wise interaction terms [19]. Their magnitude seems to indicate a relatively strong solute–solute–solvent interaction in these systems.

If the dipeptides of this investigation and that of earlier studies [9,10] are taken as a model for amino acid residues in the interior of globular proteins, while DMF provides the model environment [9,10,20], then, from the B_2^h coefficients one can derive an estimate for the stabilization of the native structure of proteins due to the presence of OH groups. When a hydroxylated residue interacts with another residue or with the backbone of the polypeptide chain, one would tentatively predict that the interaction is stabilised by at least 110–300 J mol^{-1} per residue, relative to unhydroxylated residues. Neglecting the effect of conformation (entropy), this would imply that rat-skin collagen, which contains 92 Hyp residues per 1000 residues [21], is stabilized energetically by an amount of 9–27 kJ per 1000 residues. Accordingly, a protein-like ribonuclease-S, which contains 15 Ser and 10 Thr residues [22], would be stabilized by 2.5–7.5 kJ mol^{-1}. These values are by no means negligible when one realises that the energy which is required to bring a folded protein to some denatured state is of the order of 20–80 kJ mol^{-1} [23].

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