Biophysical Chemistry 15 (1982) 149–155
Elsevier Biomedical Press

MASS-SPECTROMETRIC INVESTIGATIONS ON HYDRATION OF NUCLEIC ACID COMPONENTS IN VACUUM

I. ALKYLATED URACILS

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Received 17th August 1981
Revised manuscript received 2nd December 1981

Key words: Mass spectrometry; Alkylated uracil; Hydration; Enthalpy

Association reactions between water and alkylated uracils, occurring under field-ionization conditions in a mass spectrometer at the tungsten point emitter surface, were studied at several temperatures. The origin of peaks observed in the mass spectra at *m/e* ratios corresponding to M^+H and $M^+H \cdot H_2O$ were attributed to $M \cdot H_2O$ and $M \cdot (H_2O)_2$ hydrates, respectively, hydrogen-bonded via carbonyl groups of the diketopyrimidines (M) investigated. The appearance of these ions is explained in terms of the field-ionization mechanism of the neutral hydrates involving intramolecular H^+ transfer and concomitant release of the $OH^{\cdot}$ radical. Measurements of the relative peak intensities allowed the calculation of apparent equilibrium constants, K_{ass}, for the association reactions, and hence the respective van't Hoff enthalpies of hydration. The latter are discussed in connection with the available quantum-mechanical hydration energies for specific groups of respective canonical nucleic acid bases and experimental enthalpies of hydration of alkylated uracils with water. Specific hydration is estimated to contribute about 15–20% to the total enthalpy of interaction of the solutes with their hydration shells.

1. Introduction

It is known that multiple equilibria involved in hydration of nucleic acids play an important role in sustaining their various ordered forms and functional states. Therefore, both experimental [1–3] and theoretical (e.g., see refs. 4–6) studies concerning hydration of their particular components are of great interest for the elucidation of the nature and energy of interaction with water of various polar and nonpolar binding sites. We have shown previously [7] that field-ionization mass-spectroscopic studies of association between isolated bases and water under vacuum conditions may prove useful in this connection. In the present paper we report results obtained by application of this method to association reactions occurring at the field emitter surface between some alkylated uracils and water.

2. Materials and methods

All experiments were carried out on a mass spectrometer model MI-1201 (U.S.S.R.), equipped with a field-ionization source [7]. A single tungsten point emitter was used, with a radius of curvature of $r_e \approx 4000$ Å. It was formed by electrochemical etching in NaOH of the tungsten wire $\phi = 0.15$ mm in diameter.

The alkylated uracil derivatives: 1-methyluracil (m^1Ura), 1-methylthymine (m^1Thy), 1,3-dimethyluracil ($m_2^{1,3}$Ura), 1,3-dimethylthymine ($m_2^{1,3}$Thy) and 1,3-diethylthymine ($e_2^{1,3}$Thy), synthesized according to standard methods, were

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the same original samples as used in our previous studies (cf. refs. 2 and 3).

The compounds were supplied to the emitter from a heated-glass evaporator, situated at a distance of 2–3 mm from its tip. A time-stable molecular beam of water was obtained by evaporation of the structured water from crystalline $CuSO_4 \cdot 5H_2O$ placed in a glass ampoule near the emitter's tip.

3. Results and discussion

Provided that the molecular beams of water and the bases fall on the emitter simultaneously, the field-ionization mass spectrum is characterized by two groups of peaks reflecting various association reactions occurring close to its surface. The first group is assigned to autoassociates of water and the other to the hydrated bases. Below we give a more detailed description of both groups of species and discuss the possible mechanisms of their appearance.

3.1. Water autoassociates

The presence in the field-ionization mass spectrum (fig. 1) of the series of peaks at $m/e = 18$ (H_2O^+), 19 (H_2O^+H), 37 ($(H_2O)_2^+H$) and 55 ($(H_2O)_3^+H$) can be explained by the sufficiently high density of water molecules near the emitter's surface so that the frequency of intermolecular collisions becomes significant. The physical cause of such a high molecular density is the strong attractive force, F_p, directed towards the emitter's tip and equal to the gradient of the polarization energy, E_p, in the electric field of strength F:

$$F_p = \frac{dE_p}{dr} = (\mu + \alpha_F)\frac{dF}{dr},$$

where r is the distance from the point, μ the dipole moment of water, and α its polarizability.

At the point surface of the oxidized tungsten emitter, water vapor pressure can readily attain a value corresponding to condensation conditions, owing to the presence of strong adsorption forces. This leads to formation of a thin film of water covering the surface, the thickness of which can amount to a few tens of angstroms [8]. The instantaneous structure of such a film, most probably similar to that of liquid water, is characterized by the presence of a number of associates. Upon ionization, the latter appear in the mass spectrum. This concept finds support in the recently demonstrated [9] agreement between the heats of evaporation of liquid water and that of water condensed on a thread emitter obtained from field-ionization mass-spectrometric H_2O^+ peak intensity measurements as a function of temperature. The temperature dependence of the water mass-spectrum lines was also studied in our laboratory [10]. From these data we determined the binding enthalpies, ΔH, corresponding to formation of water dimers, trimers, tetramers and pentamers. It was shown that the mean value of the binding enthalpy per hydrogen bond increases gradually, approaching a value close to the heat of evaporation of liquid water.

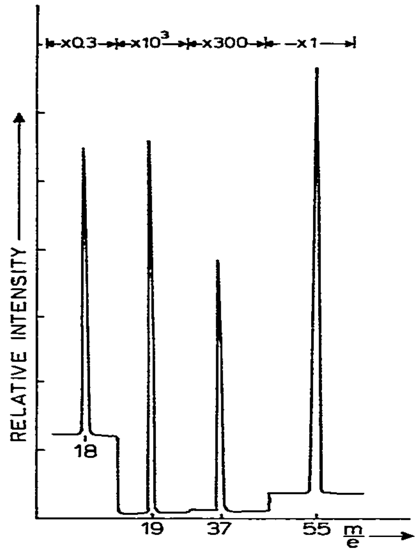

Fig. 1. Field-ionization mass spectrum of water, taken with tungsten emitter: $t_e = 20°C$, $U_e = 5$ kV.

3.2. Base-water (1:1) associates

The field-ionization mass spectra of $m_2^{1,3}$Ura, $m_2^{1,3}$Thy and $e_2^{1,3}$Thy, taken at 20°C in the

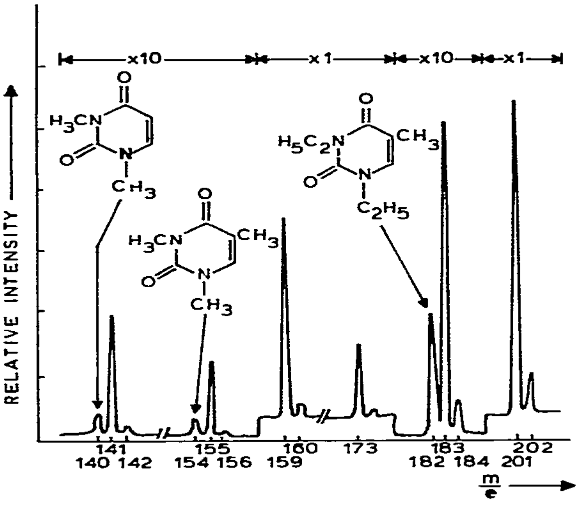

Fig. 2. Field-ionization mass spectra of the alkylated uracils indicated, taken with tungsten point emitter in the presence of water vapor.

presence of water, are shown in fig. 2. They all exhibit peaks due to the original molecular ions M^+ (m/e 140, 154 and 182, respectively), singly hydrated associates M^+H (m/e 141, 155 and 183) and doubly hydrated associates $M^+H \cdot H_2O$ (m/e 159, 173 and 201). Note that the M^+H peaks are of maximum intensity. Their origin can be attributed to the field-ionization mechanism of neutral monohydrates, which implies, as in the case of water autoassociation, occurrence of H^+ exchange. The following considerations allow more precise description. While a base molecule attaches to the surface of the thin water film, a neutral complex $M \cdot H_2O$ is formed, and is stabilized by a hydrogen bond, most probably of the $\overset{|}{\mathrm{O}}\text{–H}\cdots\mathrm{O{=}C}\Big<$ type (cf. fig. 3). Then ionization occurs by tunnelling into the metal of a lone-pair electron localized at the oxygen atom of the water molecule. Simultaneously, the strength of the O–H bond is weakened, leading in turn to the transfer of an H^+ onto the carbonyl group of the base to which it is attached and formation of M^+H. Thus, in the process described, it is the water molecule and not the base which is ionized. The ionization

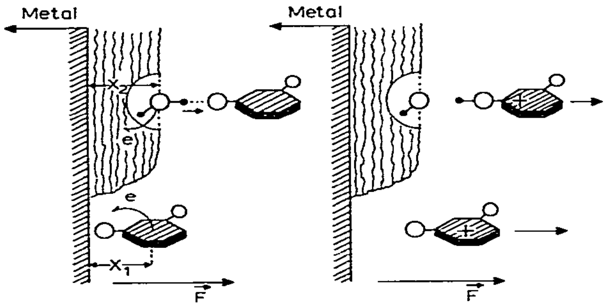

Fig. 3. Supposed mechanism of the field-ionization process for the $M \cdot H_2O$ complex. At the emitter surface there is a thin water layer with an islet structure. Note that the value of X_2 differs from that of X_1.

potentials of these two compounds are indeed substantially different: 12.61 eV for water [11], compared to 8.75 and 8.25 eV for $m_2^{1,3}$Ura and $m_2^{1,3}$Thy, respectively [12]. Therefore, their characteristic ionization coordinates, X_1 and X_2 (cf. fig. 3), should also be correspondingly different. Association itself is not expected to affect the ionization potential and X significantly.

Direct ionization of a free base molecule can thus take place only at those sites of the emitter's surface from which adsorbed water is absent. This means that the thin film of condensed water on the emitter's surface has an islet structure. In this connection, it is worth noting that replacement of the tungsten emitter by a platinum one leads to substantial changes in the mass spectrum of $m_2^{1,3}$Ura taken under similar conditions (cf. figs. 2 and 4). The ratio of the M^+ (m/e 140) and M^-H (m/e 141) peak intensities becomes inverted and there appears only a weak peak at m/e 158 due to $M^+H \cdot H_2O$. This clearly indicates that association between water and the base molecules takes place mainly in the gas phase so that the amount of condensed water on the platinum emitter's surface, as compared to that on the tungsten one, is certainly much smaller. It can thus be concluded that the mechanism of M^+ formation consists of the transfer of an outer π-electron from the base onto the metal without concomitant H^+ transfer.

Comparison of the mass spectra of variously alkylated diketopyrimidines shows that they all

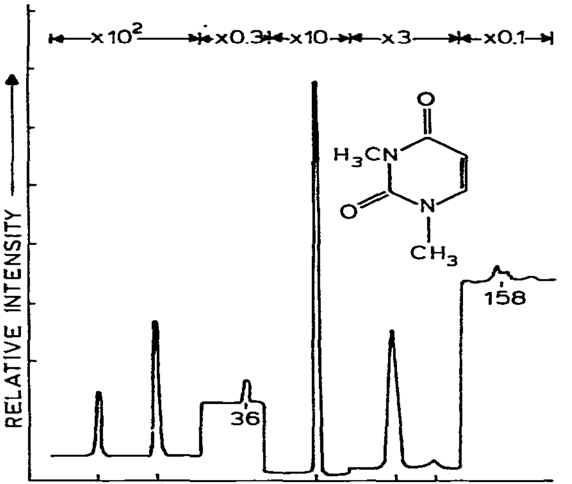

Fig. 4. Field-ionization mass spectrum of $m_2^{1,3}$Ura taken with a platinum emitter in the presence of water vapor: $t_e = 20°C$, $U_e = 5$ kV.

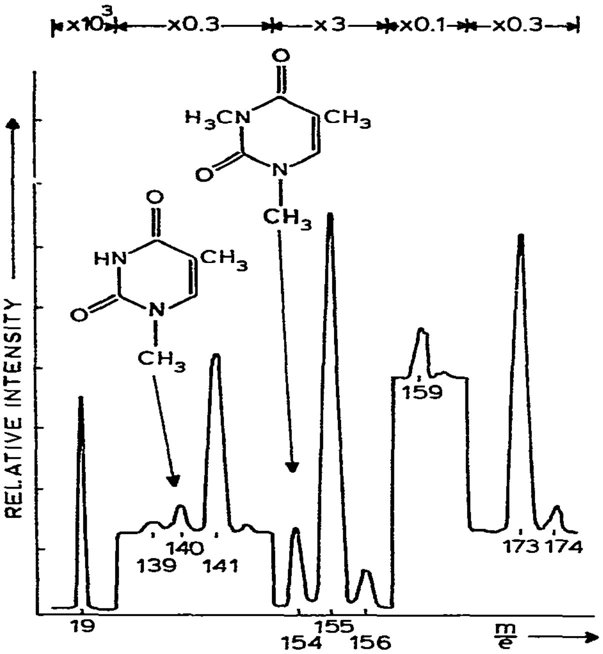

Fig. 5. Field-ionization mass spectrum of a mechanical mixture of $m_2^{1,3}$Thy and m^1Thy taken with a tungsten emitter in the presence of water vapor.

Table 1

Peak intensity ratios for hydrated ions in the mass spectra of the mixtures m^1Thy + $m_2^{1,3}$Thy (cf. fig. 5) and m^1Ura + $m_3^{1,4,4}$Cyt (cf. fig. 6)

Ratio	Compound			
	m^1Thy	$m_2^{1,3}$Thy	m^1Ura	$m_3^{1,4,4}$Cyt
M^-H/M^+	6.4	4.8	3.7	3.0
$M^+H \cdot H_2O/M^+$	0.095	0.09	0.13	–

exhibit similar relative peak intensity patterns. Replacement of N^1- and N^3-substituted methyl groups for ethyl groups, on passing from $m_2^{1,3}$Thy to $e_2^{1,3}$Thy, or the presence in m^1Thy of a free N–H hydrogen bond-donating group does not affect the relative intensity of M^+H/M^+ peaks (cf. figs. 2 and 5 and also table 1). This can be taken as evidence that one of the carbonyl groups of the diketopyrimidine skeleton is mainly involved in formation of the 1:1 base-water complexes. Should this not be the case, i.e., if the N–H group of m^1Thy could play a significant role in binding of a water molecule, then no such strong H^+ exchange accompanying ionization could occur, as inferred from the M^+H/M^+ peak intensity ratios.

3.3. Base-water (1:2) associates

As mentioned in the preceding section, in the field-ionization mass spectra of hydrated bases, peaks appear also at *m/e* 159, 173 and 201 (fig. 2) which correspond to $M^+H \cdot H_2O$ of the three bases investigated. These ions are formed according to the same field-ionization mechanism postulated for M^+H production, viz., by ionization of the neutral complexes $M \cdot (H_2O)_2$ accompanied by subsequent H^+ transfer from a water molecule onto the base:

$$M \cdot H_2O + H_2O = M \cdot (H_2O)_2 \xrightarrow{\text{in field } F} M^+H \cdot H_2O + OH + e^-$$

This implies involvement of both carbonyl groups of the uracil ring in binding of water. In order to prove this point we studied simultaneously in the presence of water vapor field-ionization mass spectra of two compounds, m^1Ura and $m^{1,4,4}$Cyt, hav-

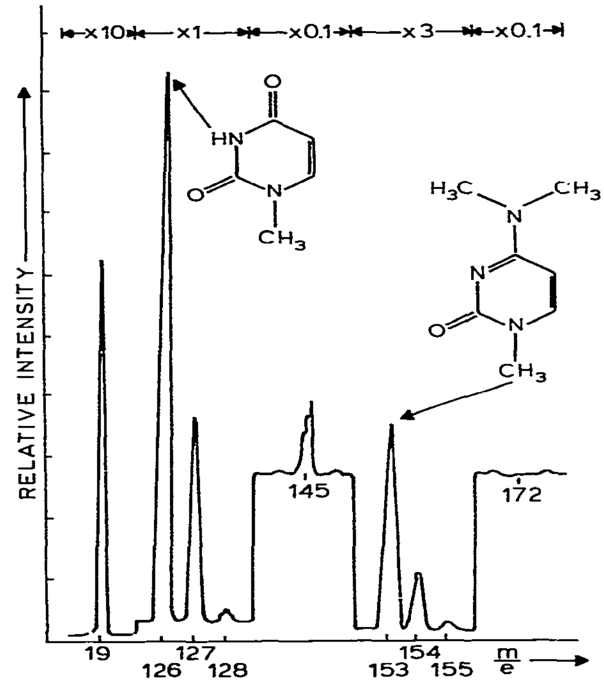

Fig. 6. Field-ionization mass spectra of $m_3^{1,4,4}$Cyt and m^1Ura taken simultaneously in the same experiment in the presence of water vapor. Tungsten emitter, $U_e = 5$ kV.

ing approximately the same volatility, but differing in the number of carbonyl groups ($m_3^{1,4,4}$Cyt contains instead of $C^4{=}O$ the $-N(CH_3)_2$ group). As seen in fig. 6, in the mass spectrum obtained under comparable base concentrations (ion currents $J_{m^1Ura} \approx J_{m_3^{1,4,4}Cyt}$), the peak at m/e 145 corresponding to $M^+H \cdot H_2O$ of m^1Ura was detected, but not that at m/e 172, expected for the $m_3^{1,4,4}$Cyt dihydrate. Similar relative M^+H/M^+ peak intensity ratios indicated that the yield of monohydrated species was approximately the same for both compounds. This confirms the validity of the reaction scheme proposed for the appearance of $M^+H \cdot H_2O$ and the involvement of both carbonyl groups of diketopyrimidines in their formation.

3.4. Determination of the enthalpies of hydration

Since the ion current J is proportional to the concentration of neutral molecules in the reaction zone, apparent association constants can be deduced for mono- and dihydrates of the bases from the ratios of the peak intensities in the mass spectrum [7,10]:

$$K_{\mathrm{ass}}^{\mathrm{M\cdot H_2O}} = \frac{[\mathrm{M\cdot H_2O}]}{[\mathrm{M}][\mathrm{H_2O}]} \approx \frac{J_{\mathrm{M^+H}}}{J_{\mathrm{M^+}} \cdot J_{\mathrm{H_3O^+}}},$$

$$K_{\mathrm{ass}}^{\mathrm{M\cdot(H_2O)_2}} = \frac{[\mathrm{M\cdot(H_2O)_2}]}{[\mathrm{M\cdot H_2O}][\mathrm{H_2O}]} \approx \frac{J_{\mathrm{M^+H\cdot H_2O}}}{J_{\mathrm{M^+H}} J_{\mathrm{H_3O^+}}}.$$

Note that in the denominators of the above equations, for the sake of higher precision, instead of the H_2O^+ peak intensity we use that for H_3O^+. This is justified by our previous observations [10] that under similar experimental conditions $J_{H_2O^+} \approx J_{H_3O^+}$.

Van't Hoff plots of log K_{ass} versus T^{-1} (K) for $m_2^{1,3}$Ura, $m_2^{1,3}$Thy and $e_2^{1,3}$Thy mono- and dihydrates are shown in figs. 7–9, respectively. The enthalpies of association, ΔH_{ass}, obtained from the slopes of the plots are listed in table 2. For comparison purposes the enthalpies of formation of water dimers and trimers [10] are also included therein. Note that the binding enthalpy for the water dimer was determined as an approximate value, because of the low intensity of the corresponding peaks in the mass spectrum.

The enthalpies of hydration of the bases studied are close to one another but they are much larger than those of water-water autoassociation. A rough estimate of the enthalpy of $m_3^{1,4,4}$Cyt hydration was made with the aid of an approximate formula [7]: $\Delta H_1 - \Delta H_2 \approx RT \ln(K_{\mathrm{ass}}^1/K_{\mathrm{ass}}^2)$, valid at a given temperature for any two reactions reflected in the mass spectrum. From the mass spectra recorded in fig. 6 at 320 K we obtained: $\ln K_{\mathrm{ass}}^{m^1Ura}/K_{\mathrm{ass}}^{m_3^{1,4,4}Cyt} = 0.33$, which indicates that the difference between the enthalpies of respective

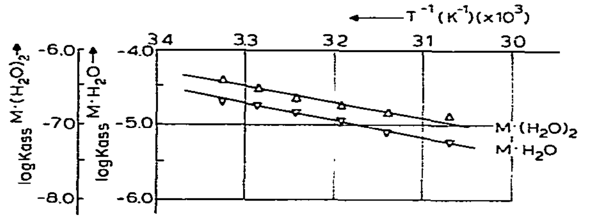

Fig. 7. Van't Hoff plot of K_{ass} for hydrated $m_2^{1,3}$Ura.

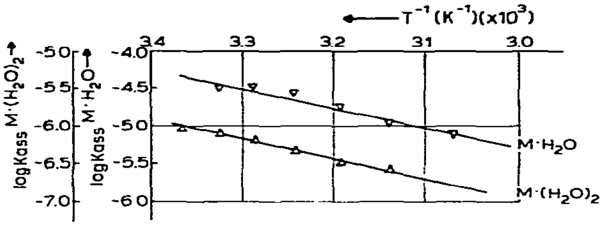

Fig. 8. Van't Hoff plot of K_{ass} for hydrated $m_2^{1,3}$Thy.

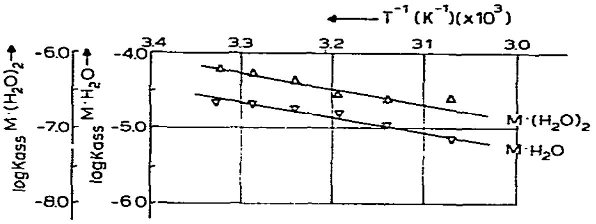

Fig. 9. Van't Hoff plot of K_{ass} for hydrated $e_2^{1,3}$Thy.

monohydrate formation remains well within the limits of experimental uncertainty. Assuming reasonably the similarity of specific hydration schemes for m^{1}Ura and m^{1}Thy, we evaluated ΔH_{ass} for monohydration of the latter compound, and thus also for $m_3^{1,4,4}$Cyt (table 2), with the aid of the foregoing equations and the mass-spectrometric data shown in fig. 5.

It is interesting to compare our experimental ΔH_{ass} data with some quantum-mechanical predictions of hydration energies. For instance, Pullman et al. [13] calculated for linear dimers $H_2O \cdots H_2O$ and trimers $H_2O \cdots H_2O \cdots H_2O$ the binding energies −20.9 and 27.6 kJ mol^{-1}, respectively. They agree well with the experimental data found for water associated under vacuum [10]. No such direct comparison with the theory of our ΔH_{ass} data is possible because quantum-mechanical calculations have been limited thus far to hydration of the canonical nucleic acid bases. Nevertheless, one can observe that the energies of binding of some specifically located water molecules, calculated for polyhydrated thymine [4], are very close indeed to the experimental ΔH_{ass} values obtained by us for N,N-alkylated thymines. For example, the most advantageous hydration site, located between the N^1–H and C^2=O groups of the diketopyrimidine ring, is characterized by a binding energy of −44.1 kJ mol^{-1}, as compared to $\Delta H_{ass} = -36.4$ kJ mol^{-1} for the $m_2^{1,3}$Thy monohydrate and $\Delta H_{ass} = -42.7$ kJ mol^{-1} for the dihydrate (cf. table 2). It would of course be very interesting to study theoretically the effects of an alkyl group substituted at the ring nitrogen on the energy of binding of a water molecule to an adjacent carbonyl group. It is known [3] that in aqueous solutions each –CH$_2$– group added on alkylation of uracil contributes as much as about 20 kJ mol^{-1} to the total energy of interaction of the solutes with their hydration shells. Our present data, however, do not provide any useful information as to this point.

The high enthalpies of water binding found under vacuum conditions, ΔH_{vac}, in solution should be noticeably reduced by the value of ΔH_{red}

Table 2

Enthalpies of association in vacuum, ΔH_{ass}, of the bases (M) with the first and second water molecules, and for water autoassociation [10]

Associate	$-\Delta H_{ass}$ (kJ mol^{-1})			
Base:	$m_2^{1,3}$Ura	$m_2^{1,3}$Thy	$e_2^{1,3}$Thy	$m_3^{1,4,4}$Cyt
M·H$_2$O	54.0±5.8	36.4±3.8	45.2±2.1	36.4
M·(H$_2$O)$_2$	45.2±3.3	42.7±4.2	43.1±3.8	–
(H$_2$O)$_2$	16.7			
(H$_2$O)$_3$	26.4±1.7			

owing to the weakening of hydrogen-bond interactions between the bases and water by the solvent environment [14]: $-\Delta H_{\mathrm{sol}} = -\Delta H_{\mathrm{vac}} + \Delta H_{\mathrm{red}}$. For instance, in the case of base-base association in chloroform [7], ΔH_{vac} is reduced by a factor of 2.2. In a polar solvent this reduction should be even larger. Indeed, comparison of the enthalpies of addition of a third water molecule to the water dimer in liquid water [15] with that for the same process in a vacuum (table 2) gives a reduction factor $\Delta H_{\mathrm{sol}}/\Delta H_{\mathrm{vac}} = 2.5$. This factor was used for estimation of the hydration energies of carbonyl groups (referred to later as specific hydration [16]) of the bases in aqueous solution. Hence, the enthalpies of formation of dihydrates of the bases in an aqueous environment were evaluated as follows: $\mathrm{m}_2^{1,3}$Ura, -39.7 kJ mol^{-1}; $\mathrm{m}^{1,3}$Thy, -31.4 kJ mol^{-1}; and $\mathrm{e}_2^{1,3}$Thy, -35.6 kJ mol^{-1}. Comparison of these values with the total enthalpies of interaction of the solutes with their hydration shells, ΔH_{int}, obtained from the heats of solution and sublimation of crystalline compounds [3] indicates that the contribution of the specific hydration of alkylated uracils to ΔH_{int} is quite appreciable and amounts to about 15–20%. The remaining part of the hydration energy is due to a less specific polar interaction and the hydrophobic van der Waals' interactions involving a number of water molecules in successive hydration layers. In the light of recent Monte Carlo calculations of hydration of the nucleic acid bases [5], in the first hydration shell of thymine there are at least 18 more or less specifically located water molecules. This agrees semiquantitatively with the preceding discussion based on energy considerations.

Further studies along this line concerning hydration under vacuum of a series of alkylated cytosines and adenines should help in the estimation of the energy of specific hydration of the amino group and its contribution to the total energy of interaction of aminopyrimidines and aminopurines with the solvent in aqueous solution.

Acknowledgments

The authors are most grateful to Professor B.I. Vierkin for his constant interest in this work and encouragement and to Miss K. Bolewska for synthesis of alkylated uracils. This work was performed under the realm of the CMEA programme on collaboration in the field of biophysics.

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