

Stacking Interaction of Nucleobases: NMR Investigations

III. Molecular Aspects of the Solvent Dependence*

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Abstract. The stacking interaction between nucleic acid bases has been investigated by the determination of the self-association of 6-methylpurine in various mixtures of water and nonaqueous solvents in order to elucidate the solvent effect. The parameters of stacking association as well as of local solvent-solute interactions have been measured by means of NMR technique. The influences of local hydration and of solvent-solvent interactions on the stacking ability are discussed.

Key words: Stacking — Solvent-mixtures — 6-methylpurine — Self-association.

Introduction

Numerous results about the stacking interaction of nucleic acid bases have been presented during the last years. In general, the parameters of the thermodynamic equilibrium of self-association as well as of mixed associations between various purines and pyrimidines have been studied (Ts'o, 1974); the mechanism of base stacking and the special role of water, however, have not been solved yet.

Since the solvent water seems to be an indispensable requirement for the stacking interaction a contribution of hydrophobic interaction to stacking has been suggested (Plesiewicz, 1976; Ts'o, 1969). Since, however, hydrophobic forces are considered to be special van der Waals forces a distinction of the stacking interaction in regard to hydrophobic or van der Waals type seems to be a rather artificial judgement (Gaarz, 1976). In any case, the main stabilizing forces of stacked complexes seem to be dipole-induced dipole and London dispersion forces depending on the charge distribution and the π -electron polarizability of the interacting molecules (Schimmack, 1975 II.; Lawaczek, 1974; Pörschke, 1972; Ts'o, 1969) as well as on the structure of the surrounding solvent (Kreishman, 1976; Scruggs, 1972). The

* Parts I and II see Schimmack 1975

contribution of the solvent to the stacking energy has also been emphasized by theoretical considerations (Sinanoglu, 1964).

Furthermore, denaturation of DNA, e.g., caused by nonaqueous solvents, has been assumed to be a consequence of a reduced stacking energy (McGhee, 1977; Lowe, 1972; Herskovits, 1972). In order to elucidate the stabilizing effect of water on stacking the solvent water has been replaced by mixtures of water and non-aqueous organic solvents. These solvents might interfere with the water-water interaction and the interaction between water and the solute molecules, 6-methylpurine, thus, influencing the stacking self-association of this compound. The stacking association as well as the local solvent-solute interaction have been determined by nuclear magnetic resonance (NMR) method.

Experimental Methods

6-Methylpurine ($m^6\text{Pur}$) has been obtained from Serva, Heidelberg, Germany. The deuterated solvents have been purchased from Sharp and Dohme, München, Germany, the other solvents from Merck, Darmstadt, Germany. All substances have been used without further purification.

The NMR spectra were recorded on a Varian HA-100 spectrometer operating in the field-sweep mode. The non-deuterated portion of the solvents investigated provided the field-frequency-lock signal. The probe temperature was 25° C in all experiments controlled by a Varian temperature controller V 4343.

Molecular parameters of stacking interaction, detectable by the NMR method, are the apparent self-association constant K , the monomer chemical shift δ_M (chemical shift extrapolated to zero concentration), and the dimer complex shift Δ_2 (proton chemical shift of a molecule associated with another one relative to δ_M). These parameters have been calculated according to the isodesmic model described recently (Schimmack, 1975 I.). Using the assumption that the complex shift of protons inside of a stacking associate is influenced twice as much as that one of the protons located at the ends (Dimicoli, 1973), the following equation can be derived (Sapper, 1976)

$$\frac{\Delta}{\Delta_2} = 2 + \frac{1 - \sqrt{1 + 4K \cdot a_0}}{K \cdot a_0}$$

$$\approx 2K \cdot a_0 - 4K^2 \cdot a_0^2 + \dots \text{ (provided that } Ka_0 < 0.25 \text{)}$$

Δ : chemical shift observed relative to δ_M

a_0 : total solute concentration

It can be seen that the slope of the function $\Delta(a_0)$ at $a_0 = 0$ is given by $2K \cdot \Delta_2$. This quantity is largely independent of the model of evaluation and, in the concentration range used, is more accurate than the values K and Δ_2 taken separately as has been pointed out by Deranleau (1969) in the similar case of optical spectroscopy. Furthermore, this quantity should be a very appropriate indication for the destacking effect in the case when K , Δ_2 , or both of these parameters decrease concomitantly with a reduction in stacking.

The calculations of $2K \cdot \Delta_2$ as well as of K and Δ_2 , and the extrapolation of δ_M were performed on a CD 3300 computer, Hochschulrechenzentrum, Gießen, using a nonlinear least square fit procedure. The asymptotic standard deviations of the parameters given by the computer program have been found to be less than $\pm 5\%$ for the quantity $2K \cdot \Delta_2$ and up to $\pm 20\%$ for K and Δ_2 . The monomer chemical shifts have been computed to an accuracy of better than ± 0.005 ppm.

Results

In earlier studies it has been shown that the self-association of 6-methylpurine ($m^6\text{Pur}$) in aqueous solution is caused by vertical stacking (Sapper, 1975; Kelly, 1974; Marenchic, 1973; Stoesser, 1967). This stacking interaction is shown, in the NMR spectrum, by an upfield shift of the resonance signals induced by the ring current effect of the associating molecules. The concentration dependence of this upfield shift can be used for calculating the association parameters. It is less expressed if nonaqueous solvents, e.g., dioxane, are added to an aqueous $m^6\text{Pur}$ solution as can be seen in Figure 1 (the addition is expressed in mol-% and not in vol-% because molecular qualities rather than volume qualities of the solvents are considered). In the mixtures the slope of the curves $\Delta(a_0)$ at $a_0 = 0$ representing the quantity $2K \cdot \Delta_2$ is decreased. The change in the concentration dependence as well as in the corresponding $2K \cdot \Delta_2$ -values is shown in Figure 2 for the cases in which 9 mol-% of formamide or dioxane had been added to D_2O .

The influence of the concentration of the nonaqueous solvent (e.g., formamide, methanol, and dioxane) on the $m^6\text{Pur}$ self-association (as based on the H-2 proton resonance) is shown in Figure 3. No decrease has been observed with methanol concentrations of up to 5 mol-%. In the case of dioxane, the self-association was too small for evaluation if more than 30 mol-% were added to D_2O . The examples shown in Figure 3 demonstrate that the destacking due to the addition of organic solvents seems to be qualitatively similar and that the greatest differences between the various solvents can be expected at about 9 mol-%.

Therefore, the quantity $K \cdot \Delta_2$ evaluated from the H-2 signal (abbreviated as $K \cdot \Delta_2^2$) as well as from the H-8 signal (abbreviated as $K \cdot \Delta_2^8$) is listed in Table 1 for a 9 mol-% mixture of various solvents with water.

Without considering molecular processes of the solvents added, $K \cdot \Delta_2^2$ as well as $K \cdot \Delta_2^8$ should decrease with increasing volume content of the nonaqueous solvents if the solvent water is an essential condition for stacking. As anticipated, these values decrease below 60% (always in comparison to the value obtained for pure D_2O) in an 18.0 vol-% mixture with formamide, below 40% in a 22.6 vol-% mixture with acetonitrile or in a 29.0 vol-% mixture with acetone, below 30% in a 32.2 vol-% mixture with dioxane, and below 20% in a 34.6 vol-% mixture with *t.* butanol. Other solvents, however, such as methanol, glycol, and dimethylsulfoxide (DMSO) don't seem to reduce the stacking ability in the expected degree and the $K \cdot \Delta_2$ -values remain e.g., above 60% in a 23.4 vol-% mixture with glycol.

The ratio Δ_2^2/Δ_2^8 which is independent on K should give an indication for possible changes in stacking geometry. If this ratio of the complex shifts remains unchanged it may be concluded that also the geometry of the association remains the same as in

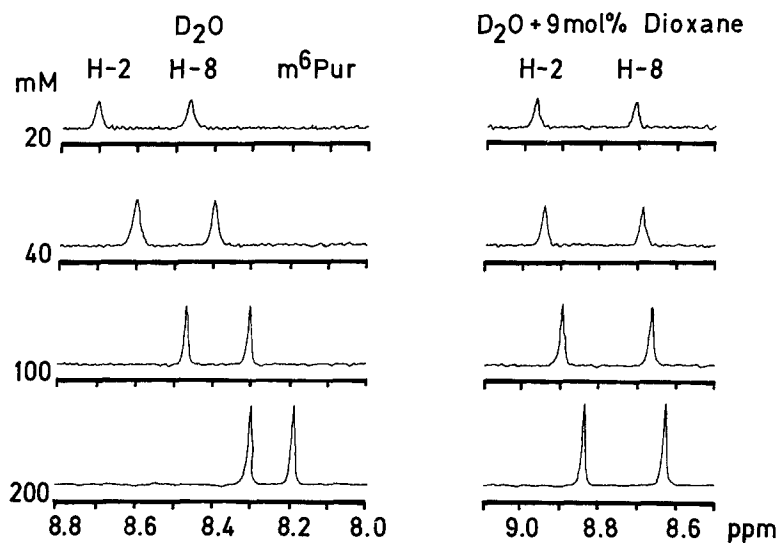


Fig. 1. Concentration dependence of H-2 and H-8 resonances of $m^6\text{Pur}$ in D_2O and in a 9 mol-% dioxane- D_2O mixture

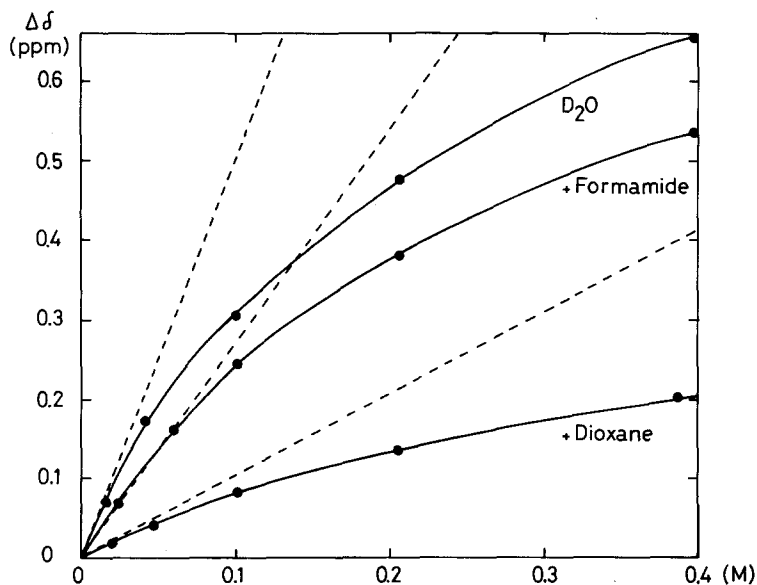


Fig. 2. Influence of 9 mol-% formamide and dioxane added to D_2O on the concentration dependent chemical shift of H-2 of $m^6\text{Pur}$; the quantity $2K \cdot \Delta_2$ is represented by the slopes (dashed lines) of these curves at zero $m^6\text{Pur}$ concentration

Fig. 3. Influence of methanol, formamide, and dioxane on $K \cdot \Delta_2$ of H-2 relative to the value obtained in D_2O

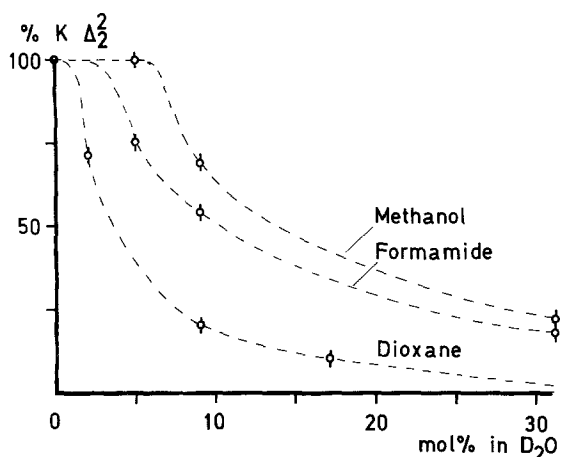


Table 1. Molarities m_s of some organic solvents and their vol-% required for obtaining a 9 mol-% mixture with D_2O . The values of $K \cdot \Delta_2$, evaluated from the H-2 signal (abbreviated as $K \cdot \Delta_2^2$) and the H-8 signal (abbreviated as $K \cdot \Delta_2^8$), resp., and the values of the ratio Δ_2^2/Δ_2^8 are given in per cent of those ones obtained with pure D_2O

Solvent	m_s [M]	Vol.-%	$K \cdot \Delta_2^2$ [ppm/M]	$K \cdot \Delta_2^8$ [ppm/M]	Δ_2^2/Δ_2^8
D_2O + 9 mol-%	55.5	100	2.46	1.59	1.55
[%], $D_2O \cong 100\%$					
Formamide	25.2	18.0	54	58	93
Methanol	24.7	18.4	69	68	101
Acetonitrile	19.1	22.6	35	31	113
Glycol	17.9	23.4	63	63	100
DMSO	14.1	28.2	56	55	102
Acetone	13.6	29.0	34	31	110
Dioxane	11.7	32.2	20	26	77
t. Butanol	10.5	34.6	18	13	138

D_2O [that is an almost antiparallel stacking with only a slight displacement of the ring systems (Sapper, 1975)]. This association pattern has been observed in mixtures in which an unexpected small destacking has been found, that is in the mixtures with methanol, glycol, and DMSO (Table 1). In the other solvent mixtures the stacking pattern might be changed in such a way, that either the H-2 proton (in the case of acetonitrile and t. butanol) or the H-8 proton (in the case of formamide and dioxane) exhibit a relatively large complex shift, that is, it is located more adjacent to the center of the ring current of the interacting m^6Pur molecules.

In order to get some more detailed information on the solvent dependence of stacking, the parameters K and Δ_2 have been computed separately and are listed in Table 2. The monomer shifts are also shown as influenced by the solvents added.

Table 2. Self-association constant K , complex shift Δ_2 for H-2 and H-8, resp., and the monomer shifts δ_M for H-2 and H-8, resp., in D₂O and 9 mol-% mixtures of various solvents with D₂O

Solvent	K [M ⁻¹]	Δ_2^2 [ppm]	Δ_2^8 [ppm]	δ_M^2 [ppm]	δ_M^8 [ppm]
D ₂ O + 9 mol-%	3.2	0.77	0.61	8.80	8.54
Formamide	1.8	0.74	0.57	8.88	8.61
Methanol	3.3	0.50	0.34	8.76	8.50
Acetonitrile	1.8	0.47	0.31	9.02	8.74
Glycol	3.0	0.52	0.36	8.75	8.49
DMSO	2.6	0.53	0.37	8.88	8.62
Acetone	2.0	0.42	0.25	8.87	8.62
Dioxane	1.4	0.35	0.17	9.01	8.75
t. Butanol	1.0	0.43	0.25	8.79	8.53

It can be seen that the K -values (as evaluated from the H-2 signal) in the mixture with methanol or glycol are very similar to those ones obtained with pure D₂O (3.2 M⁻¹), whereas in the case of DMSO this value is slightly less. All other values are 2.0 M⁻¹ or less. The order of these results is very similar to that one obtained with $K \cdot \Delta_2$ with the exception of acetone, in which case either the $K \cdot \Delta_2$ -value might be too small or the K -value too large.

The complex shifts behave somewhat differently. The smallest Δ_2 -values have been observed in the case of dioxane whereas in the case of t. butanol the values are larger and resemble those ones of acetone. In the case of formamide the complex shifts are very similar to those ones obtained with pure D₂O, although the corresponding K -value is relatively small. In all other cases, the Δ_2 -values are considerably smaller. It is interesting to note that the complex shifts are the smallest the more the ratio Δ_2^2/Δ_2^8 (Table 1) differs from the value obtained with pure D₂O, e.g., in the mixtures with t. butanol, dioxane, acetonitrile, and acetone.

The so-called monomer chemical shift δ_M of a solute molecule is influenced, in general, by the solvent, since it has to be considered as a complex shift of a solute-solvent complex. The complexation between a monomer m⁶Pur molecule and water in aqueous solution should resemble that one of the monomer adenine in water which has been calculated by Port and Pullman (1973). According to these calculations the hydration should occur essentially adjacent to the aromatic ring system whereas a hydrophobic area should be located above and below the ring system. Both N-3 and H-9 should be the preferred hydration site utilizing the bifunctional acid-base behaviour of water. Other sites may be the N-1 and N-7 atoms (Fig. 4).

These latter sites, however, may be less preferred in m⁶Pur than in adenine where a complexation via the protons of the NH₂-6 group is possible. Therefore, non-aqueous solvents will compete with water first at N-1 and N-7 whereas the water molecule remains bound strongly at N-3 and H-9. Thus, solvents acting as donors for hydrogen bonds may compete easier with water than other solvents simulating, in part, the hydration of m⁶Pur.

For the conformation of this assumption the monomer shifts δ_M have been computed by an extrapolation of the observed chemical shifts to $a_0 = 0$ (Table 2).

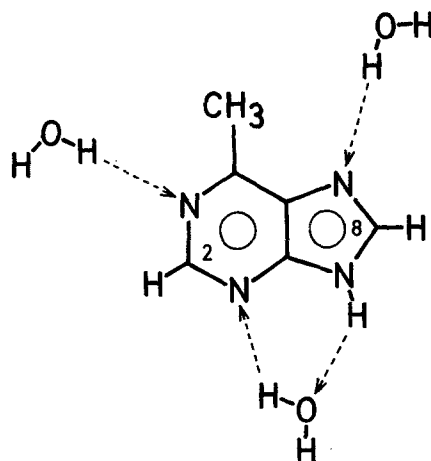


Fig. 4. Hydration of the monomer $m^6\text{Pur}$ [comparable to that one of adenine which has been calculated by Port and Pullman (1973)]

For an exact comparison of the monomer shifts obtained in various solvent mixtures, an appropriate reference should be used. External as well as internal reference substances, however, give rise to difficulties because of the magnetic susceptibility corrections needed or possible additional interactions. Therefore, the chemical shifts were referred to the water signal (δ_{HOD}) measured for all solvent mixtures in a separate probe without $m^6\text{Pur}$. Then, 4.83 ppm (chemical shift of water at 25° C) were added in order to obtain the ppm-scale relative to tetramethylsilane.

By this procedure, unfortunately, it cannot be distinguished whether δ_M or δ_{HOD} is shifted by the addition of nonaqueous solvents. Nevertheless, it can be seen that the various solvents behave differently. The mixtures containing alcohols like methanol, glycol, and *t.* butanol cause an upfield shift of δ_M (or likewise a downfield shift of δ_{HOD}) which might be interpreted by a solvation of $m^6\text{Pur}$ similar to that one obtained in pure water but reduced concerning the strength of hydrogen bonds preferentially at N-1 and N-7. On the contrary, the other solvents cause a downfield shift of δ_M (or likewise an upfield shift of δ_{HOD} , up to 0.22 ppm in the case of acetonitrile). If this fact would be explained by a solvation of $m^6\text{Pur}$ stronger than in pure water δ_M^8 should be more influenced than δ_M^2 since the H-donor site H-9 is located adjacent to H-8. This effect, however, has not been observed. Therefore, it might be concluded that the observation can be explained by the upfield shift of δ_{HOD} rather than by the downfield shift of δ_M . The upfield shift of the water signal indicates, then, a decrease in hydrogen bond structure of water.

Discussion

The results described above indicate that the stacking self-association of $m^6\text{Pur}$ depends essentially on the kind and composition of the solvent mixture used. The destacking caused by the addition of a nonaqueous solvent seems to depend on both molecular solvent-solvent and solvent-solute interactions.

The solvent-solute interaction of $m^6\text{Pur}$ in water seems to be caused by the combined interaction of N-3 and H-9 via hydrogen bonds and, furthermore, on a

van der Waals attraction due to the CH_3 -group substituted at C-6. This suggestion is confirmed by the fact that the self-association of unsubstituted purine in aqueous solution is smaller [$K = 2.2 \text{ M}^{-1}$ at 25°C (Dimicoli, 1973)] than the one obtained with m^6Pur [$K = 3.2 \text{ M}^{-1}$ (Table 2)] but larger than the one obtained with 9-methylpurine [$K = 1.3$ to 1.9 M^{-1} (Gaarz, 1976; Helmkamp, 1968)]. The kinetics of stacking interaction is influenced essentially by the solvent and depends, in general, on the properties of the solvent shell surrounding the solute molecule (Pörschke, 1972).

On the molecular level, the addition of solvents which can act like water as donors for hydrogen bonds (e.g., methanol, glycol, and *t.* butanol) to the aqueous solution of m^6Pur may cause a partial displacement of the bound water. Consequently, the self-association of the m^6Pur molecules will decrease due, probably, to stereochemical reasons. That means, if rather voluminous solvent molecules replace the small water molecules bound to m^6Pur , the distance between the interacting ring systems of m^6Pur becomes larger. Because of the dependence of the van der Waals forces on $1/r^6$, both the association constant and the complex shift should decrease. The sequence of the destacking ability should, then, depend on the size of the solvent molecules used. This is confirmed by the following order obtained experimentally: *t.* butanol > glycol > methanol. This result agrees well with the observation that the effectiveness of denaturing alcohols increases with increasing hydrocarbon content (Herskovits, 1972).

In solvents acting only as acceptors for hydrogen bonds, e.g., DMSO, acetone, or dioxane, the interaction between the solvents might be more pronounced than a displacement of water from hydration sites. This results in a partial disruption of the water structure as can be seen from the upfield shift of the water signal and in a decrease of the contribution of the surrounding water to the interaction between the stacked m^6Pur molecules. These results confirm, thus, findings obtained by Kreishman et al. (1976) according to which the structure of the bulk water is an essential condition for the stacking of purines. Therefore, it might be concluded that the solvent molecules bound have to be regarded as an integral part of the solute molecules interacting with each other. This applies, especially, to the relatively small but polar water molecules which may enhance the polarization and/or the polarizing ability of the solute molecules.

From the results obtained it can be concluded that the stacking interaction of nucleobases seems to be determined, to a great extent, by specific local interactions of the water molecules. In order to describe the stacking association in detail, solute-solute as well as solvent-solute, and solvent-solvent interactions have to be considered, therefore, at the molecular level.

Acknowledgements. The preparation of the computer programs by Dipl. Phys. H. H. Paul is kindly appreciated. This work was supported in part by a Euratom grant.

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