THERMOCHEMISTRY OF AQUEOUS SOLUTIONS OF ALKYLATED NUCLEIC ACID BASES. VI. ENTHALPIES OF HYDRATION OF 2-ALKYL-9-METHYLADEINES

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Received 17 December, 1986

Enthalpies of solution in water, $\Delta H_{\text{sol}}^0$, and vant'Hoff enthalpies of sublimation, $\Delta H_{\text{subl}}^0$, were determined experimentally for a number of crystalline 2-alkyl derivatives of 9-methyladenine: m$_2^{19}$Ade, c$_2^{19}$Ade, pr$_2^{19}$Ade and but$_2^{19}$Ade. Standard enthalpies of hydration, $\Delta H_{\text{hydr}}^0$ derived from these data were corrected for the calculated cavity terms, $\Delta H_{\text{cav}}^0$, to yield enthalpies of interaction, $\Delta H_{\text{int}}^0$, of the solutes with their hydration shells. The apparent residual contribution of alkyl groups, R, to the enthalpy of interaction $\delta\Delta H_{\text{int}}^0 (R)$ was found to increase linearly with the number of CH$_2$ groups added upon alkyl substitution, whereas this contribution calculated per unit area of the water-accessible molecular surface, $S_R$, of alkyl residues $\delta\Delta H_{\text{int}}^0 (R)$: $\delta S_R(R)$ appeared constant over the whole series of the compounds investigated. This indicates that alkyl groups substituted at the C(2) carbon atom of the adenine contribute additively to the van der Waals' part of the enthalpy of interaction and do not affect the electrostatic part of the energy of interaction of the solutes with their hydration shells.

The knowledge of hydration of biological macromolecules is a prerequisite for the understanding of intra- and intermolecular interactions involved in their complex folding, binding and aggregation processes. In order to

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** This work was supported by the Polish Academy of Sciences within the Project 09.7 and 01.3, and performed within the realm of the CMEA programme on collaboration in the field of biophysics.
better characterize hydration properties of nucleic acids we studied the thermochemistry of aqueous solutions of alkylated pyrimidine and purine bases [1-5] and the thermodynamics of formation of their simple hydrates in the gas phase [6, 7]. In particular, enthalpies of hydration, $\Delta H_{\text{hydr}}$, were obtained for 5-alkyluracils [3, 4] and N-methylated adenines [5] from experimental enthalpies of solution in water, $\Delta H_{\text{sol}}^0$, and of sublimation $\Delta H_{\text{subl}}^0$ of crystalline compounds. Corresponding enthalpies of interaction of the solutes with their hydration shells, $\Delta H_{\text{int}}^0$, were evaluated by correction of $\Delta H_{\text{hydr}}^0$ for the enthalpy of cavity formation, $\Delta H_{\text{cav}}^0$, calculated according to Sinanoglu [8]. Values of residual enthalpies of interaction, $\delta \Delta H_{\text{int}}^0$ (R), within a given homologous series of alkyl (R) derivatives of a base were considered to reflect a net result of compensation of a favourable contribution to $\Delta H_{\text{int}}^0$ from the additive van der Waals’ interactions, and a loss in the energy of electrostatic interactions, proportional to the number and energy of binding of water molecules displaced from their most favourable positions in the hydration shell by alkyl substituents. The normalized increments of the enthalpy of interaction per unit area of the water-accessible molecular surface $S_B$ of the alkyl group substituted, $\delta \Delta H_{\text{int}}^0(R):\delta S_B(R)$, can be compared with one another within a given homologous series of derivatives as well as with those obtained for different parent compounds [5]. In this paper we report experimental enthalpies of hydration for a series of 2-alkyl substituted 9-methyladenines and discuss enthalpies of hydration derived therefrom in connection with the data obtained previously [5] for N-methylated adenines.

**MATERIALS AND METHODS**

*Compounds.* The 2-alkyl derivatives of 9-methyladenine investigated: 2,9-dimethyladenine (m$_2^9$Ade), 2-ethyl-9-methyladenine (e$_2^9$Ade), 2-propyl-9-methyladenine (pr$_2^9$Ade) and 2-butyl-9-methyladenine (but$_2^9$Ade) were synthesized by “CHEMIPAN”, Warszawa, according to standard methods and purified by repeated crystallization and vacuum sublimation. Their
melting properties and calorimetric purity were characterized by differential scanning calorimetry using a DuPont model 910 calorimeter and model 1090B thermal analyser. The data obtained for samples kept in hermetic ampoules are collected in Table 1.

_Determination of enthalpies of sublimation and solution in water._ Vant' Hoff enthalpies of sublimation, \( \Delta H_{\text{sub}} \), were measured by the low temperature quartz-resonator method using the classical Knudsen cell as evaporator [9].

Heats of solution in water were measured as described previously [3, 4] with the use of a calorimeter equipped with an 80 cm³ steel vessel and two 150-Ω thermistors. The calorimeter was calibrated by the electric Joule effect and by determination of the heat of solution of KCl. Values of the enthalpy of solution obtained for a series of solute concentrations were numerically extrapolated back to an infinitely diluted solution to yield \( \Delta H_{\text{sol}}^{0} \).

_Evaluation of enthalpies of cavity formation._ The enthalpies of cavity formation, \( \Delta H_{\text{cav}}^{0} \), corresponding to the energy required to make a cavity in liquid water able to receive a solute molecule, were evaluated according to a general formula derived by Sinanoglu [8] from the thermodynamic properties of pure liquids and dilute solutions:

\[
\Delta H_{\text{cav}}^{0} = k'_{1} (\Phi_{1B}^{-1/3}) \cdot S_{B} \cdot \gamma_{1} \left[ 1 - \frac{\partial \ln \gamma_{1}}{\partial \ln T} - \frac{2}{3} \alpha_{1B} \cdot T \right] \ldots (1),
\]

where \( \gamma_{1} \) is the macroscopic surface tension of the solvent, \( S_{B} \) solvent-accessible molecular area of the solute B, \( \alpha_{1B} \) the coefficient of thermal expansion appropriate to the cavity and closely approximated [10] by the solvent expansion coefficient, and \( k'_{1} (\Phi_{1B}^{-1/3}) = 1 + \Phi_{1B}^{2/3} (k'_{1}/1 - 1) \) is a constant, dependent on the volume fraction \( \Phi_{1B} = V_{1}/V_{B} \), which adjust the planar surface energy to highly curved microscopic dimensions. The following values of the solvent constants were used for water: \( \gamma_{1} = 72 \) dyne cm\(^{-1}\) at 25°C, \( \alpha_{1B} = 0.235 \times 10^{-3} \) K\(^{-1}\), and \( k'_{1} (1) = 1.277 \) [10].
Water-accessible molecular area, \( S_B \), was calculated by the method of static steric accessibility of atoms developed by Lavery et al. [11] using a computer programme kindly made available to us by Dr. R. Lavery. In this method all hydrogen atoms are taken explicitly into consideration and the solvent-accessible areas are computed at the surface itself of the van der Waals’ spheres of the atoms forming the molecule. The attacking water molecule can be represented as a sphere of the radius \( r_{\text{HOF}} = 1.2 \) Å or \( r_{\text{HOH}} = 1.4 \) Å depending on the atom (indicated by a bar) which makes contact with the acceptor atom. Since the compounds considered are highly substituted with alkyl groups a hydrophobic model of the hydration shell was assumed and the effective solvent-accessible areas were calculated as average values for \( r = 1.2 \) and 1.4 Å. Since the method used does not permit to calculate corresponding molecular volumes, the solvent-accessible molecular volumes, \( V_B \), were obtained from extrapolated linear correlation between \( V_B \) and \( S_B \), calculated according to the method of Finney [5, 12]. The calculated values of \( S_B \), \( V_B \) and \( \Delta H^0_{\text{cav}} \) for the compounds investigated are collected in Table 2.

### Table 2

**Water-accessible molecular areas (\( S_B \)) and volumes (\( V_B \)), and enthalpies of cavity formation (\( \Delta H^0_{\text{cav}} \)) at 25°C**

<table>
<thead>
<tr>
<th>Compound</th>
<th>( 10^{16} S_B ) (cm(^2))</th>
<th>( 10^{24} V_B ) (cm(^3))</th>
<th>( \Delta H^0_{\text{cav}} ) (kJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>m(^1) Ade</td>
<td>77.7</td>
<td>69.3</td>
<td>62.3</td>
</tr>
<tr>
<td>m(^2) Ade</td>
<td>84.8</td>
<td>75.7</td>
<td>67.4</td>
</tr>
<tr>
<td>e(^2)m(^1) Ade</td>
<td>90.3</td>
<td>80.6</td>
<td>71.5</td>
</tr>
<tr>
<td>pr(^1)m(^1) Ade</td>
<td>97.6</td>
<td>87.1</td>
<td>76.7</td>
</tr>
<tr>
<td>but(^1)m(^1) Ade</td>
<td>104.9</td>
<td>93.6</td>
<td>82.0</td>
</tr>
</tbody>
</table>

DISCUSSION AND RESULTS

The experimental enthalpies of solution in water at 25°C, \( \Delta H^0_{\text{sol}} \), and vant’ Hoff enthalpies of sublimation, \( \Delta H^0_{\text{subl}} \), of crystalline 9-methyladenine [5] and of its 2-alkyl derivatives investigated in this work (Table 3) correlate linearly with the number of methylene groups \( n_{\text{CH}} \), added upon alkyl substitution at the carbon C(2) atom of the adenine moiety (correlation coefficients –0.974 and 0.922, respectively). The enthalpy of solution decreases while the enthalpy of sublimation increases with the length of alkyl substituent. As a result, the negative enthalpy of hydration: \( \Delta H^0_{\text{hydr}} = \Delta H^0_{\text{sol}} - \Delta H^0_{\text{subl}} \) increases in the same order. It corresponds to the process of transfer
Table 3

Enthalpies of sublimation, enthalpies of solution in water, and enthalpies of hydration

Vant Hoff enthalpies of sublimation, $\Delta H^0_{\text{subl}}$, were measured over the temperature range $\Delta T(K)$ indicated in parentheses, whilst enthalpies of solution in water, $\Delta H^0_{\text{sol}}$, were determined at $25^\circ C$ by measurements of heat of solution in the range of solute molalities, $10^3 \text{m}$, shown in parentheses; enthalpies of hydration, $\Delta H^0_{\text{hydr}}$, were calculated at $25^\circ C$ on the assumption that $\Delta H^0_{\text{subl}}$ is to a first approximation independent of temperature down to 298.15 K.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\Delta H^0_{\text{subl}}$</th>
<th>$\Delta H^0_{\text{sol}}$</th>
<th>$\Delta H^0_{\text{hydr}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>m&quot; Ade</td>
<td>$121.3 \pm 4.6$ (381 - 411)</td>
<td>$25.1 \pm 0.8$ (2.6 - 6.4)</td>
<td>$-96.2$</td>
</tr>
<tr>
<td>m&quot; Ade</td>
<td>$126.0 \pm 0.8$ (338 - 368)</td>
<td>$22.6 \pm 0.4$ (2.2 - 4.9)</td>
<td>$-103.4$</td>
</tr>
<tr>
<td>e&quot;m&quot; Ade</td>
<td>$130.4 \pm 1.3$ (328 - 361)</td>
<td>$16.3 \pm 0.3$ (1.6 - 5.1)</td>
<td>$-114.9$</td>
</tr>
<tr>
<td>pr&quot;m&quot; Ade</td>
<td>$128.5 \pm 0.6$ (331 - 358)</td>
<td>$14.2 \pm 0.4$ (1.6 - 6.8)</td>
<td>$-114.3$</td>
</tr>
<tr>
<td>but&quot;m&quot; Ade</td>
<td>$137.7 \pm 0.8$ (320 - 360)</td>
<td>$12.5 \pm 0.2$ (1.1 - 3.5)</td>
<td>$-125.2$</td>
</tr>
</tbody>
</table>

* Linear correlation coefficient for $\Delta H^0_{\text{subl}}$ ($b_{\text{CH}_2}$): $r = 0.922$; $b = -0.974$ for $\Delta H^0_{\text{sol}}$ ($b_{\text{CH}_2}$); $c$ Data from ref. [5].

of one mole of the solute from the gas phase to the infinitely diluted solution, and contains thus the cavity term, $\Delta H^0_{\text{cav}}$, proportional to the water-accessible molecular area $S_b$ of the solute (cf. eq. 1). When corrected for this term it gives the enthalpy of interaction of a solute with its hydration shell: $\Delta H^0_{\text{int}} = \Delta H^0_{\text{hydr}} - \Delta H^0_{\text{cav}}$. The values of the latter were obtained with the use of $\Delta H^0_{\text{cav}}$ calculated according to Sinanoglu & Halicinoglu [8, 10] as described in the experimental section. They are collected in Table 4.

The water-accessible areas $S_b$, computed according to the method of Lavery et al. [11], correspond to the van der Waals' surface of the molecules. They are therefore considerably smaller than those calculated previously [3 - 5] by the Finney method [12] for the surfaces determined by the loci of the center of the attacking water sphere. For instance the $S_b$ values obtained for m°Ade by those two methods are $77.7 \times 10^{-16}$ cm² (Table 2) and $164.5 \times 10^{-16}$ cm² [5], respectively. Consequently, proportionally smaller are also the enthalpies of cavity formation and of interaction with water. The latter is now to a larger extent governed by the experimental enthalpy of hydration.

As pointed out previously [4, 5], alkyl groups can be used a thermochemical probes of anisotropy of the electrostatic molecular potential of water binding around polar solutes. For this purpose apparent residual contributions of alkyl (R) groups to the total enthalpy of interaction of a given solute: $\delta \Delta H^0_{\text{int}}$ (R) and corresponding normalized increments of $\Delta H^0_{\text{int}}$ per unit
area of the water-accessible surface of the alkyl group substituted: \( \delta \Delta H^0_{\text{int}} \) (R)/\( \delta S_B \) (R), are considered. The latter can be directly compared with one another within a given group of homologous derivatives and also with those derived from \( \delta \Delta H^0_{\text{int}} \) (R) data for different parent compounds. In order to avoid an influence of experimental errors involved in determination of \( \Delta H^0_{\text{hydr}} \) on the relatively small values of \( \delta \Delta H^0_{\text{int}} \) (R), the two increments were calculated with the use of \( \Delta H^0_{\text{int}} \) obtained from linearly interpolated \( \Delta H^0_{\text{hydr}} \) values (Table 4, in brackets).

**Table 4**

**Enthalpies of solute-water interaction and the apparent residual contributions of alkyl groups**

Enthalpies of interaction, \( \Delta H^0_{\text{int}} \), shown in parentheses were obtained with the use of linearly interpolated \( \Delta H^0_{\text{hydr}} \) and applied in calculations of apparent residual contributions of alkyl groups (R) to \( \Delta H^0_{\text{int}} \) (\( \delta \Delta H^0_{\text{int}} \)(R)) and normalized increments of \( \Delta H^0_{\text{int}} \) per unit area of the water-accessible molecular surface \( S_B \) (R) of alkyl substituent (\( \delta \Delta H^0_{\text{int}} \) (R):\( \delta S_B \))(R)

<table>
<thead>
<tr>
<th>Compound</th>
<th>(- \Delta H^0_{\text{int}} ) (kJ mol(^{-1}))</th>
<th>(- \delta \Delta H^0_{\text{int}} ) (R) (kJ mol(^{-1}) A(^{-2}))</th>
<th>(- \delta \Delta H^0_{\text{int}} ) (R):( \delta S_B ) (R) (kJ mol(^{-1}) A(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>m(^9) Ade</td>
<td>158.5 (158.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>m(^2) Ade</td>
<td>170.8 (170.9)</td>
<td>(12.4)</td>
<td>(1.75)</td>
</tr>
<tr>
<td>e(^2) Ade</td>
<td>186.4 (182.3)</td>
<td>(23.8)</td>
<td>(1.88)</td>
</tr>
<tr>
<td>pr(^2) ade</td>
<td>191.0 (194.8)</td>
<td>(36.3)</td>
<td>(1.82)</td>
</tr>
<tr>
<td>but(^2) ade</td>
<td>207.2 (207.4)</td>
<td>(48.9)</td>
<td>(1.80)</td>
</tr>
</tbody>
</table>

The data in Table 4 indicate that the apparent residual contribution of alkyl residues to \( \Delta H^0_{\text{int}} \) (column 2) increases linearly with \( n_{CH} \), whereas the normalized increment (column 3) remains practically constant over the whole series of compounds. In order to rationalize these observations we followed the earlier proposed interpretation of \( \delta \Delta H^0_{\text{int}} \) (R) [4, 5]: that it is made of two opposing contributions: (i) a positive one from the additive van der Waals’ interactions and (ii) a negative contribution due to a loss in energy of electrostatic interactions caused by displacement by an alkyl substituent of some water molecules from their most favourable positions in the hydration shell of solute molecule. The invariability of \( \delta \Delta H^0_{\text{int}} \)(R)/\( \delta S_B \) (R) increment in the light of this interpretation means that alkyl substitution at C(2) ring carbon atom of adenine does not affect the electrostatic part of the energy of interaction of water molecules with the purine moiety, perhaps with the exception of methyl group. Namely, it has been shown in the preceding paper of this series [5] that in N-methylated adenes variously substituted methyl groups contribute differently to \( \Delta H^0_{\text{int}} \). Using \( S_B \) values computed by the Lavery et al. method [11] we recalculated previously reported
$\Delta H^0_{\text{int}}$ data for N-methylated adenines [5] and obtained $\delta\Delta H^0_{\text{int}} (R)/\delta S_R (R)$ values for the m$^2$, N-m$^6$ and m$^9$ methyl groups 1.75, 1.37 and 1.14 kJ·mol$^{-1}$·Å$^{-2}$, respectively. The order of these increments remains in good agreement with the quantum chemical hydration schemes of adenine [13, 14] and of N-methyladenines [15], according to which the two strongest binding centers for water are located between (i) the N(7) ring nitrogen and the amino group, and (ii) the N(3) and N(9) ring nitrogen atoms.

The largest increment observed for the m$^2$ methyl group indicates that methyl substitution at C(2) ring carbon atom causes the smallest loss of the electrostatic energy of interaction of the hydration shell with the adenine moiety. This explains why a further increase in the size of the 2-alkyl group apparently exerts but little effect on this energy.

It was found recently (K. L. Wierczowski, unpublished) that $\delta\Delta H^0_{\text{int}} (R)/\delta S_R (R)$ remains constant not only for aliphatic hydrocarbons, what could be easily expected, but also for a series of N-n-alkylamides. In this connection it is necessary to recall that so far non-linear and diminishing increase of residual contribution of alkyl groups to $\Delta H^0_{\text{int}}$ has been found only in the case of 5-alkyluracils [4]. Further thermochemical studies carried out currently in our laboratories on hydration of 8-alkyladenines and 6-alkyluracils should help to better judge validity of the molecular model underlying the present interpretation of residual enthalpies of interaction for alkylated nucleic acid bases.

The authors are greatly indebted to Dr. R. Lavery for the computer programme for computation of water-accessible areas, to Dr. P. Zielenkiewicz for adaptation of the programme to SM-4 and CDC CYBER 73 computer and for help in computations, to Dr. M. Kamiński for characterization of the melting properties of the compounds and to Mrs. Natalia M. Archangelova for assistance in measurements of the enthalpies of sublimation.

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