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Base stacking of simple mRNA cap analogues

Association of 7,9-dimethylguanine, 7-methylguanosine and 7-methylguanosine 5′-monophosphate with indole and purine derivatives in aqueous solution

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Equilibrium constants for the association of different ionic forms of 7,9-dimethylguanine, 7-methylguanosine and 7-methylguanosine 5′-monophosphate with indole, caffeine and various methylated adenines have been determined by distributing the latter compounds between an organic solvent and aqueous solutions of the 7-methylguanine derivatives. The data are compared to those obtained for the association of unsubstituted purine with the same cosolutes. The stacking affinity of both cationic and zwitterionic forms of the 7-methylguanine ring correlates with the ring polarizability rather than the polarizing power of the cosolute. The cationic species stacks usually more efficiently. The chemical nature of the *N*9-substituent has only a moderate influence on the base-stacking properties.

1. Introduction

The 5′-terminal nucleoside in eukaryotic mRNAs is 7-methylguanosine linked by a 5′,5′-triphosphate bridge to the next nucleoside, which is often a methylated purine nucleoside [1,2]. Both ^{1}H-NMR [3,4] and fluorescence measurements [5] have revealed that the 5′-terminus of mRNA adopts a spatial configuration, where the purine bases stack with each other. The enthalpy and entropy changes for the intramolecular stacking of 7-methylguanine with adjacent adenine base have been reported [5] to be -18 kJ mol^{-1} and -60 J K^{-1} mol^{-1}, respectively.

Besides intramolecular stacking, intermolecular interactions may also play a role in the attachment of mRNA to 40 S ribosomal subunits. This process is enhanced by cap-binding proteins, which specifically recognize the cap structure [2,6]. Since the tryptophan content of cap-binding proteins is high [7], and the indole moiety of tryptophan is known to stack rather efficiently with purines [8,9], it has been speculated [10–12] that the protein-nucleic acid adducts might be stabilized by stack formation between 7-methylguanine and indole residues. Stacking of 7-methylguanine nucleosides and nucleotides with various monomeric derivatives of indole has been demonstrated by X-ray crystallography in the solid state [10,13,14] and by ^{1}H-NMR [9,11] and fluorescence spectroscopy [11] in aqueous solution. It has also been shown that hydrogen bonding between the 7-methylguanine base and anionic carboxylate groups enhances association with aromatic amino acids [12] or their small peptides [11]. However,

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the available thermodynamic data on stacking in aqueous media remain scanty. The present report is aimed at furthering the understanding of factors that influence the stacking specificity of cap analogues. For this purpose, a phase distribution technique [15] has been applied to determine the stability constants for the adducts that cationic and zwitterionic forms of 7-methylguanine derivatives form with aromatic nitrogen bases. The cosolutes were selected in such a manner that they cover a wide range of polarizability on the one hand and polarizing power on the other. The results are compared to those obtained with unsubstituted purine, used as a model of nonalkylated nucleic acid bases.

2. Results and discussion

Table 1 records the equilibrium constants obtained by a phase distribution technique [15] for the association of 7,9-dimethylguanine (**1**), 7-methylguanosine (**2**), 7-methylguanosine 5′-monophosphate (**3**) and purine (**4**) with various cosolutes in aqueous solution at pH 5.3 and 8.2. The pK_a values of 7-methylguanosine and its 5′-monophosphate have been reported to be 6.7 and 7.1 at low ionic strength (0.02 mol dm^{-3}) [16], and 7.2 and 7.4 at high values (1.0 mol dm^{-3}) [17]. The pK_a value of 7,9-dimethylguanine may be expected to be about 0.5 units

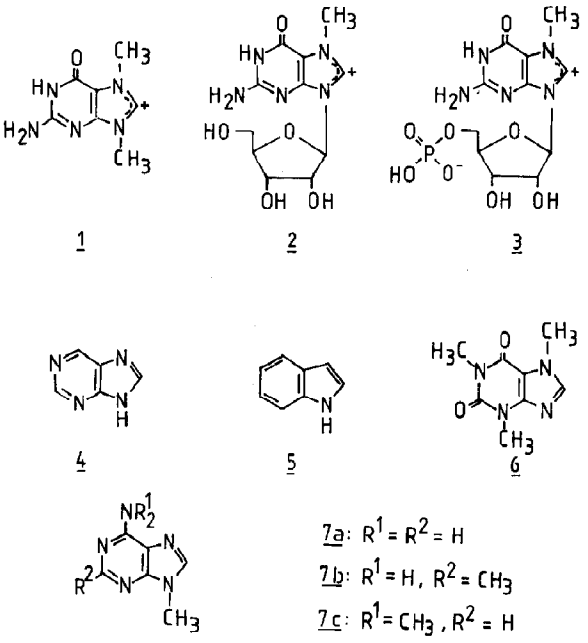

Fig. 1. Structural formulae of **1**–**7c**.

Table 1

Equilibrium constants for the association of 7,9-dimethylguanine (**1**), 7-methylguanosine (**2**), 7-methylguanosine 5′-monophosphate (**3**) and purine (**4**) with various cosolutes in aqueous solution at 298.2 K [a]

Cosolute	pH	K (dm^3 mol^{-1})			
		1	**2**	**3**	**4**
Indole (**5**)	5.3	13 (2) [b]	15 (1)	[c]	3.6 (4)
	8.2	7 (2)	6.3 (4)		
Caffeine (**6**)	5.3	4.5 (5)	3.8 (5)	1.8 (3)	15 (1)
	8.2	6.3 (5)	4.8 (9)	2.3 (7)	
9-Methyladenine (**7a**)	5.3	9.6	10 (1)	10 (1)	12 (1) [d]
	8.2	7.6	8.5 (6)	6.3 (8)	
2,9-Dimethyladenine (**7b**)	5.3	14 (1)	11 (1)	13 (1)	17 (1) [d]
	8.2	12 (1)	6.4 (4)	5.1 (3)	
N^6,N^6,9-Trimethyladenine (**7c**)	5.3	18 (2)	17 (1)	14 (1)	47 (2) [d]
	8.2	18 (1)	12 (1)	9 (1)	

[a] Calculated for a 1:1 adduct. The upper limits of the concentrations employed were 0.075 mol dm^{-3} for **1** and 0.2 mol dm^{-3} for **2**–**4**. Concentrations of cosolutes were of the order of 10^{-3} mol dm^{-3}.

[b] Standard deviations of mean for 4 measurements given in parentheses.

[c] Determination of the indole concentration in the aqueous phase failed.

[d] From ref. 15.

greater than that of the corresponding nucleoside [18–20]. The base moiety of compounds **1**–**3** is thus predominantly in a form that is cationic at pH 5.3 and zwitterionic at pH 8.2. The phosphate group of **3** is monoanionic at pH 5.3 and dianionic at pH 8.2 [17].

It has been suggested previously [5,13] that the protonated form of the 7-methylguanine ring stacks more efficiently than its zwitterionic counterpart. The data in table 1 partly corroborate this proposal. The association constants evaluated at pH 5.3 are usually greater than those at pH 8.2, the largest difference being in stacking with indole (**5**). However, the situation is reversed in the case of caffeine (**6**), indicating that the relative stacking affinities of the cationic and zwitterionic forms depend on the chemical nature of the cosolute.

As seen from table 1, all of the 7-methylguanine derivatives studied exhibit rather similar stacking affinities at pH 5.3, and at pH 8.2 the ability to undergo association slightly decreases on going from 7,9-dimethylguanine to the corresponding nucleoside and nucleotide. In contrast, the stability constant determined by ^{1}H-NMR spectroscopy for the adduct of **3** and tryptophan methyl ester (21.8 $dm^3\ mol^{-1}$) was considerably larger than that observed for **2** (8.0 $dm^3\ mol^{-1}$) [9]. This apparent discrepancy most probably results from the fact that tryptophan methyl ester is positively charged under conditions of neutrality, whereas the cosolutes used in the present study are neutral molecules. As suggested by Kamiichi et al. [9], electrostatic or hydrogen-bonding interactions between the tryptophan side chain and 5′-phosphate group of **3** may enhance the association of these compounds. It is also noteworthy that the NMR spectroscopic measurements were carried out at low solute concentrations (0.01 mol dm^{-3}), while in our measurements the concentrations of **2** and **3** were varied from 0.02 to 0.2 mol dm^{-3}. Since guanine derivatives are known to form ordered octameric structures at high concentrations, [21–23], it is also possible that stacking of the cosolutes with aggregates of 7-methylguanosine 5′-monophosphate contributes to the association constants described in the present report. Nevertheless, it seems clear that the stacking properties of **1**, **2** and **3** are comparable, and that the ribofuranosyl and 5′-phosphate moieties usually slightly retard the association with neutral molecules.

The data in table 1 reveal that the stacking specificity of 7-methylguanine derivatives differs markedly from that of unsubstituted purine (**4**). The most striking difference is that **1** and **2** associate much more efficiently with indole (**5**) than with caffeine (**6**), while the reverse of this situation occurs for purine. This difference in binding behaviour may be rationalized by the concept of dipole-induced dipole-dipole interactions [24]. According to this theory, stacking affinity depends on the mutual polarization of interacting bases. Bond moments of one base polarize the π-electron system of the other, resulting in attraction between the aromatic rings. Polarizing power has its origin in bond moments caused by ring nitrogens and especially by oxo and amino groups, while polarizability correlates with the π-electron density in the ring. Lawaczek and Wagner [24] concluded, from a comparison of the intermolecular shielding parameters and the sum of X-ray bond lengths, that the aromaticity, and hence ring polarizability, decrease in the series: indole > adenine ~ purine > guanine > caffeine. By contrast, the polarizing power, which was assumed to correlate with the number of CN and CO bonds in the bases, decreases in the order: caffeine > guanine > adenine > purine > indole. In other words, indole is a molecule with high polarizability and low polarizing power, while the opposite is true for caffeine. Indole does not stack efficiently with purine, since the polarizing power of both of these species is low, and hence marked mutual polarization cannot occur. The fact that indole associates much more readily with 7-methylguanine derivatives may be regarded as an indication of the rather strong polarizing ability of the latter compounds. Caffeine, in turn, has low polarizability and high polarizing power, and thus its binding affinity is governed by the polarizability of the molecule with which it associates. Because this cosolute stacks less efficiently with 7-methylguanine derivatives than with purine, the polarizability of **1**–**3** appears to be rather low. In conclusion, 7-methylguanine derivatives should be treated as solutes with high polarizing power and

low polarizability in rationalizing their stacking specificity.

As mentioned above, N1 deprotonation slightly enhances the association of **1–3** with caffeine, but retards that with indole. Since stacking with indole, which is governed by the polarizing ability of the 7-methylguanine derivative, is diminished on going from pH 5.3 to 8.2, zwitterion formation appears to reduce the polarizing power of the 7-methylguanine ring. At the same time, the reverse of this trend takes place in ring polarizability, because association with caffeine, which is at least partly limited by the polarizability of **1–3**, is enhanced.

It is also noteworthy that 7-methylguanine derivatives exhibit almost equal binding affinities to indole and methylated adenines (**7a–c**), whereas purine stacks much more efficiently with adenines. This difference is also consistent with the concept of dipole-induced dipole-dipole interactions. Since the polarizing power of **1–3** is high, their binding affinity is mainly limited by the ring polarizability of the cosolute molecule. In contrast, heteroassociation of purine results from mutual polarization of the interacting molecules, and hence adenine derivatives, having a marked polarizing power, form more stable adducts.

In summary, the stacking behaviour of **1–3** indicates that the polarizing power of 7-methylguanine derivatives is high compared to normal purine nucleosides, and protonation of N1 reinforces the influence exerted by this property. Accordingly, cationic 7-methylguanine rings exhibit an unusually high binding affinity to the readily polarizable residues of tryptophan. These stacking interactions may well play a role in the selective recognition of cap structure by proteins.

3. Experimental

3.1. Materials

7-Methylguanosine (**2**) and purine (**4**) were purchased from Sigma, indole (**5**) and caffeine (**6**) being obtained from Aldrich. They were used as received after checking the purity by HPLC. 7,9-Dimethylguanine (**1**) was prepared as described earlier [25]. The preparation of 7-methylguanosine 5′-monophosphate (**3**) [17] and methylated adenines (**7a–c**) [15] has been reported previously. The solvents employed were of reagent grade.

3.2. Phase distribution measurements

Stability constants for the 1 : 1 adducts of **1–4** with **5–7** were obtained by the phase distribution method described earlier [15]. The organic solvents employed were: 2,2,4-trimethylpentane (for **5**), tetrachloromethane (**6**, **7c**) and dichloromethane (**7a**, **7b**). The volumes of the organic and aqueous phases were 1.0 and 0.5 cm^3, respectively. The concentration of **1** was varied from 0 to 0.075 mol dm^{-3} and of **2–4** from 0 to 0.2 mol dm^{-3}. The concentrations of cosolutes (**5 – 7**) were of the order of 10^{-3} mol dm^{-3}. Equilibrium between the phases was attained after 2 h vigorous shaking. The solute concentrations in both phases were determined by HPLC on an RP-18 column (Techopack) using a mixture of acetonitrile and acetic acid buffer (pH 4.3) as eluent. The intercepts of the straight lines K_d/K_0 (ratio of the distribution coefficients of **5–7** in the presence and absence of **1–4**) vs. c(**1–4**) were always equal to unity within the limits of experimental error, and the correlation coefficients were > 0.98.

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