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THERMOCHEMISTRY OF AQUEOUS SOLUTIONS OF ALKYLATED NUCLEIC ACID BASES

IV. ENTHALPIES OF HYDRATION OF 5-ALKYLURACILS

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Enthalpies of sublimation, $\Delta H^{\circ}_{\mathrm{subl}}$, and of solution in water, $\Delta H^{\circ}_{\mathrm{sol}}$, were determined for a series of crystalline 1,3-dimethyluracil derivatives substituted at the C^5-ring carbon atom with alkyl groups ($-C_nH_{2n+1}$, $n = 2$–4) and some of their $C^{5,6}$-cyclooligomethylene analogues ($-(CH_2)_n-$, $n = 3$–5). From these data, enthalpies of hydration $\Delta H^{\circ}_{\mathrm{hydr}} = \Delta H^{\circ}_{\mathrm{sol}} - \Delta H^{\circ}_{\mathrm{subl}}$, were calculated and corrected for energies of cavity formation in pure liquid water in order to obtain enthalpies of interaction, $\Delta H^{\circ}_{\mathrm{int}}$, of the solutes with their hydration shells. The latter are discussed together with the recalculated $\Delta H^{\circ}_{\mathrm{int}}$ for variously methylated uracils, obtained previously according to a simplified correction procedure, in terms of perturbations in the energy and scheme of hydration of the diketopyrimidine ring brought about by alkyl substitution. It was found that each $-CH_2-$ group added with an alkyl substitution contributes favorably about -20 kJ mol^{-1} to $\Delta H^{\circ}_{\mathrm{int}}$. This contribution is partially cancelled by the unfavorable contribution to $\Delta H^{\circ}_{\mathrm{int}}$ connected with removal of some water molecules bound in the first and subsequent hydration layers by an alkyl substituent. This is particularly evident on substitution at the polar side of the diketopyrimidine ring on which water molecules are expected to be bound specifically.

1. Introduction

Hydration of nucleic acid bases plays an important role in their interactions and thus in the spatial organization of polynucleotide chains in aqueous solutions. However, as yet little is known experimentally about the hydration scheme and thermodynamics of hydration of particular bases. In view of the chemical nature of the bases, both hydrophilic and hydrophobic hydration can be implicated simultaneously. Involvement of hydrophobic hydration was in fact demonstrated indirectly on the basis of our recent heat capacity measurements for a series of alkylated uracils [1] and aminopurines [2]. In the preceding paper of this series [3], a first attempt was made to elucidate the enthalpies of hydration for a group of C- and N-methylated uracil derivatives from enthalpies of solution and sublimation of crystalline compounds. These were corrected for the positive enthalpies of cavity formation contained therein, calculated according to Sinanoglu [4], in order to obtain enthalpies of interaction of the solute molecules with their hydration shells. In this paper, we present results of our further studies in this direction concerning the determination of enthalpies of interaction of alkylated uracils, with particular reference to the effects of substitution at the C^5-ring carbon atom with $-C_nH_{2n+1}$ ($n = 1$–4) alkyl

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and $C^{5,6}$-$(CH_2)_n$- ($n = 3$ and 4) cyclooligomethylene groups thereon, and more accurate calculation of enthalpies of cavity formation. Since in the first paper [3] the latter were calculated according to a simplified version of the formula given by Sinanoglu, the enthalpies of interaction then determined for methylated uracils were recalculated and are discussed in connection with the results of the present work.

2. Materials and methods

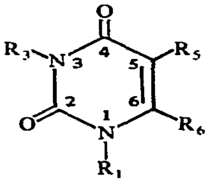
5-Alkyl- and 5,6-cyclooligomethylene-substituted derivatives of 1,3-dimethyluracil: 5-ethyl- ($m_2^{1,3}e^5$Ura), 5-propyl- ($m_2^{1,3}pr^5$Ura), 5-butyl- ($m_2^{1,3}but^5$Ura), 5,6-trimethylene- ($m_2^{1,3}(CH_2)_3^{5,6}$Ura), 5,6-tetramethylene- ($m_2^{1,3}(CH_2)_4^{5,6}$Ura) and 5,6-pentamethylene- ($m_2^{1,3}(CH_2)_5^{5,6}$Ura), synthesized according to known methods [5–8], were kindly supplied by Dr. M. Dramiński of the Military Medical Academy, Łódź. They were purified by repeated vacuum sublimation and their purity was checked chromatographically and by melting-point determination.

Van't Hoff enthalpies of sublimation, ΔH°_{subl}, were measured as described elsewhere [9] by the quartz-resonator method and field mass spectrometry, using the classical Knudsen cell as an evaporator. The results are shown in table 2 as mean values from 6–7 separate experiments.

Heats of solution were determined similarly, as described in detail previously [3], except that the calorimeter employed was slightly modified to ensure accurate measurements of the relatively smaller heats of solution for highly alkylated compounds. The 150 cm^3 measuring steel vessel was replaced by one of 80 cm^3 equipped with two 150-Ω thermistors instead of the platinum resistance thermometer used previously. It was calibrated by the electric Joule effect and by determination of the heat of solution of KCl in the same way as before. The values of enthalpies of solution obtained for a series (9–14) of solute concentrations (the range of concentrations used is specified in table 2) were numerically extrapolated back to an indefinitely diluted solution to yield ΔH°_{sol}.

Molecular areas and volumes of the compounds studied were calculated using the Voronoi polyhedron programme developed by Finney [10,11]. The effective 'hydrated' van der Waals' radii of the diketopyrimidine ring and substituents atoms, used in these calculations (table 1), were suggested by Dr. B. Gellatly of the Department of Crystallography, Birkbeck College, London. They include the 1.7 Å radius of a hypothetical solvent molecule added to all possible tetrahedral surface sites. The average bond length and dihedral angles used in construction of the diketopyrimidine skeleton were taken from the published crystallographic X-ray diffraction data [12]. Molecular areas of 5-alkyl derivatives were calculated for minimal volume conformations with side-chain atoms lying in the plane of the diketopyrimidine ring.

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



R_1, R_3: H, CH_3, C_2H_5
R_6: H, CH_3
R_5: C_nH_{2n+1} ($n = 1$–4)
$R_{5,6}$: -$(CH_2)_n$ ($n = 3$–5)

Scheme 1.

Table 1

Assumed van der Waals' radii of hydrated diketopyrimidine atoms

Atom groups	Radius[a] (Å)
All tetrahedral C of alkyl substituents at C^5 and C^6	3.8
Tetrahedral C of N-substituted CH_3, ring C^5 and C^6 and carbonyl C^2 and C^4	3.4
Carbonyl O	2.7
Ring N	2.7

[a] Includes the 1.7 Å radius of the H_2O molecule in contact with the given atom or group of atoms.

Table 2

Enthalpies of sublimation, $\Delta H^{\circ}_{\mathrm{subl}}$ (van't Hoff, determined in temperature range ΔT (K) indicated in parentheses), enthalpies of solution in water, $\Delta H^{\circ}_{\mathrm{sol}}$, and enthalpies of hydration, $\Delta H^{\circ}_{\mathrm{hydr}}$

Compound	Sublimation			Solution in H_2O		$\Delta H^{\circ}_{\mathrm{hydr}}$ [a] at 298.15 K (kJ mol^{-1})
	$\Delta H^{\circ}_{\mathrm{subl}}$ (kJ mol^{-1})		Average	$\Delta H^{\circ}_{\mathrm{sol}}$ at 298.15 K (kJ mol^{-1})	Concentration range (mmol kg^{-1})	
	Mass spectrometry	Quartz resonator				
$m_2^{1,5}e^5$Ura		110.0±1.2 (319–349)	-	8.7±0.04	6.8–22.5	−101.3
$m_2^{1,3}pr^5$Ura	100.4±0.4 (300–317)	115.1±0.4 (323–350)	107.7	12.4±0.13	3.5–22.4	−95.3
$m_2^{1,3}but^5$Ura	104.2±2.1 (314–324)	98.3±2.1 (321–344)	101.3	16.5±0.13	3.3–16.3	−84.8
$m_2^{1,3}(CH_2)_3^{5,6}$Ura	113.4±0.4 (298–314)	114.2±0.4 (331–365)	113.8	7.6±0.08	2.2–26.3	−106.2
$m_2^{1,3}(CH_2)_4^{5,6}$Ura	114.6±3.8 (323–338)	115.1±2.1 (326–376)	114.9	9.7±0.17	3.2–11.2	−105.2
$m_2^{1,3}(CH_2)_5^{5,6}$Ura	108.8±5.0 (323–338)	113.4±1.3 (340–370)	111.1	-	-	-
Anthracene [b]	103.8±2.1 (293–305)	101.7±0.4 (338–368)	102.8			

[a] Hydration enthalpies were calculated with the assumption that enthalpies of sublimation are to a first approximation independent of temperature down to 298.15 K. Variation of the interal energy of the crystals with temperature in the range indicated remains within the experimental error of $\Delta H^{\circ}_{\mathrm{subl}}$ determinations.

[b] $\Delta H^{\circ}_{\mathrm{subl}} = 102.09 + 1.2$ kJ mol^{-1} [20].

3. Results and discussion

Enthalpies of hydration $\Delta H^{\circ}_{\mathrm{hydr}}$ (table 2) of the studied compounds, which correspond to the hypothetical thermodynamic process of transfer of 1 mol of solute from the gas phase to liquid water resulting in formation of an infinitely diluted solution, were calculated from the experimental heats of solution and sublimation, $\Delta H^{\circ}_{\mathrm{hydr}} = \Delta H^{\circ}_{\mathrm{sol}} - \Delta H^{\circ}_{\mathrm{subl}}$, collected also in table 2. They consist of two terms of opposite sign and comparable magnitude: (i) a positive one, $\Delta H^{\circ}_{\mathrm{cav}}$, related to the energy required to form a cavity in liquid water of surface area equal to the molecular area of the solute, and (ii) a negative term, $\Delta H^{\circ}_{\mathrm{int}}$, which describes changes in the heat content accompanying interaction of the solute molecule with its liquid environment. The latter is the one sought after as being directly related to the thermodynamics of dilute aqueous solutions, which can be compared with hydration energies of the solutes in question, calculated quantum mechanically. Proper evaluation of $\Delta H^{\circ}_{\mathrm{cav}}$ is thus a crucial step in derivation of credible enthalpies of interaction from experimental data.

The energy required to make a cavity in liquid water that will accommodate a solute molecule B is proportional to the surface energy of such a cavity and can be calculated from a general formula derived by Sinanoglu [4] from the thermodynamic properties of pure liquids and dilute solutions:

$$\Delta H^{\circ}_{\mathrm{cav}} = k^{\mathrm{e}}_{1}\varphi_{1\mathrm{B}}^{-1/3} S_{\mathrm{B}}\gamma_{1}\left(1 - \frac{\partial \ln \gamma_{1}}{\partial \ln T} - \tfrac{2}{3}\alpha_{1\mathrm{B}}T\right) \qquad (1)$$

where $\gamma_1[1 - \partial \ln \gamma_1 / \partial \ln T)]$ is the energy part of the macroscopic surface tension of the solvent, S_{B} the molecular area of the molecule B, $\alpha_{1\mathrm{B}}$ the coefficient of thermal expansion appropriate to the cavity and closely approximated [13] by the solvent expansion coefficient, and $k^{\mathrm{e}}_{1}(\varphi_{1\mathrm{B}}^{-1/3}) = 1 + \varphi_{1\mathrm{B}}^{2/3}(k^{\mathrm{e}}_{1}(1) - 1)$ is a constant, dependent on the volume fraction $\varphi_{1\mathrm{B}} = V_1/V_{\mathrm{B}}$, which adjusts the planar surface energy to highly curved microscopic dimensions; $k^{\mathrm{e}}_{1}(1)$ the microscopic cavity factor for the solvent alone in the case of liquid water is equal to 1.277 [13].

For molecules of nucleic acid bases of large size as compared to the size of the water molecule, $k^{\mathrm{e}}_{1}(\varphi_{1\mathrm{B}}^{-1/3})$ is of the order of 1.1 (see table 3) and slowly approaches unity as the molecular volume of the solute increases. The largest unknown factor in eq. 1 is thus the molecular area S_{B} which mainly determines $\Delta H^{\circ}_{\mathrm{cav}}$. For crystalline compounds its value can be evaluated from X-ray diffraction data on the assumption of spherical shape of the molecules [13], as has been done also in our previous work [3] concerning determination of enthalpies of interaction for methylated uracil derivatives. Since for the presently studied group of uracil derivatives no crystallographic data of this sort are available, we decided to calculate their molecular areas using the Voronoi polyhedral construction according to the procedure proposed by Finney [10,11]. The area calculated in this way is a plane approximation to the 'true' molecular area given by the sum of areas on the spherical van der Waals' surfaces of particular atoms. It is estimated [11] to be about 5–10% lower than the true molecular area. Since not all component atom surfaces exposed to the solvent environment are directly accessible to water molecules (reentrant areas) of the hydration shell, the actual surface of the cavity which accommodates the solute molecule can be even somewhat larger than its calculated molecular area. Another source of a possible discrepancy between the true and calculated area is the semiarbitrary nature of van der Waals' radius assumptions [11]. The calculated values of S_{B} (table 3) should thus be considered as absolute values with all these reservations. Much to our surprise, however, the molecular areas of methylated uracils obtained from crystallographic data [3] proved to agree within a few per cent with those calculated by the Voronoi polyhedron approach (see table 3).

The enthalpies of interaction, $\Delta H^{\circ}_{\mathrm{int}} = \Delta H^{\circ}_{\mathrm{hydr}} - \Delta H^{\circ}_{\mathrm{cav}}$, obtained from the enthalpies of hydration corrected for the energy of cavity formation, are listed in table 3 together with those obtained previously [3] for methylated uracils, recalculated for the sake of consistency according to the presently adopted procedure of evaluation of $\Delta H^{\circ}_{\mathrm{cav}}$. In the former simplified calculation of $\Delta H^{\circ}_{\mathrm{cav}}$ [3], the coefficients $[k^{\mathrm{e}}_{1}\varphi_{1\mathrm{B}}^{-1/3}$ and $1 - (\partial \ln \gamma_1 / \partial \ln T) - 2\alpha_{1\mathrm{B}}T/3]$ were omitted.

We were particularly interested in the effects of

Table 3

Molecular areas, S_B, conversion constant of planar surface energy to microscopic dimensions, $k_1^c(\varphi_{1B}^{-1/3})$, enthalpies of cavity formation, ΔH^o_{cav}, and of base-water hydration shell interaction, ΔH^o_{int}

Compound	S_B (cm^2) ($\times 10^{-16}$)	$k_1^c(\varphi_{1B}^{-1/3})$.	ΔH^o_{cav} at 298.15 K (kJ mol^{-1})	$-\Delta H^o_{int}$ at 298.15 K (kJ mol^{-1})
Uracil	113.7 (115.6) [a]	1.133	89.1	180.3 [b]
m^1Ura	129.8 (128.2) [a]	1.119	100.5	189.5 [b]
$m_2^{1,3}$Ura	143.3 (147.8) [a]	1.109	110.0	196.0 [b]
Thymine (m^5Ura)	130.9 (133.9) [a]	1.118	101.3	201.9 [b]
m^1Thy	147.0 (150.3) [a]	1.107	112.6	210.5 [b]
$m_2^{1,3}$Thy	160.5	1.099	123.5	220.0 [b]
$m_3^{1,3,6}$Ura	160.5	1.099	122.1	216.5 [b]
$m_2^{1,3}e^5$Ura	177.0	1.091	133.7	235.0
$m_2^{1,3}pr^5$Ura	194.7	1.085	146.2	241.5
$m_2^{1,3}but^5$Ura	212.7	1.079	158.8	243.6
$e_2^{1,3}$Thy	198.7	1.083	148.9	234.4 [b]
$m_2^{1,3}(CH_2)_3^{5,6}$Ura	180.5	1.088	135.9	242.1
$m_2^{1,3}(CH_2)_4^{5,6}$Ura	193.8	1.083	145.2	250.4

[a] Values of S_B deduced from crystallographic X-ray diffraction data [3].

[b] Experimental ΔH^o_{subl} and ΔH^o_{sol} values used in calculation of ΔH^o_{int} were taken from our earlier paper [3].

Table 4

Residual contributions $\delta\Delta H^o_{int}(R)$ of alkyl substituents to the total enthalpy of interaction, calculated as differences between ΔH^o_{int} of respective members of homologous series of alkyluracils

Alkyl substituent, R (atom substitution, molecule)	$-\delta\Delta H^o_{int}(R)$ (kJ mol^{-1})	$-\delta\Delta H^o_{int}(CH_2)$ [a] (kJ mol^{-1})	
		Average	Consecutive -CH_2- groups
-CH_3 (C^5, Thy)	21.6	21.8	
-CH_3 (C^5, m^1Thy)	21.0		
-CH_3 (C^6, $m_3^{1,3,6}$Ura)	20.5		
-CH_3 (C^5, $m_2^{1,3}$Thy)	24.0		
-C_2H_5 (C^5, $m_2^{1,3}e^5$Ura)	39.0	19.5	15.0
-C_3H_7 (C^5, $m_2^{1,3}pr^5$Ura)	45.5	15.2	6.5
-C_4H_7 (C^5, $m_2^{1,3}but^5$Ura)	47.6	11.9	2.1
-$(CH_2)_3$- ($C^{5,6}$, $m_2^{1,3}(CH_2)_3^{5,6}$Ura)	46.1	15.4	8.5
-$(CH_2)_4$- ($C^{5,6}$, $m_2^{1,3}(CH_2)_4^{5,6}$Ura)	54.6	13.65	
-CH_3 (N^1, Ura)	9.2		
-CH_3 ($N^{1,3}$, Ura)	7.8 [b]		
-CH_3 (N^1, Thy)	8.6		
-CH_3 ($N^{1,3}$, Thy)	10.3 [b]		
-C_2H_5 ($N^{1,3}$, Thy)	16.3 [b]		5.9 [b]

[a] Differences between $\delta\Delta H^o_{int}(R)$ of consecutive members of the 5-alkyl-1,3-dimethyluracil and $N^{1,3}$-dialkylthymine series.

[b] Average value for N^1- and N^3-substituted -R.

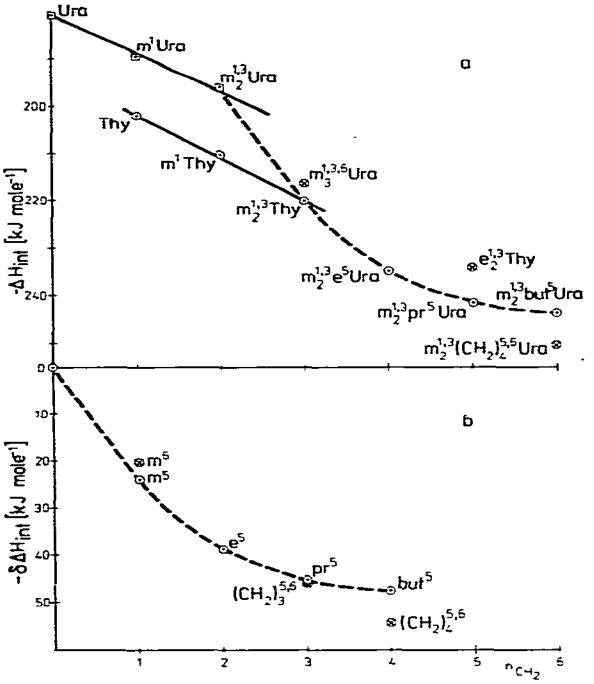


Fig. 1. Plots of $\Delta H^{\circ}_{\mathrm{int}}$ (a) and $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{R})$ (b) versus the number n_{CH_2} of methylene groups added upon alkyl substitution of uracil.

alkyl substitution on the enthalpy of interaction of uracil with its hydration shell. Therefore, $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{R})$ and $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH}_2)$ increments were calculated (table 4) as differences between $\Delta H^{\circ}_{\mathrm{int}}$ values for respective pairs of compounds in a given homologous series of uracil derivatives. The data shown in tables 3 and 4, and plotted in fig. 1 versus the number, n_{CH_2}, of -CH_2- groups added on alkyl substitution, allow the formulation of the following general observations:

(i) Every alkyl substitution brings about an increase in the enthalpy of interaction, (ii) effects of methyl or ethyl substitution at C^5 on $\Delta H^{\circ}_{\mathrm{int}}$ are 2–3-fold larger than those of analogous N substitution, (iii) in the series of C^5-alkyl derivatives of $\mathrm{m}_2^{1,3}$Ura, $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{C}_n\mathrm{H}_{2n+1})$ increments decrease sharply with increasing n (fig. 1b) in such a way that $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH}_2)$ increments for the consecutive -CH_2- groups in the chain become 2–3-fold smaller, and (iv) in the series of 5,6-cyclooligomethylene derivatives average $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH}_2)$ increments are larger than those for related open-chain analogues and seem to decrease much more slowly with increasing n.

In order to explain these various effects of alkyl substitution on $\Delta H^{\circ}_{\mathrm{int}}$ in terms of perturbations caused by the substituents in question in the hydration scheme of uracil, let us first consider in a more general way the possible changes in intermolecular interactions between water and a polar solute molecule induced by its single alkyl substitution.

Interaction of aliphatic hydrocarbons with water molecules in aqueous solution is no doubt of purely van der Waals' nature. Using the $\Delta H^{\circ}_{\mathrm{hydr}}$ data for n-pentane and n-hexane published by Gill et al. [14] and the residual -CH_2- surface area obtained from S_{B} data for the $\mathrm{m}_2^{1,3}(\mathrm{C}_n\mathrm{H}_{2n+1})^5$Ura series, we calculated $\Delta H^{\circ}_{\mathrm{int}}$ at 298.15 K for both aliphatic hydrocarbons to be −99.95 and −119.55 kJ mol⁻¹, respectively. Thus, an average increment of the van der Waals' energy of interaction per -CH_2-group, $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH}_2)$, can be set equal to −19.6 kJ mol⁻¹. On substitution with a methyl group of a polar molecule such as diketopyrimidine, $\Delta H^{\circ}_{\mathrm{int}}$ of the latter can therefore be expected to increase appropriately by this amount or by its multiplicity for larger substituents.

The presence of an alkyl group may also affect interaction of the diketopyrimidine ring with water in two ways: (i) by virtue of an inductive effect and (ii) sterically. One can expect that the inductive effect would increase $\Delta H^{\circ}_{\mathrm{int}}$ somewhat because the ionization potential of uracil (9.0 eV) drops by about 0.4 eV on either C or N substitution with a methyl group. For larger alkyl substituents of C^5 of $\mathrm{m}_2^{1,3}$Ura, the ionization potential remains constant within experimental error [15]. The magnitude of the inductive effect brought about by alkyl substitution at C^5 should thus be constant throughout the homologous series of uracil derivatives.

The steric effect of an alkyl group can be envisaged as consisting of the removal of a number of water molecules from their energetically most favorable positions in the first and subsequent layers of the hydration shell surrounding the polar diketopyrimidine ring, accompanied by relaxation of the whole hydration shell into a new dynamic equilibrium state. This effect must be necessarily connected with a decrease in $|\Delta H^{\circ}_{\mathrm{int}}|$ owing to a loss in the energy of polar interactions. The observed change in $\Delta H^{\circ}_{\mathrm{int}}$ on alkyl substitution is thus a net result of compensation of: (i) a favorable contribution to the energy of water binding resulting from the always attractive and additive van der Waals' interactions and (ii) an unfavorable contribution due to the steric effect.

With this general model of possible perturbations brought about by alkyl substitution in the scheme and energy of hydration of a polar molecule, we can now proceed to a more detailed analysis of $\Delta H^{\circ}_{\mathrm{int}}$ for variously substituted alkyl uracils.

Let us first consider the effects of substitution on the apolar side of the diketopyrimidine skeleton, i.e., at C^5- and C^6-ring carbon atoms. Replacement of the hydrogen atom at C^5 by a methyl group in uracil and $\mathrm{m^1Ura}$ or at C^6 in $\mathrm{m_2^{1,3}Ura}$ results in an increase in the absolute value of $\Delta H^{\circ}_{\mathrm{int}}$ by $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH_2}) \approx 21$ kJ mol^{-1}, very close indeed to that evaluated for aliphatic hydrocarbons (table 4). Only in the case of $\mathrm{m_2^{1,3}Thy}$ ($\mathrm{m_3^{1,3,5}Ura}$) is this increment apparently larger. Bearing in mind, however, the relatively large experimental errors involved in determination of $\Delta H^{\circ}_{\mathrm{subl}}$ (1–3 kJ mol^{-1}), we do not regard the difference between $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH_2})$ for $\mathrm{m_2^{1,3}Thy}$ and other C^5-methylated compounds in this series as meaningful. Thus, it can be concluded that the increase in $|\Delta H^{\circ}_{\mathrm{int}}|$ on substitution with a methyl group at the apolar side of the diketopyrimidine skeleton reflects for the most part a positive contribution from the van der Waals' interactions of that group with water. A small positive contribution from the inductive effect, if any, remains apparently within the experimental uncertainty of $\Delta H^{\circ}_{\mathrm{int}}$ data, or is compensated by the steric effect. The latter seems to be also negligible. Otherwise a larger value of $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH_2})$ should be attributed to $\mathrm{CH_3}$-substitution than that calculated for normal aliphatic hydrocarbons. The only source of such an increase in $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH_2})$ might be a slight inductive polarization of the $\mathrm{CH_3}$- group. However, in the case of substitution with an ethyl group, the average $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH_2})$ (-19.5 kJ mol^{-1}) also corresponds closely with that expected for normal hydrocarbons. Therefore, we can safely conclude that not only $\mathrm{CH_3}$- but also the larger $\mathrm{C_2H_5}$- group at C^5 can be accommodated within the hydration shell of $\mathrm{m_2^{1,3}Ura}$ without any significant loss in the energy of polar interactions. Most probably, removal of relatively weakly and nonspecifically bound water molecules from the apolar side of the uracil ring is compensated by the concomitant relaxation of the whole hydration shell to a new dynamic equilibrium. Further increase in the size of the alkyl substituent already manifests itself in the appearance of a steric effect as the average $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH_2})$ for $\mathrm{C_3H_7}$- and $\mathrm{C_4H_9}$-substituents decreases to -15.2 and -11.9 kJ mol^{-1}, respectively. In the case of $\mathrm{m_2^{1,3}pr^5Ura}$, it can be evaluated as being equal to about 15 kJ mol^{-1}, while in that of $\mathrm{m_2^{1,3}but^5Ura}$ it amounts to about 32 kJ mol^{-1}. Cyclic chains of the respective 5,6-oligomethylene derivatives, characterized by a more compact conformation, induce an appropriately smaller steric effect (cf. table 4). This becomes even more apparent when one takes into account their smaller molecular areas. As compared with open-chain analogues they contain two hydrogen atoms fewer and their $\mathrm{-CH_2}$- groups are less accessible to water. Also, a difference in the strain energy between five- and six-membered 5,6-cyclooligomethylene rings should be taken into account. Being lower by about 0.8 units, the pK of $(\mathrm{CH_2})_3^{5,6}\mathrm{Ura}$ [7] suggests an appropriately larger contribution to $\Delta H^{\circ}_{\mathrm{int}}$ from polar interactions in the case of $\mathrm{m_2^{1,3}(CH_2)_3^{5,6}Ura}$ than in that of its open-chain analogue. If so, then the calculated $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH_2})$ for the former compound would in fact be somewhat smaller. To summarize, one can expect that in this series of compounds, $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH_2})$ is somewhat lower than that characteristic of open-chain hydrocarbon substituents.

In connection with the above discussion, it is worth mentioning that the apparent molar heat capacity ϕC_p of the series of 5-alkyl derivatives of $\mathrm{m_2^{1,3}Ura}$ in water was found to be the same linear function of the number of hydrogen atoms present in the molecules [16], as in the case of the whole group of alkylated uracils [1] and aminopurines [2]. This indicates that each $\mathrm{-CH_2}$- group, irrespective of the length of the alkyl chain, contributes equally to the hydrophobic hydration of molecules and hence corroborates the validity of our interpretation of the observed diminution of $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH_2})$ in terms of the steric effect.

As pointed out before [3], the gain in $\Delta H^{\circ}_{\mathrm{int}}$ on substitution with a $\mathrm{CH_3}$- group at an amide ring nitrogen, i.e., on the polar side of the diketopyrimidine ring, is much smaller than that resulting from substitutions at C^5 or C^6. This difference was then interpreted as being the net result of changes brought about by N methylation in the scheme and energy of water binding in the first hydration layer, proposed in the light of quantum-mechanical studies of polyhydration of thymine [17]. According to this scheme, replacement of each of the amide hydrogen atoms by a $\mathrm{CH_3}$- group is expected to remove at least one water molecule from its energetically preferred binding site, located with in the proximity of a given amide carbonyl group. A concomitant loss in hydration energy is thus more than compensated by the gain caused by van der Waals' interactions of the hydrophobically hydrated $\mathrm{CH_3}$-group. If we assume reasonably that this gain is equal to that connected with methyl substitution at C^5 ($\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{CH_2}) = -21$ kJ mol^{-1}), then the loss in $\Delta H^{\circ}_{\mathrm{int}}$ due to the steric effect would amount to about 10–13 kJ mol^{-1}, according to the parent compound in the uracil series for which it is calculated. If we repeat the former calculation for the case of N-ethyl substitution using the respective $\delta\Delta H^{\circ}_{\mathrm{int}}(\mathrm{R})$ data, we obtain for the steric effect about 23 kJ mol^{-1}. In this way, the $\Delta H^{\circ}_{\mathrm{int}}$ data point directly to the differences in the specificity and energy of binding of water molecules on the polar and nonpolar sides of the uracil ring, much in qualitative agreement with the quantum-mechanical predictions [17,18]. From a quantitative point of view, however, the results of recent Monte Carlo calculations [18] of binding energies at 300 K of the binding of 40-water molecule clusters by uracil and thymine do not find support in our data. Neither can the large difference between thymine and uracil in the average interaction energy of the water molecules with the base (−154.77 and −322.92 kJ mol^{-1}, respectively) nor that in the net balance ($\Delta\bar{U}$) of the energies of base-water and water-water interactions (191.6 and 8.4 kJ mol^{-1}, respectively) be understood in the light of our findings. $\Delta\bar{U}$ and $|\Delta H^{\circ}_{\mathrm{int}}| = 201.9$ kJ mol^{-1} for thymine agree rather fortuitously, since the difference in $\Delta\bar{U}$ by as much as about 23-fold between thymine and uracil remains in open contradiction with the contribution of only about 10% of the $\mathrm{CH_3}$- group to $\Delta H^{\circ}_{\mathrm{int}}$ of thymine.

The present discussion of semiexperimental $\Delta H^{\circ}_{\mathrm{int}}$ data demonstrates their usefulness in gaining a deeper insight into the nature and energetics of hydration interactions. Further studies along this line for alkylated cytosines [19] and adenines are underway.

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