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CALORIMETRIC DETERMINATION OF CHIRAL INTERACTIONS IN AQUEOUS SOLUTIONS.
Part 2 N-ACETYL-LEUCINAMIDE AT 298.15 K.

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SUMMARY

The heats of dilution in water of the binary and ternary solutions of the two enantioneric forms of N-acetyl-leucinamide have been determined at 298.15 K using a microcalorimetric procedure. The excess enthalpies obtained by these data, and expressed as virial expansion series, permit the evaluation of the pairwise homotactic and heterotactic enthalpic coefficients. As for the chiral forms of other N-acetyl-peptidoamides and of a few monosaccharides, the etherotactic coefficient for the interactions between the D- and L- enantiomers is slightly but significantly different from the corresponding homotactic coefficients . A weak solvent-mediated chiral recognition can be assumed to still exist in a polar screening solvent, such as water.

INTRODUTION

Aqueous solutions containing aminoacids and simple peptides have received increasing attention in the last years (refs.1-15). These studies have been stimulated by the hope of gaining an insight into the factors determining intramolecular interactions in proteins and naturally occurring peptides. Lilley suggested (refs.3,6,14) to use, as a model for polypeptides, of uncharged molecules with the general formula:

$$CH_3\left[CO- \quad -NHCHRCO-\right]_n -NHR'$$

with n = 1,2,3...etc., R being the side chain of an amino acid and $R' = -H, -CH_3$. A series of di- and tripeptidoamides with specific sequences have also been synthesized in his laboratory

The aim of the present work, however, is to study these amino acid derivatives not as model molecules, but with regards to the possibility of using calorimetry to find evidence of chiral recognition in aqueous solutions between pairs of enantiomeric molecules. In a crystal the properties of a racemate differ markedly from those of the pure D- and L- enantiomers; this is primarily due to differences in intermolecular distances and topological correlations between the groups of atoms, which produce interactions of different intensity. Often, for reasons of symmetry, the interactions are more effective in the racemate, resulting in a decrease of solubility. In solution the interactions between solute molecules are made weaker by solvation. This is particularly effective in the case of a polar solute and a polar solvent such as water. The screening of an optically inactive solvent of this kind could make the solute-solute interactions partially ineffective, especially in a dilute solution. However, we were successful in finding evidence of a chiral recognition or discrimination in the unfavorable case of the D- and L-pentoses xylose and arabinose (ref.16), whose solutions are prevailingly characterized by solute-solvent interactions (ref.17).

As a part of a program on the calorimetric study of the chiral interactions in water, we present here the results obtained on binary and ternary aqueous solutions of the N-acetyl-leucinamide (NALA) enantiomers. As in the first paper of this series (ref.10) concerning N-acetyl-alaninamide enantiomers, the excess enthalpies $H^E(m_D, m_L)$, $H^E(m_D)$ and $H^E(m_L)$ have been determined by measuring the heats of dilution of the corresponding ternary and binary solutions. The leucine derivatives were studied because it is important to investigate how chiral interactions are modified by the dimension and shape of the alkyl chains linked to the chiral carbon atom C_α . The results show that, beyond the 95% confidence limits, the interaction between the D- and L- enantioners is less endothermic (about 5% with respect to the D-D or L-L interactions). This is exactly the opposite of what was found by Lilley (ref.11) and by us (ref.10) for N-acetyl-alaninamide. It will be discussed in terms of solvated functional group interactions .

EXPERIMENTAL

Materials

Preparation of the N-acetyl-L-leucinamide has been described by Blackburn (ref.3) and the synthesis of the D-enantiomer is a modification of this procedure. The methyl ester of D-leucine was obtained by treating the aminoacid with methanol in the presence of a small excess of $SOCl_2$. The methyl ester hydrochloride so obtained, was crystallized from methanol-ether mixtures. The purified product was dissolved in dry pyridine at 0°C and acetic anhydride was added. After a few hours of stirring at 0°C the excess of solvent was removed and the dry N-acetyl-methyl ester of D-leucine was dissolved in dry methanol, saturated with gaseous NH_3, and gently shaken at room temperature. The solvent was removed <u>in vacuo</u> and the product repeatedly crystallized from methanol-ether mixtures. The accomplishment of each step was controlled by t.l.c. and polarimetry. The purity of the product was verified by melting point. All the aqueous solutions were freshly prepared with deionized, twice distilled and degassed water.

Calorimetry

The heats of dilution of binary and ternary solutions were determined at 298.15 ± 0.02 K with a LKB 10700-1 standard flow microcalorimeter as reported reported in detail in preceding papers (refs.9,18,19).

THERMODYNAMICS

The excess enthalpies of an asymmetric mixture of nonelectrolytes (such as the aqueous solutions of solid organic compounds) can be defined as follows (refs.1,20,21):

$$H^E(\bar{m}) = H(\bar{m}) - H^{\ominus} - \sum_{x=1}^{n} m_x \bar{H}_x^0 \qquad (1)$$

where $H(\bar{m})$ and $H^E(\bar{m})$ refer to an amount of solution containing 1 kg of water and a total amount of $\bar{m}$ moles of all the solute species, so that $\bar{m} = \sum m_x$. $H_w^{\ominus}$ is the standard enthalpy of 1 kg of pure water and $\bar{H}_x^0$ are the limiting standard partial molal enthalpies of each solute species. Then, H^E represents the deviation from the behaviour of an athermal, ideal solution and its value is determined by the nature and intensity of all molecular interactions.

According to Mc Millan-Mayer solution theory (ref.21), as adapted to the aqueous non-electrolite solutions by Kauzmann and Friedman (refs.20,23), the excess enthalpies per kg of solvent of a mixture of two enantiomeric solutes, can be represented as:

$$H^E(m_D,m_L) = h_{DD}m_D^2 + 2h_{DL}\, m_D\, m_L + h_{LL}m_L^2 + h_{DDD}\, m_D^3 + 3h_{DDL}\, m_D^2\, m_L + \ldots \quad (2)$$

For the binary solutions of each enantioner Eq.2 assumes simpler forms

$$H^E(m_D) = h_{DD}m_D^2 + h_{DDD}m_D^3 + \ldots \quad (3)$$

and

$$H^E(m_L) = h_{LL}\, m_L^2 + h_{LLL}m_L^3 + \ldots \quad (4)$$

The coefficients of Eqs. (2)-(4) are the enthalpic contributions to the corresponding Gibbs free energy coefficients g_{DL} ,g_{DDL} etc., these in turn are a measure of the interactions between pairs, triplets and higher numbers of solute molecules .

As for the overall thermodynamic properties the correlation between the g and h coefficients is the classic one:

$$h_{xy} = -\left[\partial(g_{xy} / T) / \partial(1 / T)\right]_P \quad (5)$$

Moreover h_{xx} is correlated to g_{xx} and the corresponding coefficient e_{xx} of the excess internal energy $E^E(m)_x$ by the relationship :

$$h_{xx} = e_{xx} + \alpha RT^2\left[g_{xx} - \left(RT\, M_w / 2000\right) - (\phi^o_{Ex} / \alpha\, V^{*}_w)\right] \quad (6)$$

where α is the cubic expansion coefficient, M_w the molecular weight of the solvent and ϕ^o_{Ex} the limiting partial molal expansibility of the solute x and V^{*}_w the volume of 1 kg of pure water (ref.20). In principle, the virial coefficients can be evaluated (ref.24) by certain integrals of the radial distribution function, g(r), that in a dilute solution, can be assumed to be simply correlated to the potential of average force W(r) by:

$$g(r) = \exp\left[-W(r) / kT\right] \quad (7)$$

However, for a non spherical solvent, quantities $\langle g(r)\rangle$ and $\langle W(r)\rangle$ must be considered. They indicate an averaging procedure on the orientation of all the solvent molecules. For non spherical solute particles the averaging procedure must be repeated for all possible reciprocal orientations of two solutes particles: in this case, $\langle g(r,\Omega)\rangle$ and $\langle W(r,\Omega)\rangle$ are to be used, where Ω is a set of angles expressing their reciprocal orientations. This is the reason for which both the free energy and the enthalpy coefficients implicitely account for the variation of the solute-solvent and solvent-solvent interactions, as compared to the standard (infinitely diluted) solutions (ref.24).

TREATEMENT OF THE DATA

The homotactic virial coefficients h_{DD}, h_{LL} etc.are evaluated by fitting ther heats of dilution for binary solutions, with polynomial expansions as follows:

$$\Delta_{dil}H\ (m_{iD} \longrightarrow m_D) = -\ (dQ/dt)/P_w =$$

$$= h_{DD}(m_D - m_{iD})\ m_D + h_{DDD}(m_D^2 - m_{iD}^2)\ m_D + \ldots \tag{8}$$

where dQ/dt is the heat evolved in the unit time in the dilution process and P_w is the total mass flow rate of water flowing into the calorimeter. The m_{iD} and m_D are, the molalities of the solution before and after dilution respectively.

The heterotactic coefficients h_{DL}, h_{DDL} etc. are, viceversa, evaluated by using an auxiliary function ΔH^{**}, defined as (ref.19):

$$\Delta H^{**} = \Delta_{dil}H(D,L) - \Delta_{dil}H(D) - \Delta_{dil}H(L) \tag{9}$$

where $\Delta_{dil}H$, the heat of dilution of a ternary solution, is more explicitely given by :

$$\Delta_{dil}H\left[(m_{iD}\ ,\ m_{iL}) \longrightarrow (m_D\ ,\ m_L)\right] = -(dQ/dt)'\ /P_w \tag{10}$$

The ΔH^{**} is related to the heterotactic coefficients by the following expression (ref.17):

$$\Delta H^{**} = 2h_{DL}m_L(m_D-m_{iD}) + 3h_{DDL}m_L(m_D-m_{iD})(m_D+m_{iD}) + 3h_{DLL}m_L(m_D-m_{iD})(m_L+m_{iL})+\ldots \tag{11}$$

The fitting of Eqs. (11) and (14) by a least square method leads to the evaluation of the homotactic and heterotactic coefficients.

RESULTS

In Tables 1-2 the experimental values of the heats of dilution of the binary solutions of each enantiomeric D and L form of N-acetyl leucinamide (NALA) are reported, along with the initial and final molalities for each experiment.

In Table 3 the heats of dilution of the ternary solutions of the mixtures of the two enantiomers are reported together with the corresponding values of ΔH^{**} and the initial and final aquomolalities of the two solutes for each experiment. No particular effort was made to obtain equimolecular solutions.

TABLE 1

Heats of dilution of solutions of D-N-acetyl -leucinamide (D-NALA) in water at 298.15 K

m_i	m	$-\Delta_{dil}H$ (J/kg)
0.08550	0.04044	74.9
0.08617	0.04074	83.6
0.08829	0.04174	85.9
0.09988	0.04711	99.1
0.1578	0.07420	156
0.1660	0.07798	166
0.1850	0.08617	175
0.1818	0.08550	178
0.1898	0.08829	190
0.1963	0.09177	190
0.2085	0.09763	202
0.2158	0.09988	210
0.2506	0.1171	249
0.2703	0.1256	276
0.3161	0.1465	324
0.3578	0.1660	369
0.3779	0.1749	390
0.3936	0.1818	415
0.4263	0.1963	450
0.4539	0.2085	469
0.5435	0.2506	585

TABLE 2

Heats of dilution of solutions of L-N-acetyl -leucinamide (L-NALA) in water at 298.15 K

m_i	m	$-\Delta_{dil}H$ (J/kg)
0.06801	0.03213	76.1
0.07987	0.03782	81.6
0.09105	0.04200	93.2
0.1477	0.06801	155
0.1739	0.07987	177
0.1986	0.09105	197
0.1946	0.09140	201
0.2035	0.09554	204
0.2098	0.09809	213
0.2037	0.09530	214
0.2096	0.09823	215
0.2284	0.1088	228
0.2299	0.1063	240
0.2607	0.1217	274
0.3214	0.1492	335
0.3682	0.1700	391
0.4298	0.1982	443
0.4515	0.2098	478
0.4257	0.1895	479
0.4529	0.2037	507
0.5034	0.2290	543

TABLE 3

Heats of dilution of ternary solutions of D- and L-acetyl -leucinamide

m_{iD}	m_D	m_{iL}	m_L	$-\Delta_{dil}H$(J/kg)	H**(J/kg)
0.02198	0.01043	0.02436	0.01153	0.996	0.475
0.04204	0.01992	0.03464	0.01643	2.66	1.22
0.03380	0.01598	0.04129	0.01952	2.73	1.36
0.04972	0.02347	0.03105	0.01465	3.08	1.40
0.03681	0.01727	0.04164	0.01971	2.95	1.45
0.03873	0.01833	0.04052	0.01971	3.01	1.49
0.05096	0.02401	0.03182	0.01499	3.26	1.50
0.03835	0.01810	0.04338	0.02047	3.13	1.51
0.04803	0.02265	0.03571	0.01684	3.28	1.54
0.04332	0.01997	0.04427	0.02088	3.49	1.67
0.04016	0.01893	0.04308	0.02030	3.42	1.74
0.04762	0.02239	0.03876	0.01823	3.59	1.76
0.04449	0.02104	0.04653	0.02200	3.78	1.77
0.04133	0.01946	0.04675	0.02202	3.66	1.78
0.04489	0.02116	0.04816	0.02270	4.02	1.92
0.04352	0.02052	0.04923	0.02321	4.02	1.94
0.05780	0.02727	0.04298	0.02028	4.62	2.10
0.04566	0.02150	0.05165	0.02432	4.49	2.19

TABLE 4

Enthalpic pairwise interaction coefficients for D- and L-NALA and for D- and L-NAAA in water at 298.15 K

NALA	NAAA
$h_{DD} = 1919 \pm 28$	$h_{DD} = 278 \pm 5$
$h_{LL} = 1969 \pm 24$	$h_{LL} = 273 \pm 5$
$h_{DL} = 1822 \pm 41$	$h_{DL} = 294 \pm 5$

Units: J/mol (mol/kg). The given uncertainities are the 95% confidence limits.

In Table 4 the experimental homotactic and heterotactic interaction coefficients are reported for the three systems studied. Due to the limited range of concentration explored, only the pairwise parameters are needed for the interpolation of Eqs.(11) and (14), giving values for h_{DD} and h_{LL} differing significantly, beyond the 95% confidence limits. The values for D- and L-NAAA are also reported for comparison . It appears from the values given in TABLE 4 that for NALA the value of the heterotactic coefficient, h_{DL} = 1822(41) (where the number in parentheses is the 95% confidence limit) is distinctly different from those of the homotactic ones, h_{DD} = 1919(28) and h_{LL} = 1969(24), which are practically the same, within the limits of the experimental errors. The result obtained in this work for L-NALA is a little different from that obtained by Lilley and coworkers: 1714(94) (ref.3).

The positive value of the h_{DD} , h_{LL} and h_{DL} coefficients must be attributed to the prevailing hydrophobic interactions occurring in the aqueous solutions of this kind of solutes. As for alcohols (refs.20,21), alkylamides (ref.25) and alkylureas (refs.18,26), the excess thermodynamic properties of aqueous solutions of amidopeptides seem to be determined by the existence of weak, non-bonding, water-mediated interactions (refs.9-15,21). It must be remembered, while the polar interactions directly give a negative contribution to the excess free energy coefficients, the hydrophobic interactions, instead, are negative and favourable for the overwhelming entropic terms, the enthalpic contributions being by themselves unfavourable. However, as already outlined by Savage and Wood (ref.25), an important role is also played, by the "mixed interactions" (polar-apolar) in determining the positive values of h_{xx} for this kind of "mixed" solutes bearing both alkyl and polar groups.

The lower value of h_{DL} (respect to that of h_{DD} and h_{LL}) for NALA indicates a less unfavourable enthalpic contribution to the solute-solute pairwise interaction for the heterotactic pair. However, unless free energy data suggest different possibilities, it is reasonable to assume that the lower value of h_{DL} is due to a slightly less favourable hydrophobic interaction for the chiral as respect to the homotactic pairs. At all events, the differences ($h_{DL} - h_{DD}$) or ($h_{DL} - h_{LL}$) give a measure of the chiral recognition in solution. As can be seen from the data reported in Table 4, these differences, or their averages, change sign on passing from NAAA to NALA. The same effect has been found for a series of aminoacids (ref.27).

The key to the rationalization of these results stands from the consideration that the prevalence of the dipolar-dipolar or hydrophobic effect, is in turn responsible for the favourable interactions. This fact enhances one or another particular juxtaposition of a pair of solute molecules by coupling their polar or vicecersa hydrophobic faces. Repulsive polar-apolar interaction, then, can play an opposite role in the homotactic or in the heterotactic interactions, when lower or higher homologues of a series are considered.

A very interesting and important point is that calorimetry is demonstrated to be a powerful tool for providing the extremely subtle specific interactions in solution, not found chiroopticallу or spectroscopically.

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