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THERMOCHEMISTRY OF AQUEOUS SOLUTIONS OF ALKYLATED NUCLEIC ACID BASES. II. APPARENT MOLAR RELATIVE ENTHALPIES OF DILUTION OF SOLUTIONS CONTAINING URACIL AND THYMINE DERIVATIVES

Anna ZIELENKIEWICZ
Institute of Physical Chemistry, Polish Academy of Sciences, 01-224 Warszawa, Poland

Ewa PLESIEWICZ and Kazimierz L. WIERZCHOWSKI
Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-532 Warszawa, Poland

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Apparent molar relative enthalpies of dilution ϕ_L of aqueous solutions of a series of alkylated diketopyrimidines: m^1Ura, $m_2^{1,3}$Ura, $m_2^{1,3}$Thy, $m_3^{1,3,6}$Ura and $e_2^{1,3}$Thy were measured as a function of concentration of the solutes at three temperatures 298.15, 308.15 and 318.15 K. Dilution proved to be an endothermic process over the whole range of molalities m and temperatures studied for all compounds except the $e_2^{1,3}$Thy solution, the dilution of which, with the exception of the lowest concentrations ($m < 0.2$–0.3) was an exothermic process. Partial molar relative enthalpies of dilution $\bar{L}_2(m)$ derived from $\phi_L(m)$ functions were analysed as if they were composed of two additive contributions: an endothermic one $\bar{L}_2$, (m_1) and an exothermic one $\bar{L}_2$, (m_{as}), owing to the presence in the solutions of a free monomer m_1 or associated species m_{as}, respectively. Partial molar heat capacities of the solutes, evaluated by differentiation of $\bar{L}_2(m)$ functions in respect to temperature, decreased with the rise of concentration in the order of the tendency of the solutes to stacking association. Changes in heat content and in heat capacity of solutions upon their dilution are interpreted in terms of involvement of hydrophobic hydration and association of the solutes.

1. Introduction

In the first paper of this series [1] apparent molar heat capacities of aqueous solutions of several alkylated diketopyrimidines were reported. Their abnormally large values and their linear dependence on the number of hydrogen atoms covalently bonded was taken as a evidence of hydrophobic hydration of the solute molecules. The increments of partial molar heat capacity per each hydrogen atom were indeed very close to those found for homologous series of hydrocarbons [2], aliphatic amides [3] and various hydrocarbon derivatives bearing polar groups [4].

In this work the results of heat dilution studies over a broad range of solute molalities at several temperatures are presented and discussed in terms of involvement of hydrophobic hydration and interaction between solute molecules.

2. Materials and methods

All compounds investigated were the same as used previously in apparent molar heat capacity measurements [1]. Further characterization of their purity with the aid of the dynamic cryometer model TE-37/74 described elsewhere [5] indicated that they were at least 99.7–99.9 per cent pure.

Heats of dilution q_i/h_i in calories per mole of the solutes were measured in an LKB 10700-2 batch microcalorimetre. The procedure employed was to dilute the given solution containing n_i moles of a solute stepwise by using finite amounts of distilled water. Relative apparent molar enthalpies of dilution ϕ_L were then calculated by summing up the q_i/n_i values obtained for a given series of dilutions and correcting to infinite dilution. The correction term was evaluated by linear extrapolation to zero molality with the use of several q_i/n_i

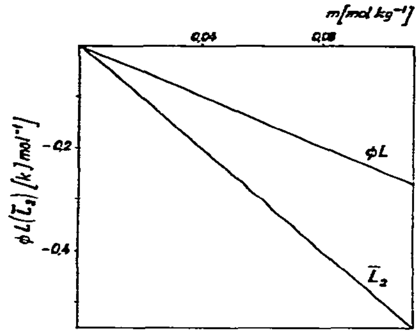

Fig. 1. Isotherms $\phi_L(m)$ and $\bar{L}_2(m)$ at 318.15 K for m^1Ura: range of dilution 0.117 m–0.026 m.

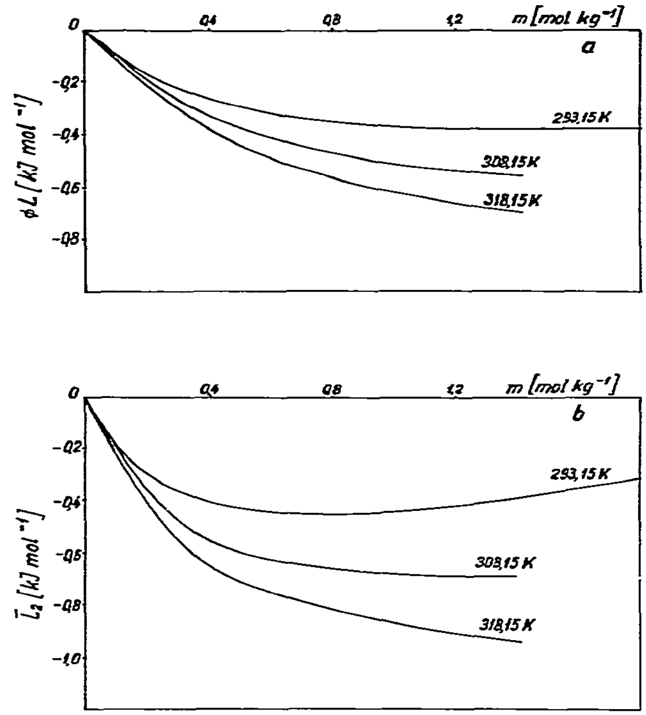

Fig. 2. Isotherms (a) $\phi_L(m)$ and (b) $\bar{L}_2(m)$ for $m_2^{1,3}$Ura; range of dilution: at 293.15 K from 1.830 m to 0.073 m, at 308.15 K from 1.430 m to 0.053 m and 318.15 K from 1.430 m to 0.068 m.

values measured at the highest dilutions. For each compound 2–3 dilution series were measured at 298.15, 308.15 and 318.15 K.

The microcalorimetre was calibrated by measurements of heats of dilution of aqueous sucrose solutions according to IUPAC recommendations [6]. The sample of sucrose used was obtained from the NBS Office of Standard Reference Materials. The measurements performed at 303 K in the concentration range of 0.043–0.209 mol kg^{-1} for two dilution series gave average values which could be fitted to an empirical equation: ϕ_L (cal mol^{-1}) = 140.4 m–8.09 m^2. Comparison of measured and standard [6] ϕ_L values showed that, at low molalities up to about 0.3, their agreement was better than within 0.1 per cent. At higher concentrations the measured values were somewhat smaller and deviated systematically from the calibration curve with a rise in m up to about 1 per cent at 1.4 m and 1.7 per cent at 2.2 m.

The heats of dilution measured for m^1Ura, $m_2^{1,3}$Ura and $m_2^{1,2}$Thy solutions were comparable to those obtained for sucrose at concentrations below 0.3 m, while those for $m_3^{1,3,6}$Ura and $e_2^{1,3}$Thy fell within the range of heats of sucrose dilution at initial concentrations between 0.3 and 1.4 m.

The microcalorimetre was also standardized after each measurement by means of the Joule effect.

The $\phi_L(m)$ isotherms (figs. 1, 2a–5a) were plotted according to smoothed and interpolated experimental ϕ_L data by means of the SMOCUB (PS-1004) procedure [7] based on the use of cubic splines. As an end condition for the interpolating function the zero value of its second derivatives in respect to molality was assumed. The smoothing procedure was modified in such a way as to permit several iterative calculations of ϕ_L data before their final interpolation. The condition was set up that smoothed ϕ_L values should not differ more than by 5 per cent from the experimental data.

$\partial\phi_L/\partial m$ were obtained by analytical differentiation of $\phi_L(m)$ functions evaluated as described in the preceding paragraph. Partial molar relative enthalpies of dilution $\bar{L}_2$ were eventually calculated from interpolated ϕ_L values and their derivatives in respect to molality: $\bar{L}_2 = \phi_L + m(\partial\phi_L/\partial m)$. Derivatives in respect to temperature: $\partial(\partial\phi_L/\partial m)/\partial T = \partial\phi C_p/\partial m$ and $\partial\bar{L}_2/\partial T = \bar{C}_{p2} - \bar{C}^0_{p2}$ were obtained by graphical differentiation.

All numerical calculations were performed on a CDC CYBER 73 60-bit word computer of series CDC 6400.

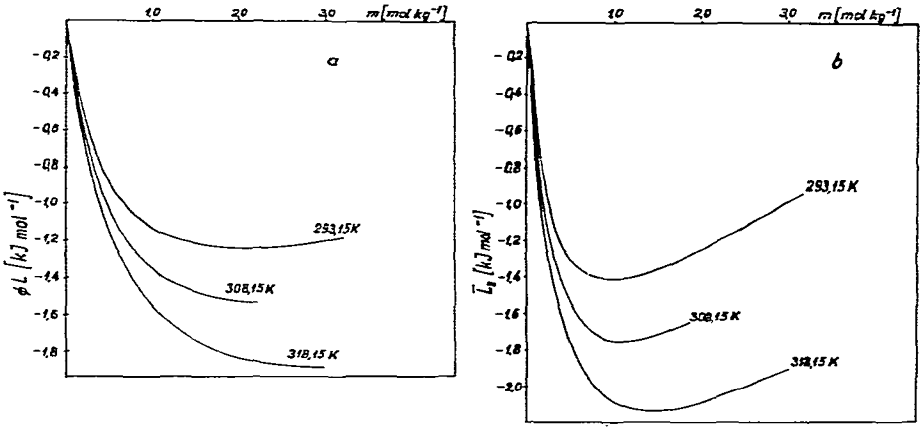

Fig. 3. Isotherms (a) $\phi_L(m)$ and (b) $\bar{L}_2(m)$ for $m_3^{1,3,6}$Ura; range of dilution: at 293.15 K from 3.531 m to 0.035 m, at 308.15 K from 2.206 m to 0.024 m and at 318.15 K from 3.336 m to 0.038 m.

3. Results and discussion

The isotherms $\phi_L(m)$ shown in figs. 1—4a indicate that (i) dilution of solutions containing m^1Ura, $m_2^{1,3}$Ura, $m_3^{1,3,6}$Ura and $m_2^{1,3}$Thy (i.e. $m_3^{1,3,5}$Ura) is an endothermic process, viz. $\Delta H_{m\to 0}/n_2 = -\phi_L > 0$, within the whole range of molalities and temperatures studied, (ii) only at the lowest initial concentrations corresponding to $m \lesssim 0.1$ ϕ_L varies almost linearly with m. Then ϕ_L concentration dependence becomes progressively less pronounced and $\phi_L(m)$ functions tend to attain a shallow minimum (cf. figs. 2a and 3a). (iii) As the temperature increases the net heat effects of dilution become more endothermic and the $\phi_L(m)$ functions begin to deviate from linearity at higher concentrations. In the case of $e_2^{1,3}$Thy, however, $\phi_L(m)$ isotherms (fig. 5a) follow this pattern only at the lowest initial molalities $m \lesssim 0.2$—0.3 and at temperatures above 298.15 K when dilution is still slightly endothermic; at 308.15 K and 318.15 K outside this concentration range and within the whole concentration range at 298.15 K dilution is an exothermic process with an almost linear dependence of ϕ_L on m at $m \gtrsim 0.2$—0.3.

The overal pattern exhibited by $\phi_L(m)$ isotherms becomes even more pronounced after their transformation to $\bar{L}_2(m)$ functions of partial molar relative enthalpy of dilution of the solutes (figs. 1—5b), owing to the relatively large $m(\partial\phi_L/\partial m)$ terms at low molalities which add to ϕ_L yielding $\bar{L}_2$.

The observed heat effects of dilution suggest that

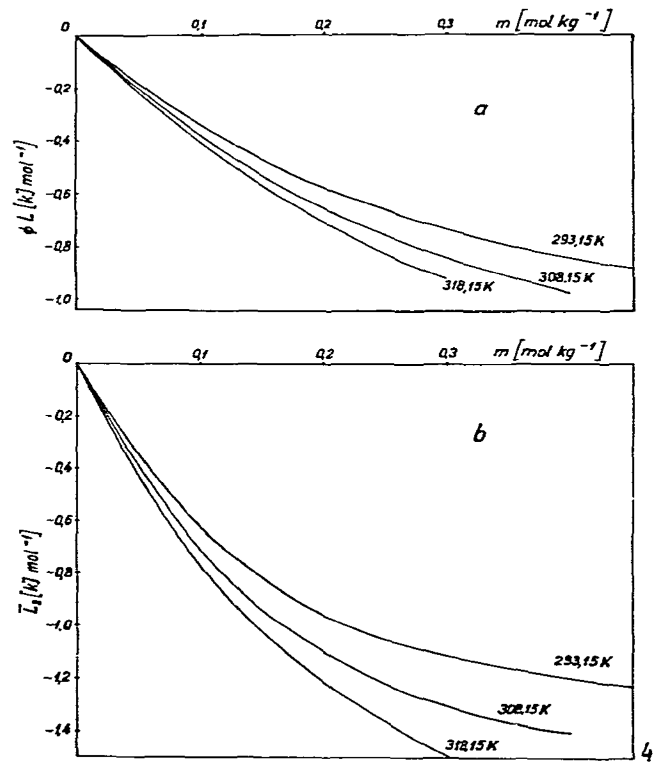

Fig. 4. Isotherms (a) $\phi_L(m)$ and (b) $\bar{L}_2(m)$ for $m_2^{1,3}$Thy ($m_3^{1,3,5}$Ura); dilution range at 293.15 K from 0.455 m to 0.033 m, at 308.15 K from 0.449 m to 0.018 m and at 318.15 K from 0.480 m to 0.025 m.

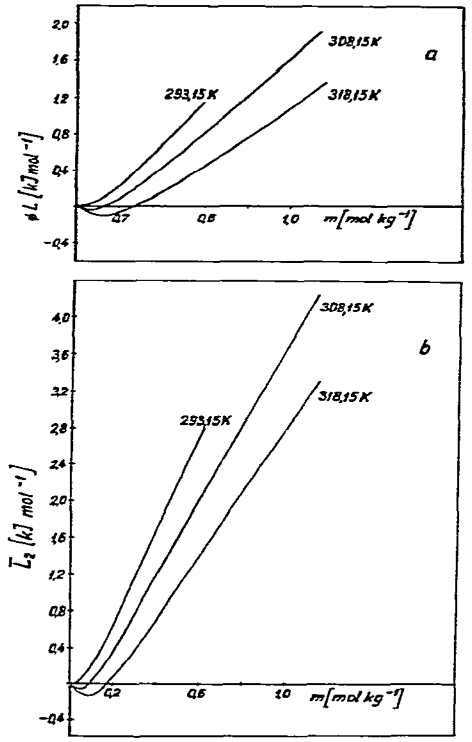

Fig. 5. Isotherms (a) $\phi_L(m)$ and (b) $\bar{L}_2(m)$ for $e_2^{1,3}$Thy; dilution range at 293.15 K from 0.649 m to 0.053 m, at 308.15 K from 1.195 m to 0.058 m and at 318.15 K from 1.195 m to 0.026 m.

dilution is accompanied by two composite processes of opposite sign of enthalpy changes, of which the endothermic one prevails at low solute concentrations and at higher temperatures, while the exothermic one begins to manifests itself at higher concentrations and at lower temperatures. The relative contributions of both processes vary from compound to compound but do not seem to follow their order in the homologous series. In order to provide a basis for molecular interpretation of the two thermochemical processes implicated, we took into account the results of vapour pressure osmometric studies of self-association of alkyluracils in water [8]. They demonstrated that in the first approximation the isodesmic model of infinite association, which assumes equality of all consecutive equilibrium constants and enthalpies of association, could be fitted to the experimental osmotic coefficients.

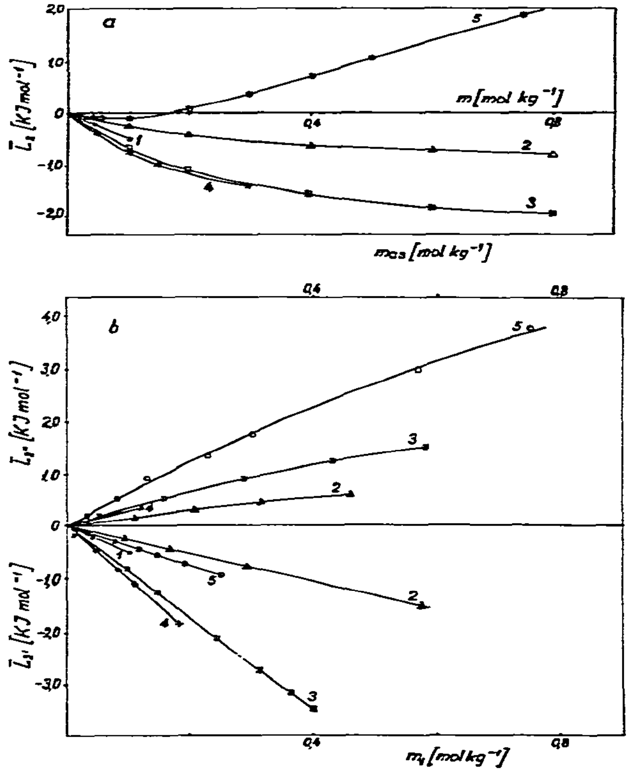

Fig. 6. Comparison of (a) $\bar{L}_2(m)$ isotherms at 318.15 K for m^1Ura(1), $m_2^{1,3}$Ura(2), $m_3^{1,3,6}$Ura(3), $m_2^{1,3}$Thy(4) and $e_2^{1,3}$Thy(5), and (b) of respective $\bar{L}_2'(m_1)$ and $\bar{L}_2''(m_{as})$ isotherms derived from $\bar{L}_2(m)$ functions as explained in the text.

Using association equilibrium constants thus derived we calculated the molal concentrations of solutes present in solutions as monomers m_1 and as associated species $m_{as} = m - m_1$ at different stoichiometric molal concentrations m of various solutes studied, and then analysed the $\bar{L}_2$(m) isotherms as if they were made up by addition of two components $L_2'(m_1)$ and $L_2''(m_{as})$ the first of which would correspond to the endothermic process of dilution of monomers and the other one to an effectively exothermic composite process of dilution and dissociation of aggregates. In doing so we assumed that the initial slopes $\partial\bar{L}_2/\partial m$ of $\bar{L}_2(m)$ isotherms corrected, if necessary, for the presence of minute amounts of associated species resemble closely linear $\bar{L}_2(m_1)$ functions. The results of such analysis of $\bar{L}_2(m)$ isotherms at 318 K are shown in fig. 6.

Let us first consider family of $\bar{L}_2'(m_1)$ plots. Molecules of alkyluracils are highly hydrated in aqueous solutions, owing to the presence of two secondary amide carbonyls and hydrophobic structuring of water around their apolar parts bearing C—H and alkyl groups [1,9]. Enthalpy of interaction, of uracil with water molecules in the hydration shell at infinite dilution, ΔH^0_{int}, is close to -60 kJ mole^{-1} and its absolute value decreases on N-methyl substitution approximately 6.5 kJ per mole of CH_2 added but becomes more negative by about the same increment on C(5) or C(6) substitution with methyl group [9]. At low solute concentrations, when practically only monomeric species are present in solutions, the solute molecules face predominantly the aqueous environment. Endothermicity of dilution of such solutions can be thus attributed to a decrease in the extent of structure promotion in the solvent network contained between solute molecules, owing to long-range hydrophobic interactions [10] and possibly also to analogous interactions induced by carbonyl groups of molecules or, alternatively, to a shift in highly cooperative multiplet hydration equilibria involving alkyl groups [11]. Presence of long-range hydrophobic interactions can be inferred from the relative positions of the $\bar{L}_{2'}(m_1)$ isotherms for solutions of compounds of the same hydrogen-bonding capacity toward water via their carbonyl groups: $m_2^{1,3}$Ura, $m_2^{1,2}$Thy, $m_3^{1,3,5}$Thy and $m_3^{1,3,6}$Ura. Similar slopes of the isotherms for the two tri-methylated isomers viz. $m_3^{1,3,5}$Ura and $m_3^{1,3,6}$Ura and a much lower slope of that for $m_2^{1,3}$Ura support this view. Enthalpies of interaction of the solutes with their hydration shells [9] evidently correlate to some extent with the magnitude of negative heat effect upon dilution. On the other hand, steric shielding of the two amide carbonyls of adjacent N-alkyl groups, accompanied by a decrease in ΔH^0_{int}, diminishes magnitude of the heat effect; cf. $\bar{L}_{2'}(m_1)$ isotherms 1 and 2 in fig. 6b for m^1Ura and $m_2^{1,3}$Ura, respectively. Lower endothermicity of dilution of $e_2^{1,3}$Thy solutions as compared with that of $m_2^{1,3}$Thy solutions results obviously from stronger steric shielding of both carbonyls by N-ethyl than by N-methyl groups for in the former case $\Delta H^0_{\text{int}} = -30.6$ kJ mole^{-1} while in the latter $\Delta H^0_{\text{int}} = -54.0$ kJ mole^{-1} [9]. On the basis of this large difference in interactions enthalpies one would expect much larger than actually observed an effect of carbonyl groups shielding on heat of dilution of $e_2^{1,3}$Thy solutions. Steric shielding seems thus to be partially recompensated by long-range hydrophobic structuring of water between ethyl groups on different molecules. It is this interaction, which induces also entropy driven solute self-association even at its comparatively low concentrations, cf. respective isotherm $\bar{L}_{2''}(m_{\text{as}})$ in fig. 6a.

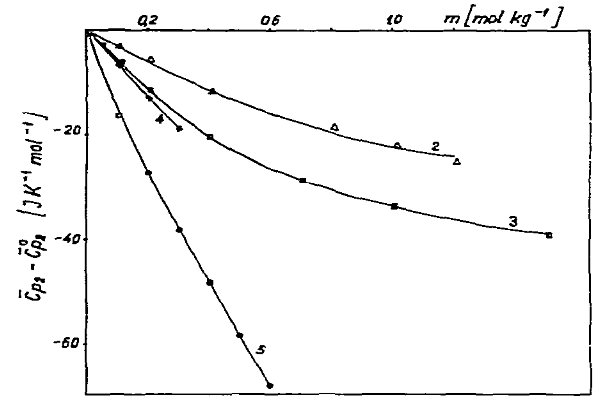

Fig. 7. Changes in partial molar heat capacities $\Delta\bar{C}_{p2} = \bar{C}_{p2} - \bar{C}^0_{p2}$ with concentration of solutes obtained by differentiation of $\bar{L}_2(m)$ functions with respect to temperature: $m_2^{1,3}$Ura(2), $m_3^{1,3,6}$Ura(3), $m_2^{1,3}$Thy(4), $e_2^{1,3}$Thy(5).

Similar analysis of isotherms $\bar{L}_2(m)$obtained at 298.5 and 308.15 K confirmed the earlier conclusions concerning the temperature dependence of endothermic and exothermic processes based on inspection of the isotherms solely (figs. 1b—5b), viz. the increase of the endothermicity of the monomer dilution and decrease of the exothermicity of dilution and dissociation of aggregates. The temperature dependence of $\bar{L}_{2'}(m_1)$ functions seems thus to remain in agreement also with the postulated involvement of the long-range through-solvent interactions between solute molecules, aided by structure promotion in the water bulk. As the temperature increases more water bulk molecules with one or more broken hydrogen bonds become available for their specific orientation and structuring around and in between the molecules of hydrophobic solutes. This should result in a larger negative heat effect upon dilution, what is actually observed (cf. figs. 1—5), and in an increase of the partial molar heat capacity of solutes $\bar{C}^0_{p2}$ with temperature, as shown previously [1] to be also the case.

Exothermicity of dilution and dissociation of aggregates (cf. $\bar{L}_{2''}(m_{\text{as}})$ plots in fig. 6b) can be most easily rationalized as a net effect of inherently endothermic aggregate dilution and dissociation processes and the exothermic effect of hydrophobic and hydrogen bond hydration processes of monomer molecules formed upon dissociation. The latter processes should decline with a rise of temperature, as actually observed. This explanation is consistent with the interpretation of the predominantly entropy-driven aggregation of essentially hydrophobic solutes [10]. It finds additional support in the changes of partial molar heat capacities obtained by differentiation in respect to temperature of the $\bar{L}_2(m)$ functions: $\partial \bar{L}_2 / \partial T = \bar{C}_{p2} - \bar{C}^0_{p2}$ (fig. 7). The negative values of differences between partial molar heat capacities at a given molality, $\bar{C}_{p2}$, and those at infinite dilution, $\bar{C}^0_{p2}$, in the whole range of concentrations studied indicate that always $\bar{C}_{p2} < \bar{C}_{p2}$. Furthermore, $\bar{C}_{p2}$ decreases with a rise in concentration of alkyluracils in the order of the growing tendency of the compounds to self-association [8] in water: $m_2^{1,3}$Ura $< m_3^{1,3,5}$Ura $\approx m_3^{1,3,6}$Ura $< e_2^{1,3}$Thy. Large partial molar heat capacities of alkyluracils in aqueous solutions, proportional to the number of hydrogen atoms owing to hydrophobic hydration of the solute molecules [1], decrease thus upon association because of partial reversal of hydration of the molecules contained in aggregates.

At lowest solute molalities, when concentration of associated species is very small [8], $\bar{C}_{p2}$ also decreases with growing m. This may indicate that either the effect of hydrophobic structuring of water on $\bar{C}_{p2}$ has the same sign or it is hidden by a larger self-association negative effect.

Partial molar heat capacity, however, is not a sensitive function of hydrophobic self-association of alkyluracils, for changes in $\bar{C}_{p2}$ do not exceed 10 per cent at concentrations corresponding to molar fractions of associated monomers as high as 0.6–0.7. The same conclusion follows from the temperature dependence of $\partial \phi_L / \partial m$ derivatives: $\partial(\partial \phi_L / \partial m) / \partial T = \partial \phi C_p / \partial m$ (table 1). At concentrations of the order of 0.01–0.2 m the $m(\partial \phi C_p / \partial m)$ terms contribute but little to the measured apparent molar heat capacities ϕC_p [1], what explains their apparent insensitivity to concentration. Such highly conservative behaviour of the degrees of freedom in the solutions studied, in spite of their extensive deviations from ideality, suggests that solute molecules associate probably in such a way that most of their hydrogen atoms remain surrounded by water so that an approciable reversal of hydrophobic hydration does not occur. Stacking self-association implicated previously [8] as the main mode of association of variously substituted diketopyrimidines in water may satisfy this condition.

Table 1
Values of $\partial(\partial \phi_L / \partial m) / \partial T = \partial \phi C_p / \partial m$ derivatives obtained by graphical differentiation of $\partial \phi_L / \partial m$ versus T linear plots in the temperature interval of 298.15–318.15 K.

Solute	Derivative (J mole^{-2} K^{-1})	
$m_2^{1,3}$Ura	–10.0	(apparently independent of the solute concentration m)
$m_3^{1,3,6}$Ura	–26.8	
$m_2^{1,3}$Thy	–33.4	
$e_2^{1,3}$Thy	–50.0 – (–) 63.0	(varies gradually in the concentration range of 0.05–0.6 m)

Attempts at fitting of an equation [12] relating apparent molar relative heats of dilution ϕ_L, isodesmic equilibrium association constants and enthalpies of association to measured ϕ_L values were not very successful. Plots of ϕ_L against $\phi_L(m)^{1/2}$ the slopes of which should yield according to the equation: $\phi_L = \Delta H - (\Delta H / K_{as})^{1/2} \phi_L(m)^{1/2}$, enthalpies of association ΔH, proved non-linear in all cases and exhibited a more pronounced downward curvature for solutions containing alkyluracils characterized by larger association constants K_{as} [8]. Enthalpies of association vary thus with temperature. A similar conclusion was arrived at when enthalpies of association were calculated directly from ϕ_L and osmotic coefficient φ [8], data: $\Delta H = \phi_L(1 - \varphi)$ [12]. The varying degree of hydration with temperature and of solute distribution among various aggregates towards more predominantly hydrophobically stabilized ones as temperature increases seem to be mainly responsible for such a variation of ΔH with temperature.

Much larger negative ϕ_L values were found in water for 6-methylpurine, $n_H = 6$ [12], and 6-dimethylaminopurine, $n_H = 9$ [13], than those measured in this work for diketopyrimidines bearing a similar number of hydrogen atoms. As compared with pyrimidines, purines exhibit in water a higher tendency to stacking association which is characterized by negative enthalpy changes larger by one order of magnitude [14], indicative of the predominately electrostatic nature of intermolecular interactions. At first sight this seems to explain the larger enthalpies of dilution referred to above. However, more systematic concentration and temperature studies on ϕ_L would be required in order to elucidate the association and hydration effects which contribute to the observed heat of dilution isotherms.

Acknowledgements

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