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# THERMOCHEMISTRY OF AQUEOUS SOLUTIONS OF ALKYLATED NUCLEIC ACID BASES. I. APPARENT MOLAR HEAT CAPACITIES OF URACIL, THYMINE AND THEIR DERIVATIVES

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Apparent molar heat capacities $\phi C_p$ of uracil, thymine and a series of their alkylated derivatives: $m^1$Ura, $m_2^{1,3}$Ura, $m_2^{1,3}$Thy, $m_3^{1,3,6}$Ura, $m_2^{1,3}e^5$Ura and $e_2^{1,3}$Thy in dilute aqueous solutions were measured in the temperature range of 293.15–388.15 K, using a differential adiabatic scanning microcalorimeter. They were found to be (i) much higher than the estimated heat capacities $C_p$(s) of solid compounds, (ii) comparable with the respective partial molar heat capacities at infinite dilution, $C_{p2}^0$, and (iii) linearly related to the number $n_H$ of hydrogen atoms covalently bound to the solute molecules. The increment thus obtained $\Delta C_{p2}^0 = 42.8$ J mole$^{-1}$ K$^{-1}$ $n_H^{-1}$ per each hydrogen atom at 298.15 K proved (i) to coincide closely with those found previously for homologous series of aliphatic amides and hydrocarbons, and (ii) to decrease with a rise of temperature. These findings imply the involvement of hydrophobic hydration of the solutes.

## 1. Introduction

Methyl and other alkyl groups on pyrimidine and purine bases are known to play an important role in the establishment of biologically active conformations of nucleic acids, tRNA and mRNA in particular, and in the recognition and modification of specific sequences thereof by various regulatory and enzymatic proteins [1,2]. They were also shown to contribute significantly to stacking interactions between both purine [3] and pyrimidine [4,5] bases in aqueous solutions. On the basis of thermodynamic self-association parameters of alkylated uracils, derived from vapour pressure osmometric studies, this contribution has been demonstrated to be due to involvement of classical hydrophobic interactions between the apolar regions of molecules [4]. The inadequacy of the assumptions underlying the molecular association models commonly used in interpretation of osmometric data led us to direct calorimetric studies of the thermochemical properties of aqueous solutions of selected alkyl-uracils and -thymines in order to obtain an independent evidence as to the involvement of hydrophobic hydration of, and interaction between the solutes. Preliminary results of these studies have already been reported [6,7]. In this paper we present results of apparent molar heat capacity measurements. Partial molar heat capacities of alkyl-substituted non-electrolytes in aqueous solutions are known [8] to reflect specific interactions of solute molecules with water and to induce thereby structuring of the neighbouring water molecules, referred to as "hydrophobic hydration". So far however, very limited data concerning heat capacities of aqueous solutions of nucleic acid bases are available. Only changes in molar heat capacity calculated from calorimetric heats of solution of thymine [9,10] uracil [11], cytosine [12], adenine [13] and of two methylated xanthines [14] have been reported.

In the second part of this paper [15] results of heat dilution studies are presented. Determination of

Table 1
Apparent molar heat capacities $\phi C_p \approx \bar{C}^0_{p2}$ (partial molar heat capacity at infinite dilution) of diketopyrimidines in aqueous solution at various selected temperatures (in parentheses standard deviation $\sigma$), parameters $a$ and $b = \Delta\bar{C}^0_{p2}$ obtained by least squares fitting of an equation $\bar{C}^0_{p2} = a + b \cdot n_H$ to the $\bar{C}^0_{p2}$ data ($n_H$-number of hydrogen atoms per molecule of a given solute), and estimated [a] heat capacities of solid compounds $C_p(s)$

| Compound | $n_H$ | $\phi C_p \approx \bar{C}^0_{p2}$ J mole$^{-1}$ K$^{-1}$ | | | | $C_p(s)$ J mole$^{-1}$ K$^{-1}$ |
|---|---|---|---|---|---|---|
| | | 298.15 K | 318.15 K | 333.15 K | 363.15 K | |
| Uracil (Ura) | 4 | 137 (5) | 167 (7) | 206 (4) | 264 (10) | 120.5 ref. [10] |
| Thymine (Thy) | 6 | 220 (8) | 250 (16) | 290 (14) | 343 (8) | 150.4 ref. [10] |
| 1-methyluracil ($m^1$Ura) | 6 | 205.0 (4.9) | 225.9 (3.2) | 238.7 (3.5) | 255.9 (3.8) | ~150 |
| 1,3-dimethyluracil ($m^{1,3}_2$Ura) | 8 | 295.0 (7.6) | 313.2 (7.4) | 325.1 (6.8) | 351.5 (7.3) | ~180 |
| 1,3,6-trimethyluracil ($m^{1,3,6}_3$Ura) | 10 | 357.2 (3.7) | 371.9 (3.5) | 380.5 (3.2) | 394.2 (3.5) | ~210 |
| 1,3-dimethylthymine ($m^{1,3}_2$Thy) | 10 | 373.0 (9.0) | 387.9 (9.9) | 398.2 (7.8) | 407.9 (6.7) | ~210 |
| 1,3-dimethyl-5-ethyluracil ($m^{1,3}_2e^5$Ura) | 12 | 473.4 (20.9) | 492.0 (20.1) | 503.3 (20.3) | 512.7 (20.3) | ~240 |
| 1,3-diethylthymine ($e^{1,3}_2$Thy) | 14 | 565.1 (8.8) | 567.3 (7.1) | 567.2 (6.6) | 568.1 (5.2) | ~270 |
| $b = \Delta\bar{C}^0_{p2}$ (all compounds) | | 42.8 ± 1.4 | | | | |
| (Ura and Thy omitted) | | 44.9 ± 1.6 | 42.9 ± 1.8 | 41.6 ± 2.1 | 39.0 ± 2.6 | |
| $a$ (all compounds) | | −43.5 ± 13.8 | | | | |
| (Ura and Thy omitted) | | −68.5 ± 16.6 | −33.4 ± 18.4 | −11.2 ± 21.7 | +27.2 ± 27.5 | |

[a] The contribution of $CH_2$ group to the heat capacity of the solid was obtained as the difference between experimental $C_p(s)$ values of solid thymine [9,10] and of uracil [11], $C_p(s) = 30$ J mole$^{-1}$ K$^{-1}$; $C_p(s)$ of $m^1$Ura was assumed comparable with that of its structural isomer-thymine, and values for higher members of the series were calculated by addition of ($n_{CH_2}C_p(s)$) products to that of $m^1$Ura.

hydration enthalpies [16], from integral heats of solution and enthalpies of sublimation, as well as thermodynamic parameters of transfer of alkyluracils from organic solvent to water, derived from solubility studies, will be the subject of forthcoming papers.

## 2. Materials and methods

Apparent molar heat capacities $\phi C_p$ of the compounds studied in aqueous solutions were obtained in the temperature range of 293.15–363.15 K, using a differential adiabatic scanning microcalorimetre, model DASM-1M. The instrument and working procedure have been described elsewhere [17]. The measurements were performed at the heating rate of 1 K min$^{-1}$. The volume of each vessel was 1.0 ml.

The actual parameter measured in this calorimeter is the compensation power $\Delta P$, which is related to $\phi C_p$ at a given temperature by the following equation: $\phi C_p = C^0_{p1} V_2 V_1^{-1} - \Delta P m_2^{-1}$, where $C^0_{p1}$ is the specific heat of liquid water, $V_1$ its specific volume, $V_2$ the specific volume of the solute and $m_2$ its mass content per 1 ml of solution.

The specific volumes of the solutes were determined pycnometrically except for uracil and thymine which were obtained using a Paar Precision Density Meter DMA-02D. Solutions of known molar concentration in the range of 0.05–0.2 m were prepared by weight with the use of carefully dried compounds and redistilled

water. For each compound several independently prepared solutions were scanned and the $\phi C_p$ shown in table 1 are average values with standard error indicated.

The alkylated compounds were obtained by routine methods [18] and thoroughly purified by repeated crystallization. Their purity was checked by melting point determinations and chromatography in several solvent systems. Quantitative thin-layer chromatographic analysis demonstrated that N,N-dialkylated species contained not more than 0.05 per cent of the respective mono-N-alkylated intermediates. Uracil (lot no. 65c-0285) and thymine (puriss.) were commercial products purchased from Sigma and Koch-Light, respectively, and used without any further purification.

## 3. Results and discussion

Values of $\phi C_p$ obtained in the concentration range of 0.05–0.2 $m$ and at a given temperature (table 1) appeared to be concentration independent within an accuracy of experimental measurements characterized by relative standard errors of 1–2.5 per cent in most cases. This observation finds an independent support in the results of calorimetric heat dilution studies presented in the accompanying paper [15]. They indicated that in the temperature interval of 298.15–318.15 K the negative values of derivatives $\partial/\partial T\,(\partial\phi_L/\partial m) = \partial\phi C_p/\partial m$ are so small that the $m(\partial\phi C_p/\partial m)$ terms contribute at most 1–3 per cent to the partial molar heat capacity: $\bar{C}_{p2} = \phi C_p + m(-\partial\phi C_p/\partial m)$ at molalities of the solutions used in $\phi C_p$ determination. Values of $\phi C_p$ are thus very close indeed to those of partial molar heat capacities: $\phi C_p \gtrsim \bar{C}_{p2}$. The latter also vary little with concentration of solutions, as evidenced by the negative values of the $\bar{C}_{p2} - \bar{C}^0_{p2}$ differences obtained from the temperature dependence of partial molar relative enthalpies of dilution: $\partial\phi_L/\partial T$. Partial limiting molar heat capacities $\bar{C}_{p2}$ are thus expected to be higher by some 2–5 per cent than the respective values of $\bar{C}_{p2}$ and 0.2 $m$ concentration of the solutes. These considerations explain why $\phi C_p$ does not vary with concentration and allows us to regard $\phi C_p \approx \bar{C}^0_{p2}$ for practical purposes further in the text.

As compared with the estimated heat capacities of crystalline solids, $C_p(s)$, partial molar heat capacities of diketopyrimidines in water are very much higher (cf. respective data in table 1). This has been also observed previously for other non-electrolytes bearing both polar and alkyl groups [19] including natural diketopyrimidine bases [9,10,11] and structurally analogous methylated xanthines [14]. Furthermore, they were found to correlate linearly with the number of hydrogen atoms, $n_H$, attached covalently to the diketopyrimidine skeleton directly and to the alkyl groups thereon (fig. 1). The slope of the plot $\bar{C}^0_{p2}$ versus $n_H$ at 298.15 K gives an increment $\Delta\bar{C}^0_{p2} = 42.8 \pm 1.4$ J mole$^{-1}$ K$^{-1}$ $n_H^{-1}$ per each hydrogen atom. It resembles closely that found recently by Sköld et al. [20] for a series of aliphatic amides in water: $\Delta\bar{C}^0_{p2} = 44.35 \pm 1.0$ J mole$^{-1}$ K$^{-1}$ $n_H^{-1}$, as well as those obtained for other essentially hydrophobic solutes [19] and various series of homologous hydrocarbons [19,21]. Heat capacities of alkylated diketopyrimidines in water are thus also governed by large, positive and approximately constant contributions of the hydrogen atom

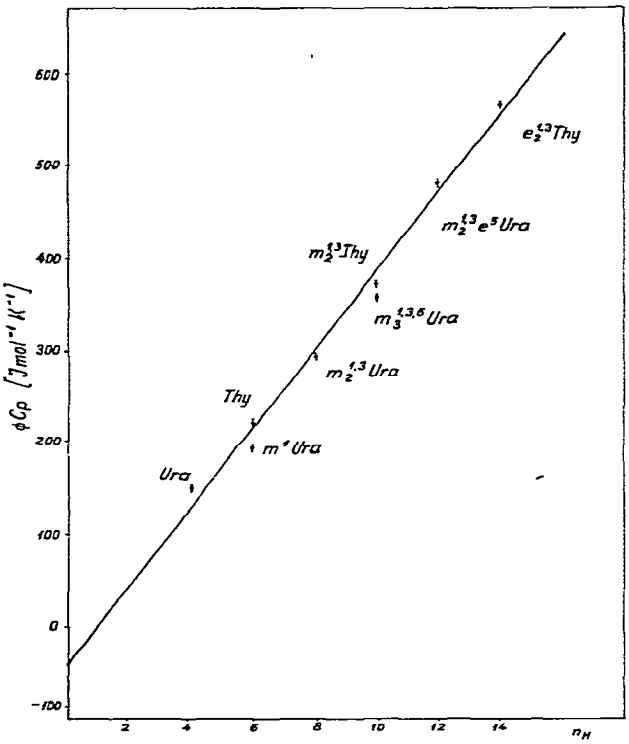


Fig. 1. Partial molar heat capacities $\phi C_p \approx \bar{C}^0_{p2}$ of diketopyrimidines in aqueous solutions at 298.15 K (table 1) plotted against the number $n_H$ of hydrogen atoms bound covalently.

heat capacities. Regardless of weather they are bound to the pyrimidine ring carbon and nitrogen atoms or to alkyl carbon atoms.

The small but significant differences found between partial heat capacities of structural isomers Thy and $m^1$Ura as well as $m_3^{1,3,6}$Ura and $m_2^{1,3}$Thy ($m_3^{1,3,5}$Ura) may be attributed to alteration in the interaction of a given group (C=O, N–H–C–H or $CH_3$) with the solvent caused by adjacent substituent group(s).

The "hydrogen effect" discussed herein was recently demonstrated in connection with the additivity correlations for the heat capacities of several groups of non-electrolytes in aqueous solutions [20]. Its origin is sought [21] in a clathrate type organization of water molecules in the solvation shells formed around hydrophobic groups of molecules. According to this model the large heat capacity effect could arise as a result of temperature-induced hydrogen bond breakage between water molecules, with a concomitant increase of the number of orientations available for hydrophobically confined water molecules. Alternatively, this large heat capacity effect could be explained in terms of a shift in a highly cooperative hydration equilibrium involving alkyl groups [22], which fluctuate between solvated and nonsolvated states [23].

Variation of $\phi C_p \approx \bar{C}^0_{p2}$ with temperature brings a new insight into the mechanism of hydrophobic hydration. $C^0_p$ of pure liquid water is known to vary but slightly with temperature and the $C^0_p(T)$ function exhibits a shallow minimum at about 308 K [24]. However, $\bar{C}^0_{p2}$ of aqueous uracil and its alkylated derivatives increased with the rise of temperature over the whole interval of temperatures studied (fig. 2) in all cases except for the $e_2^{1,3}$ Thy solution. The largest increment $\Delta\bar{C}^0_{p2}/\Delta T$ within this temperature interval was observed for uracil and thymine solutions. It corresponded to an about 92 per cent and 56 per cent rise in $\bar{C}^0_{p2}$, respectively. It decreased gradually in the series of N-alkylated derivatives with the growing number of hydrogen atoms in the molecules to reach practically zero for the last member, i.e. $e_2^{1,3}$ Thy. As it can be seen from fig. 2, variation of $\phi C_p$ with temperature for both diketopyrimidines unsubstituted with alkyl groups at amide nitrogen atoms, viz. Ura and Thy, was a distinctly steeper function than for N-alkylated members of the series. For this reason the $\bar{C}_{p2}$ data obtained at higher temperatures than 298.15 K do not correlate linearly with $n_H$ for the whole group of compounds studied. However, when Ura and Thy were omitted this correlation still appeared to hold. The increments $\Delta\bar{C}^0_{p2}$ per each hydrogen atom, obtained from linear plots $\bar{C}^0_{p2} = a + bn_H$ at higher temperatures (table 1), diminished as the latter increased. The larger the difference $\bar{C}^0_{p2} - C_p(s)$ between partial molar heat capacity of the solute in water at 298.15 K and its heat capacity in solid state (cf. table 1), the smaller was the noted temperature effect on $\bar{C}^0_{p2}$. This observation suggests strongly that the extent of hydrophobic hydration shell formation around diketopyrimidine molecules is related to the number of their hydrogen atoms and is temperature dependent. Temperature-induced breakage of hydrogen-bonded networks of water molecules in the water bulk promotes further specific ordering of solvent molecules around solutes and exerts an effect on $\bar{C}^0_{p2}$, the value of which depends on

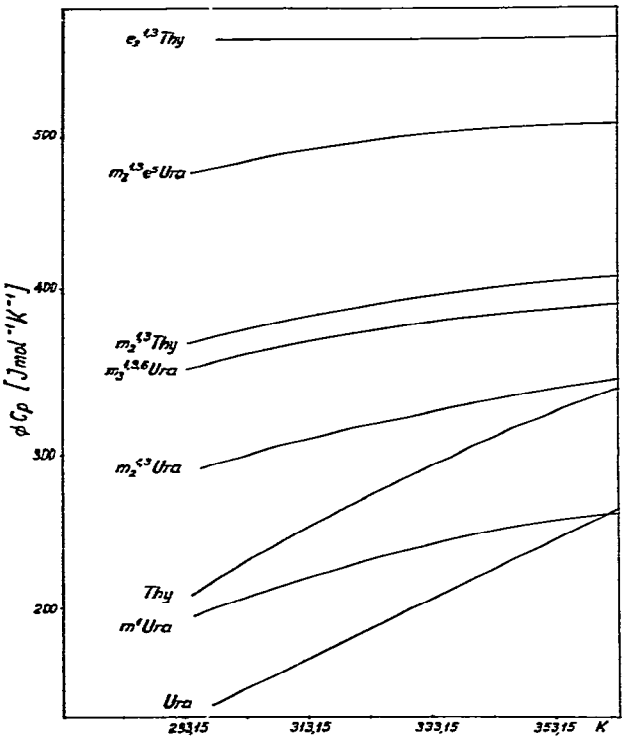


Fig. 2. Variation with temperature of apparent molar heat capacities of diketopyrimidines in aqueous solution; concentration of the solutes: Ura-0.0065 m, Thy-0.0063 m, $m^1$Ura-0.117 m, $m_2^{1,3}$Ura-0.177 m, $m_3^{1,3,6}$Ura-0.182 m, $m_2^{1,3}$Thy-0.169 m, $m_2^{1,3}e^5$Ura-0.190 m and $e_2^{1,3}$Thy-0.187 m.

the completeness of hydrophobic hydration shell formation.

At low temperatures hydration of uracil and thymine involves mainly interaction of water with their amide carbonyl groups via hydrogen bonding. Enthalpies of interaction of both compounds with their solvation shells attain largest negative values in the series of the order of 60 kJ/mole [16]. With a rise in temperature apparently a rearrangement of the hydration shells occurs towards attainment by water molecules of a new orientation more typical for hydrophobic hydration. It is this strong hydrogen-bond hydration of cyclic secondary amide groups which seems to be primarily responsible for larger temperature coefficient $\partial \bar{C}^0_{p2}/\partial T$ observed for Ura and Thy. Replacement of secondary amide hydrogen atoms by alkyl groups removes hydrogen-bond donating capacity of the compounds and diminishes their hydrogen-bond accepting capacity by "steric" shielding of the amide carbonyls by adjacent alkyl groups, as evidenced by heat of hydration data [16]. This promotes hydrophobic structuring of water around solute molecules. In solutions of highly alkylated derivatives, e.g. $e_2^{1,3}$Thy, the hydrophobic hydration shell is almost fully organized at 298.15 K.

Alvarez and Biltonen [9], on the basis of identical estimates of the changes in the heat capacity for the solution of thymine in water and in ethanol, $\Delta C_{p2}$ = 188.3 J mole$^{-1}$ K$^{-1}$ and of specific heat of thymine in solid state $C_p$(s) = 151.4 J mole$^{-1}$ K$^{-1}$ (determined in a drop-heat calorimeter), obtained an apparent heat capacity of thymine $\phi C_p$ = 339.7 J mole$^{-1}$ K$^{-1}$ at 298.15 K in both solutions. This led them to the conclusion that it is the hydrogen bonding of solvent to the solute molecules which is mainly responsible for the high heat capacity of thymine in water and that the hydrophobic contribution, if any, resulting from the presence of a methyl group at C(5) of the diketopyrimidine ring is not significant. In the light of our data, however, this contribution seems to be very important. Since $\phi C_p$ of thymine in water solution found by these authors seems to be greatly overestimated, as compared with our value of 220 J mole$^{-1}$ K$^{-1}$ as well as with that of 256 ± 10 J mole$^{-1}$ K$^{-1}$ recently reported by Kilday [10], their experimental data must be viewed with some circumspection.

Attempts at application of the additivity scheme and group parameter values $\phi Y$ [20], assigned to various functional groups in aqueous solution for prediction of the $\bar{C}^0_{p2}$ values of alkyluracils, proved unsuccessful until a new parameter was introduced, either for the whole diketopyrimidine radical $\phi(C_4H_3N_2O_2)$ = 48 J mole$^{-1}$ K$^{-1}$ or for its unsaturated carbon atom $\phi$(=C–) = 52 J mole$^{-1}$ K$^{-1}$. If not, the value of the parameter $\phi$ (NHCO) shown to hold in the series of aliphatic amides (25) must be seriously modified. Partial molar heat capacities of alkyluracils other than those studied presently can be most easily predicted by using our empirical linear equation for the $\bar{C}^0_{p2}$ ($n_H$) function.

The large partial molar heat capacities in water at 298.15 K of the two methylated xanthines: caffeine ($n_H$ = 10) and theophylline ($n_H$ = 8), 669.4 and 585.8 J mole$^{-1}$ K$^{-1}$ [12], respectively, point to the importance of hydrophobic hydration also among purine derivatives. Determination of $\bar{C}^0_{p2}$ as a function of temperature for a larger group of purines seemed thus worthwhile. Preliminary results of such studies [26] support this point of view fully.

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