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CHIRAL RECOGNITION OF ENANTIOMERIC PEPTIDES IN WATER AT 25°C BY CALORIMETRY

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The heats of dilution in water of binary and ternary solutions of the two enantiomeric forms of *N*-acetylalanineamide have been measured at 25°. The excess enthalpies, expressed as virial expansion series, permit evaluation of the pairwise self and cross enthalpic coefficients. As for the chiral forms of some monosaccharides, the cross coefficient for the interaction between the *D* and *L* forms of *N*-acetylalanineamide is slightly but significantly different from the corresponding self coefficient. A weak, water-mediated chiral recognition can be assumed to exist between pairs of amide molecules.

In aqueous solutions containing aminoacids and simple peptides, the interactions between themselves and with electrolytes and non-electrolytes have received increasing attention in recent years [1–16]. These studies are stimulated by the hope of gaining insight into the factors that determine the properties of the solutions of proteins and naturally-occurring polypeptides. This work, however, is primarily devoted to ascertaining if it is possible to find evidence in aqueous solution of the existence of a weak chiral recognition between pairs of enantiomeric molecules.

It is well known that in the solid state the properties of racemic mixtures differ markedly from those of the pure *D* and *L* enantiomeric forms. This is due essentially to the different distances and topological correlations between the group of atoms in the molecules, that generate interactions of different intensity. For reasons of symmetry, the interactions in racemic crystals are very often more effective, resulting in more stable and less soluble crystals of both enantiomers. These differ from each other only in chiroptical properties.

In the presence of a non-chiral solvent, the interactions between the solute molecules are made weaker and are overwhelmed by the solute-solvent and solvent-solvent interactions. This is particularly true in the case of water, whose molecules interact strongly with polar solute molecules and with one another. Thus, it is not obvious to expect that evidence of chiral correlations can be found in solutions in the presence of a diluting component. However, we were earlier successful in finding such evidence in the unfavourable case of the monosaccharides *D*- and *L*-

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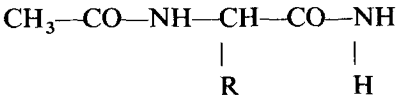
xylose and *D*- and *L*-arabinose, whose aqueous solutions, like those of other sugars, are characterized by predominant solute-solvent interactions [17].

In the present work, the interactions in binary and ternary aqueous solutions of the *D* and *L* forms of *N*-acetylalanineamide (NAAA) have been evaluated from the excess enthalpy data determined by the same technique flow microcalorimetry as employed in the previous work. The importance of the synthesis of compounds of general formula



$$CH_3{-}CO{-}NH{-}\underset{\displaystyle R}{\underset{|}{CH}}{-}CO{-}\underset{\displaystyle H}{\underset{|}{NH}}$$

is double: they are better models of peptide chains than aminoacids and, when compared to the corresponding free aminoacids, they allow study of the influence and role of charges on this kind of interaction.

Experimental

Materials

The preparation of *N*-acetyl-*L*-alanineamide has been described by Blackburn [7] and the synthesis of the *D* enantiomer is a modification of this preparation. The products were crystallized several times and dried in vacuo at room temperature. The solutions were freshly prepared with deionized, double-distilled, degassed water.

Calorimetry

The heats of dilution of binary and ternary solutions were determined at 25° with an LKB 10700–1 standard flow microcalorimeter, by the procedure reported in detail in a preceding paper [11].

Thermodynamics

Excess thermodynamic properties are an important basis for formulating hypotheses on intermolecular interactions. For operational purposes the excess enthalpy H^E of asymmetric mixtures of non-electrolytes (e.g. aqueous solutions of solid organic compounds) can be defined as [4–7, 9–13, 18]:

$$H^E = H - H_w^0 - \sum_{x=1}^{n} m_x \overline{H}_x^0 \qquad (1)$$

where H^E and H refer to an amount of solution containing 1 kg of water and $m_1 \ldots m_n$ moles of each solute species,, H^0_w is the standard enthalpy of 1 kg of pure water, and the $\bar{H}^0_x$ are the standard partial molal (limiting) enthalpies of each solute species. Therefore, H^E represents the deviation from the behaviour of an athermal, ideal solution and is determined by the nature and intensity of the molecular interactions.

The excess enthalpy per kg of solvent can be represented as a power series in solute molalities:

$$H^E = \sum_x \sum_y h_{xy} m_x m_y + \sum_x \sum_y \sum_z h_{xyz} m_x m_y m_z + \ldots \tag{2}$$

where the summation is over all solute species and over all positive, integral numbers of molecules.

Equation (2), for binary and ternary solutions, assumes the following forms, respectively:

$$H^E(m_x) = h_{xx} m_x^2 + h_{xxx} m_x^3 + \ldots \tag{3}$$

$$\begin{aligned} H^E(m_x, m_y) = {} & h_{xx} m_x^2 + 2h_{xy} m_x m_y + h_{yy} m_y^2 + h_{xxx} m_x^3 + 3h_{xxy} m_x^2 m_y + \\ & + 3h_{xyy} m_x m_y^2 + h_{yyy} m_y^3 + \ldots \end{aligned} \tag{4}$$

According to the MacMillan approach [1, 19, 20], the coefficients of Eqs (3) and (4) can be considered as the enthalpic contributions to the corresponding Gibbs free energy coefficients; these, in turn, are a measure of the interactions between pairs, triplets and higher numbers of solute molecules. However, they implicitly also account for the variation of the solute-solvent and solvent-solvent interactions as compared to the standard states [10, 11, 20].

Treatment of data

The self interaction coefficients h_{xx}, h_{xxx}, etc. are evaluated by fitting the heats of dilution for the binary solutions with the polynomial expression:

$$\Delta_{dil} H(m_i \rightarrow m_f) = h_{xx}(m_f - m_i) m_f + h_{xxx}(m_f^2 - m_i^2) m_f + \ldots \tag{5}$$

where m_i and m_f are the molalities of the solutions before and after dilution, respectively.

The cross coefficients h_{xy}, h_{xxy}, h_{xyy}, etc are evaluated by using an auxiliary function, ΔH^{**}, defined as follows:

$$\Delta H^{**} = \Delta_{dil} H(x, y) - \Delta_{dil} H(x) - \Delta_{dil} H(y) \tag{6}$$

where $\Delta_{dil}H(x, y)$ is the heat of dilution of the ternary solutions. ΔH^{**} is related to the cross coefficients as follows:

$$\Delta H^{**} = 2h_{xy}m_y^f(m_x^f - m_x^i) + 3h_{xxy}m_y^f(m_x^f - m_x^i)(m_x^f + m_x^i) + \\ + 3h_{xyy}m_y^f(m_x^f - m_x^i)(m_y^f + m_y^i) + \ldots \tag{7}$$

The self and cross coefficients are obtained through the interpolation of Eqs (5) and (7) by a least square method.

Results and discussion

Tables 1–2 report the experimental values of the heats of dilution of the binary solutions of each enantiomeric form of the peptido-amide considered, *D*- and *L*-NAAA, along with the initial and final molalities for each experiment.

Table 3 gives the heats of dilution of the ternary solutions of mixtures of the two enantiomers, together with the corresponding auxiliary function H^{**}, and the initial and final aquo-molalities of the two solutes for each experiment.

Table 1 Heats of dilution of *N*-acetyl-*D*-alaninamide (D-NAAA) in water at 25.0°

m_i	m_f	$-\Delta_{dil}H$(J/mol)
0.09619	0.04549	14.8
0.1050	0.04950	14.8
0.1639	0.07687	21.8
0.1972	0.08448	25.4
0.2106	0.09872	27.0
0.2210	0.1028	31.0
0.2238	0.1050	32.1
0.2134	0.1001	32.7
0.2573	0.1334	35.3
0.2374	0.1113	37.0
0.2765	0.1432	38.0
0.2986	0.1563	40.5
0.3262	0.1523	49.1
0.4616	0.2134	64.8
0.4547	0.2106	68.6
0.4770	0.2210	72.1
0.4845	0.2238	74.2
0.4150	0.2374	79.1

Table 2 Heats of dilution of *N*-acetyl-*L*-alaninamide (L-NAAA) in water at 25.0°

m_i	m_f	$-\Delta_{dil}H$(J/mol)
0.06951	0.03648	8.98
0.1684	0.08866	19.1
0.1327	0.06951	19.8
0.1842	0.09693	25.0
0.1623	0.07650	25.6
0.2209	0.1036	29.0
0.2293	0.1096	31.8
0.2546	0.1327	35.8
0.2670	0.1249	38.4
0.3070	0.1433	43.9
0.3301	0.1539	47.3
0.3482	0.1623	50.2
0.3807	0.1775	53.8
0.3947	0.1811	58.2
0.4118	0.1889	62.2
0.4971	0.2546	68.7
0.4770	0.2209	68.6
0.4971	0.2293	73.9

Table 3 Heat of diluiotn of ternary aqueous solutions containings both *D*- and *L*-NAAA at 25.0°

m_L^i	m_L^f	m_D^i	m_D^f	$-\Delta_{dil}H$(J/kg)	$-\Delta H^{**}$(J/kg)
0.09557	0.04509	0.09665	0.04562	2.49	1.21
0.09974	0.04700	0.1003	0.04727	2.69	1.24
0.08937	0.04609	0.09044	0.04663	2.38	1.27
0.1313	0.06361	0.1196	0.05613	4.49	2.32
0.1268	0.05970	0.1294	0.06093	4.74	2.48
0.1374	0.06450	0.1383	0.04931	5.39	2.77
0.1556	0.07264	0.1566	0.07310	6.83	3.48
0.1529	0.07183	0.1560	0.07329	7.00	3.71
0.1659	0.07770	0.1693	0.07930	8.05	4.19
0.1774	0.08507	0.1785	0.08559	8.89	4.53
0.1810	0.07881	0.1649	0.07180	8.60	4.54
0.1844	0.08937	0.1866	0.09044	9.34	4.60
0.1798	0.08430	0.1834	0.08598	9.45	4.91
0.1865	0.08701	0.1876	0.08753	9.95	5.15
0.1942	0.09077	0.1965	0.9184	10.7	5.45
0.2042	0.09557	0.2066	0.09669	11.9	6.10
0.2145	0.09974	0.2158	0.1003	13.2	6.81
0.2842	0.1313	0.2589	0.1196	21.0	10.9

Table 4 lists the experimental self and cross interaction coefficients for the three systems studied.

Due to the limited range of concentration explored, only the pairwise parameters are needed for the interpolation of Eqs (5) and (7), giving values for h_{xx} and h_{xy} significant within the 95% confidence limits.

It is clear from the values given in Table 4 that for NAAA the value of the cross coefficient, $H_{DL} = 294(5)$ (where the number in parentheses is the 95% confidence limit) is distincly different from those of the homogeneous coefficients, $h_{DD} = 273(5)$ and $h_{LL} = 279(5)$, which are the same, as expected within the limits of experimental errors. The result obtained in this work for NAAA are in good agreement with those obtained by Lilley and coworkers [14].

Table 4 Enthalpic pairwise interaction coefficients for *D*- and NAAA in water at 25.0°C

$h_{DD} = 278 \pm 5$
$h_{LL} = 273 \pm 5$
$h_{DL} = 294 \pm 5$

Units: J/mol $(\text{molkg}^{-1})^{-1}$. The given uncertainities are the 95% confidence limits

The positive values of the coefficients h_{DD}, h_{LL} and h_{DL} must be attributed to the prevailing hydrophobic interactions in aqueous solutions of this kind of solute. As for alcohols [18, 20], alkylamides [21, 22] and alkylureas [23–26], the excess thermodynamic properties of aqueous solutions of peptides seem to be determined by the existence of weak, non-bonding, water-mediated interactions [7–13]. The hydrophobic interactions, like the polar ones, make favourable contributions to the free energy coefficients, which are overall negative. However, the negative value is determined by a negative enthalpic contribution from the polar hydrophilic interactions, and by a positive entropic contribution from the hydrophobic interactions. The latter contribution is much larger than the corresponding positive enthalpic contribution. In other words, for the hydrophobic interactions:

$$g_{HI} = h_{HI} - Ts_{HI} < 0 \tag{8}$$

$$Ts_{HI} > h_{HI} > 0 \tag{9}$$

However, especially in determining the positive values of h_{xy} for such "mixed" solutes bearing both alkyl groups and polar functions, an important role is also played by the "mixed interactions" polar-apolar, as outlined by Wood and coworkers [21, 22].

The higher value of h_{DL} than those of h_{DD} and h_{LL} apparently indicates a more unfavourable enthalpic contribution to the solute-solute pairwise interaction in the case of the pair $D-L$ than for the homogeneous pairs $D-D$ and $L-L$. However, unless free energy data, when available, suggest different possibilities, it is reasonable to assume that the higher value of h_{DL} is due to slightly more favourable hydrophobic interactions in the case of the chiral pairs relative to the case of homogeneous pairs of solute molecules. This would result in an even more positive value of the entropic contributions and finally in a more negative value of the free energy coefficient g_{DL} relative to g_{DD} and g_{LL}. Since both polar and hydrophobic interactions in water seem to be solvent-mediated, an important conclusion of this work is that the hydration shells maintain a memory of the asymmetry of the solute, and this fact plays role in the overlapping of the cospheres.

It is also very interesting to point out that the fact that enantiomeric forms are able to distinguish each other even in solution has been revealed by a classical thermodynamic approach. In other words, calorimetry is demonstrated to be a powerful tool to reveal evidence of extremely subtle indications of the existence of specific molecular interactions in solution, which have not been found by using chirooptical and spectroscopic approaches.

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Zusammenfassung — Die Verdünnungswärmen von binären und ternären wässrigen Lösungen der zwei enantiomeren Formen von N-Acetylalaninamid wurden bei 25 °C gemessen. Die in einer Virialexpansionsreihe entwickelten Überschußenthalpien ermöglichen die Berechnung der „self" und „cross" Enthalpiekoeffizientenpaare. Was die optisch aktiven Formen einiger Monosaccharide anbetrifft, so ist der „cross" Koeffizient für die Wechselwirkung zwischen der D- und L-Form von N-Acetylalaninamid etwas, aber signifikant verschieden von dem entsprechenden „self" Koeffizienten. Es kann angenommen werden, daß Paare von Amidmolekülen eine schwache, durch Wasser vermittelte Unterscheidungsfähigkeit hinsichtlich der optischen Aktivität zeigen.

Резюме — При температуре 25° измерены теплоты разбавления в воде двуз- и трехкомпонентных растворов двуз энантиоморфных форм N-ацетилаланинамида. Величины избыточных энтальпий позволили оценить энтальпийные коэффициенты самоспаривания и перекрестного спаривания. значения перекрестных коэффициентов взаимодействия Д и L-форм N-ацетилаланинамида, как и в случае оптически активных форм некоторых моносахаридов, достаточно малы, но значительно отличаются от коэффициентов самоспаривания. Выдвинута гипотеза о наличии слабого взаимодействия между парами отпически активных амидных молекул.