Biophysical Chemistry 11 (1980) 17–21

THERMOCHEMISTRY OF AQUEOUS SOLUTIONS OF ALKYLATED NUCLEIC ACID BASES.
III. ENTHALPIES OF HYDRATION OF URACIL, THYMINE AND THEIR DERIVATIVES

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Received 12 July 1979
Revised manuscript received 22 August 1979

Enthalpies of sublimation ΔH^0_{subl} of crystalline uracil, thymine and their methylated derivatives as well as of N,N-diethylthymine were determinated by the quartz-resonator method and mass spectrometry. Enthalpies of solution at infinite dilution ΔH^0_{sol} in water of alkylated compounds were obtained calorimetrically. Hence the calculated enthalpies of hydration: $\Delta H^0_{\text{hydr}} = \Delta H^0_{\text{sol}} - \Delta H^0_{\text{subl}}$, were corrected for energies of cavity formation in pure liquid water to yield enthalpies of interaction ΔH^0_{int} of the solutes with their hydration shells. For uracil $\Delta H^0_{\text{int}} = -59.8$ kJ mole^{-1} was obtained in this way. This value decreased linearly on N-methyl substitution with a mean increment of about 6.5 kJ/mole CH_2^{-1}. After C(5) or C(6) ring substitution it increased by about 3 kJ. These results are discussed in connection with heat of dilution data and theoretical schemes of hydration.

1. Introduction

Knowledge of the hydration scheme and hydration energy of particular nucleic acid bases is of crucial importance in elucidation of the effect of an aqueous environment on base pairing and stacking interactions, and thus on spatial organization of polynucleotide chains. In previous papers of this series [1,2] hydrophobic hydration [3] of alkylated uracils was demonstrated on the basis of heat capacity [1] and heat of dilution [2] data. This proved to be also the case for alkylated aminopurines [4]. It seemed worth-while to investigate the effect of alkylation on the energy and the scheme of hydration. So far, however, experimental data of this kind were not available even for unsubstituted bases. Only recently application of field-ionization mass spectroscopy to studies on formation of addition complexes in gas phase between 1-methyluracil and water [5] indicated the usefulness of this technique for assessing enthalpies of hydration. The present notions concerning hydration of nucleic acid bases stem mainly from quantum-mechanical studies of their mono- and poly-hydration [6–9]. These studies indicated the preferred sites of water binding to and energetical characteristics of the interaction of a number of water molecules with the natural bases.

In this paper we present results of measurements of enthalpies of sublimation of crystalline uracil, thymine and their variously methylated derivatives along with enthalpies of dissolution in water of alkylated compounds. Recently determined enthalpies of solution of uracil [10] and of thymine [11] were taken also into consideration. Enthalpies of hydration thus obtained were corrected according to Sinanoglu [12] for energies of cavity formation in pure liquid water upon solute introduction to yield enthalpies of interaction between solutes and the surrounding water. These are compared with respective data for thymine, obtained theoretically [9].

Table 1
Enthalpies of sublimation, ΔH^0_{subl}, (determined in temperature range ΔT indicated) enthalpies of solution in water, ΔH^0_{sol}, and of hydration ΔH^0_{hydr} [a]

Compound	sublimation		solution in H_2O		ΔH^0_{hydr} 298.15 K (kJ · mole^{-1})
	ΔH^0_{subl} (kJ mole^{-1})	ΔT (K)	ΔH^0_{sol} 298.15 K (kJ mole^{-1})	concentration range (mmole kg^{-1})	
Uracil (Ura)	120.5 ± 1.3	378–428	29.29 ± 1.21 (ref. [11])	3 – 45	−91.2
1-Methyluracil (m^{1}Ura)	112.5 ± 2.6	378–418	23.5 ± 0.5	1.3– 3.9	−89.0
1,3-Dimethyluracil (m$_2^{1,3}$Ura)	101.7 ± 2.1	313–363	15.7 ± 0.2	0.9–513.3	−86.0
1,3,6-Trimethyluracil (m$_3^{1,3,6}$Ura)	106.7 ± 2.5	300–340	12.34 ± 0.04	8.9– 54.5	−94.4
Thymine (Thy)	124.4 ± 1.3	378–428	24.32 ± 0.70 (ref. [10])	5 – 35	−100.1
1-Methylthymine (m^{1}Thy)	120.1 ± 2.6	373–423	22.2 (ref. [20])	–	−97.9
1,3-Dimethylthymine (m$_2^{1,3}$Thy)	109.2 ± 2.1	313–363	10.2 ± 0.1	3.0– 41.8	−99.0
1,3-Diethylthymine (e$_2^{1,3}$Thy)	95.0 ± 2.1	307–325	9.6 ± 0.2	3.2– 15.9	−85.4

[a] Hydration enthalpies were calculated with the assumption that enthalpies of sublimation are in the first approximation independent of temperature down to 298.15 K. Variation of the internal energy of the crystals with temperature in the range indicated remains within the experimental error of ΔH^0_{subl} determinations.

2. Materials and methods

Methylated uracil and thymine derivatives were obtained according to routine methods. They were of these same crystalline stock samples used and characterized previously [1,2]. Uracil and thymine were commercial samples obtained from Sigma and Koch-Light.

Vant'Hoff enthalpies of sublimation were measured as described elsewhere [5] by the quartz-resonator method and mass spectrometry. In the temperature range employed (see table 1), always well below the melting temperature of the compound, no thermal decomposition of samples was observed.

Heats of solution were measured in a calorimeter constructed by one of the authors in the Institute of Physical Chemistry, Polish Academy of Sciences [13]. It consists of a 150 cm^3 measuring steel vessel equipped with a calibration heater (99.7 Ohm), platinum resistance thermometer (100 Ohm) ensuring determination of temperature with an accuracy of 0.003°C, and a mechanical stirrer. The calorimetric vessel is placed at a short distance in a metal shield and immersed in a water thermostat regulated with an accuracy of 0.003°C. Before each experiment the calorimeter was tested by means of the electric Joule effect. The heat effect was evaluated by the method of temperature increase with the Regnault-Pfaundler correction. The calorimeter was additionally tested by determination of heat of solution of potassium chloride (99.99% pure according to a test performed in the Dept. of Metrology, Polish Commitee of Standards, Warsaw) in water. The value 17.51 kJ mole^{-1} obtained at 298.15 K, and the final concentration of 0.287 mole kg^{-1} was very close to that of 17.54 kJ mole^{-1}, recommended as the standard (J.D. Cox, Recommenced Calibration and Test Materials for the Realization of Physico-chemical Properties, IUPAC, 1972). The values of enthalpies of solution determined at a given final concentration of the solute studied were numerically extrapolated back to an indefinitely dilute solution of yield ΔH^0_{sol} (table 1).

Mean-square errors involved in determination of ΔH^0_{subl} and ΔH^0_{sol} are indicated along with respective numerical data in table 1.

3. Results and discussion

Measured enthalpies of sublimation, ΔH^0_{subl}, and of solution, ΔH^0_{sol}, (table 1) for the two series of alkylated diketopyrimidines, viz. derivatives of uracil and thymine, can be used to evaluate changes in the heat content, accompanying transfer of the solute molecules investigated from the gas phase to liquid water, i.e. enthalpies of hydration ΔH^0_{hydr}. Positive enthalpies of solution $\Delta H^0_{\text{sol}} > 0$ can be interpreted as a net result of heat content changes associated with two thermodynamic processes: (i) transfer of molecules from crystalline solid to gas phase (sublimation), and (ii) their subsequent placement in water (hydration) to form an indefinitely dilute solution. Thus,

$$\Delta H^0_{\text{hydr}} = \Delta H^0_{\text{sol}} - \Delta H^0_{\text{subl}}.$$

The values of ΔH^0_{hydr} calculated in this way (table 1) do not reflect solely energies of interaction between the solute and water molecules contained in the solvation shell because transfer of a molecule from the gas phase to water requires energy to form a cavity. This energy can be assumed proportional to the molecular volume of the solute. Therefore,

$$|\Delta H^0_{\text{hydr}}| = |\Delta H^0_{\text{int}}| - |\Delta H^0_{\text{cav}}|,$$

where ΔH^0_{int} and ΔH^0_{cav} correspond to enthalpy changes associated with structuring of water around the solute and with cavity formation in pure liquid water, respectively.

According to Sinanoglu [12] this last term is: $\Delta H^0_{\text{cav}} = C\gamma v^{2/3}$, where $C = 1.715 \times 10^{12}$ is a constant (ΔH^0_{cav} is expressed in kJ mole^{-1}), γ is the surface tension of pure liquid water in dyne cm^{-1}, and v the molecular volume of the solute in cm^3.

Molecular volumes of most of the compounds investigated were calculated from published crystallographic data [14–18] as the ratio V/Z between the volume of the elementary crystal unit (V) and the number of molecules contained in this unit (Z). For compounds the crystal structures of which are unknown, i.e. for $\text{m}_2^{1,3}$Thy, $\text{m}_3^{1,3,6}$Ura and $\text{e}_2^{1,3}$Thy, molecular volumes were computed as follows. The volume of the CH_2 group was first calculated as the difference between molecular volumes of m^1Ura and $\text{m}_2^{1,3}$Ura: $\delta v_{CH_2} = 31.6 \times 10^{-24}$ cm^3, and an appropriate number of its equivalents was then added to the known molecular volume of the respective parent compound.

Calculated ΔH^0_{cav} values correlate linearly with the number n_{CH_2} of alkyl groups substituted on the diketopyrimidine ring (fig. 1a) with a mean increment $\delta\Delta H^0_{\text{cav}} = 4.6$ kJ (mole CH_2)$^{-1}$. This corroborates the validity of the assumptions underlying our molecular model of hydration and offers a simple way to estimate ΔH^0_{cav} for other alkylated diketopyrimidines of unknown crystal structure.

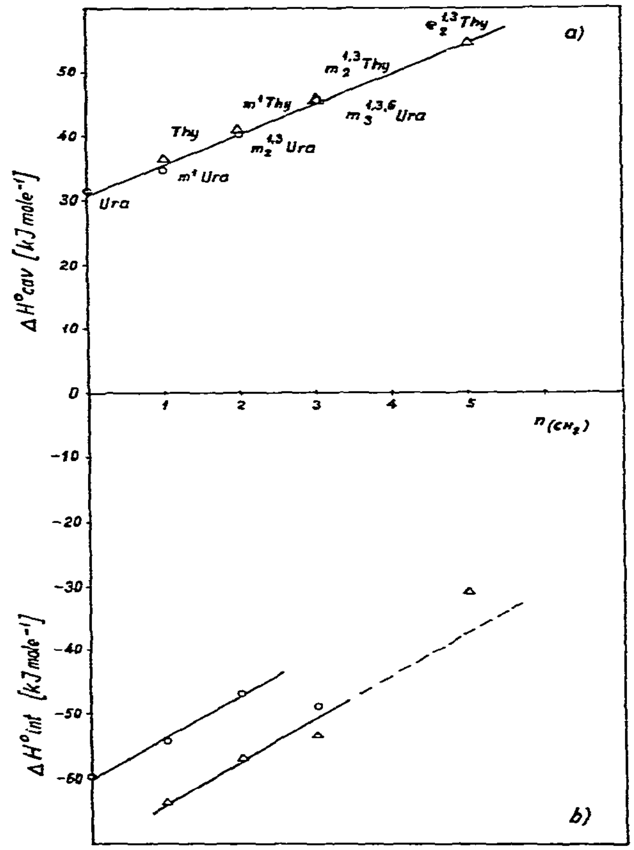

Fig. 1. Plots of (a) enthalpies of cavity formation, ΔH^0_{cav}, and (b) of enthalpies of interaction, ΔH^0_{int}, between the solute and surrounding water molecules versus the number n_{CH_2} of CH_3 groups substituted for N(H) and/or C(H) hydrogen atoms; ○ – uracil and derivatives, △ – thymine and derivatives.

Most interestingly, ΔH^0_{int} values, which represent energies of interaction of a solute molecule with water molecules in the solvation shell, vary not only with the number of CH_2 groups, but also with their position of substitution on the ring (fig. 1b). While an increase in the number of N-substituted alkyl groups brings about reduction of ΔH^0_{int} with a mean increment close to -6.5 kJ (mole CH_2)$^{-1}$ in both series of derivatives (except for $\text{e}_2^{1,3}$Thy), substitution of the

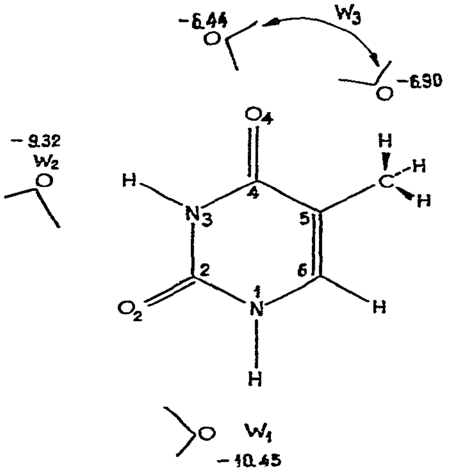

Fig. 2. Hydration scheme of thymine with three molecules of water: W_1, W_2, W_3, and their binding energies in kcal mole^{-1} according to Pullman et al. [9].

CH_3 group either at C(5) or C(6) gives an opposite effect, viz. an increase in the absolute energy of interaction on average by about 3 kJ (mole CH_2)$^{-1}$.

Quantum-mechanical computations of the hydration scheme for uracil [8] and for thymine [9] indicated that the preferred sites for binding of water molecules are located in the plane of the ring between adjacent C=O and N–H groups. This is in agreement with the qualitative picture one would expect on the basis of knowledge of a large group dipole moments $\mu_{C=O} \approx$ 4 Debye, responsible for the molecular dipole moment of uracil and its alkylated derivatives [19]. Therefore, substitution at C(5) with the CH_3 group should not affect the structure and energy of hydration on the polar side of the uracil skeleton. The observed increase of interaction energy ΔH^0_{int} with this substitution may thus be attributed to an increased number of interatomic contacts contributing to stabilization of the water molecule located close to C(4) = 0 carbonyl, as predicted by the polyhydration scheme of thymine obtained recently theoretically [9]. On the other hand, according to this hydration scheme (see fig. 2), replacement of each of the amide hydrogen atoms for the CH_3 group is expected to remove at least one water molecule from its preferred binding site, and to subsequently induce relaxation of the hydration shell to a new equilibrium structure.

Opposite effects of N-methyl and C(5 or 6)-methyl substitution on the enthalpy of interaction of the solutes with their hydration shells are also manifested indirectly in the heat of dilution data [2]. Dilution of dilute aqueous solutions of alkyluracils is an endothermic process and its endothermicity increases with C-methylsubstitution, while replacement of amide hydrogens for alkyl groups brings about its reduction. Since endothermicity of dilution was attributed to a long-range cooperative interaction of hydration shells of neighbouring solute molecules, the differences observed in ΔH^0_{int} between various alkyluracils reflect presumably also the extensiveness of structuring of water around solutes and the ability of the specifically ordered hydration shells to extend cooperatively towards each other.

The decrease in ΔH^0_{int} observed upon successive N-methyl substitution in both uracil and thymine series demonstrates the general validity of the theoretically predicted hydration scheme. A much larger incremental effect on ΔH^0_{int} of ethyl than methyl substitution, noted in the case of $e_2^{1,3}$Thy, indicates that linear correlation between ΔH^0_{int} and the length of the alkyl substitutent at ring amide nitrogen should not be expected. This is not surprising since the electrostatic part of the total interaction energy is known to play a major role in maintaining the first hydration shell [8].

Recently estimated energies of thymine hydration according to the polyhydration scheme [9], in which simultaneous interaction of a number of water molecules located in the first hydration shell is taken into account and energies of water–water interactions subtracted from the sum of the base-water interaction energies, can be compared with our experimental ΔH^0_{int} enthalpy of base–water interaction. For the systems Thy + $3H_2O$ and Thy + $4H_2O$ under the assumption of $E_{H_2O...H_2O}$ = 21.3 kJ/mole (5.1 kcal/mole), quantum-mechanical computations gave hydration energies –47.7 kJ/mole (–11.4 kcal/mole) and –41.8 kJ/mole (–10.0 kcal/mole), respectively. They are distinctly lower than our value of ΔH^0_{int} = –63.6 kJ/mole. One should, however, bear in mind that ΔH^0_{int} is obtained for a complete hydration shell. The similarity in the order of magnitude of experimental and theoretical hydration energies may indicate that the first hydration shell is responsible for a large part of the interaction energy.

Table 2
Molecular volumes (v), enthalpies of cavity formation ($\Delta H^0_{\mathrm{cav}}$) and of base–water interaction ($\Delta H^0_{\mathrm{int}}$)

Compound	$v \times 10^{-24}$ (cm^3)	$\Delta H^0_{\mathrm{cav}}$ (kJ mole^{-1})	$\Delta H^0_{\mathrm{int}}$ (kJ mole^{-1})
Ura	114.2 (ref. [14])	31.4	−59.8
m^1Ura	133.1 (ref. [15])	34.7	−54.3
$m_2^{1,3}$Ura	164.7 (ref. [17])	40.2	−45.8
$m_3^{1,3,6}$Ura	220.5 a)	45.6	−48.8
Thy	142.8 (ref. [18])	36.4	−63.7
m^1Thy	168.9 (ref. [16])	41.0	−56.9
$m_2^{1,3}$Thy	200.5 a)	45.6	−53.4
$e_2^{1,3}$Thy	263.7 a)	54.8	−30.6

a) Estimated as described in text.

Acknowledgements

This work was in part supported by the Polish Academy of Sciences within the Project 09.7. We are greatly indebted to Miss Krystyna Bolewska for syntheses of all alkylated compounds, to Mrs. Teresa Weclawska for help in heat of solution measurements, and to Dr. L.F. Sukhodub for determination of ΔH_{subl} for $m_3^{1,3,6}$Ura.

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