Short Communication

The Influence of Intramolecular Electrostatic Interactions on the Hydrolytic Stability of the *N*-Glycosidic Bond of 7-Methylguanosine 5′-Monophosphate, a Simple *Cap* Analogue

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The 5′-terminal nucleoside in eukaryotic mRNA molecules is 7-methylguanosine (**1a**).[1,2] The corresponding mononucleotide, 7-methylguanosine 5′-monophosphate (**1b**), adopts a rather rigid conformation in solution, owing to an electrostatic attraction between the cationic imidazole ring and the anionic phosphate group.[3–6] This rigidity has been suggested to play an important role in the recognition of the 5′-end of mRNA by the so-called *cap*-binding proteins.[6,7] We have shown recently that though electrostatic interactions keep the phosphate group in the proximity of the imidazole ring, retarding the nucleophilic attack of hydroxide ion on the C8 atom, they are not strong enough to affect significantly the acidity or complexing ability of the interacting moieties.[8] We now report that these interactions are also too weak to affect markedly the hydrolytic stability of the *N*-glycosidic bond.

It is widely accepted that the acidic hydrolysis of purine nucleosides proceeds by rate-limiting cleavage of the protonated substrate to the free purine base and a cyclic glycosyl oxocarbenium ion.[9] Most probably the same mechanism is also applicable to the hydrolysis of the corresponding nucleoside 5′-monophosphates. For example, the rate profiles and thermodynamic activation parameters obtained with 2′-deoxycytidine 5′-monophosphate closely resemble those for the hydrolysis of 2′-deoxycytidine.[10]

The first-order rate constants for the hydrolysis of purine nucleosides have been shown to be strictly proportional to the concentration of oxonium ion over a wide acidity range.[9,11,12] In

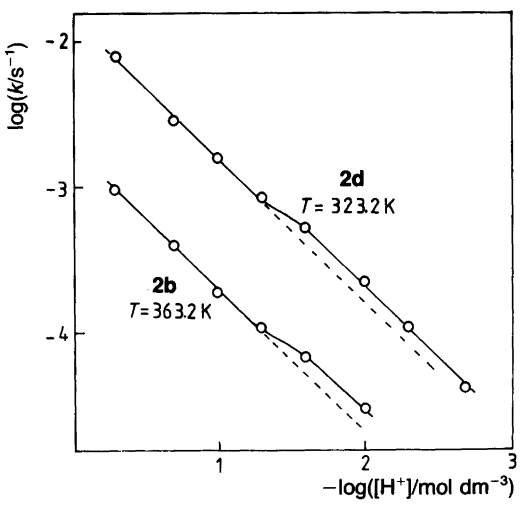

Fig. 1. Rate-profiles for the hydrolysis of adenosine 5′-monophosphate (**2b**) and its 2′-deoxy derivative (**2d**). The ionic strength was adjusted to 0.10 mol dm^{-3} at $[H^+] < 0.10$ mol dm^{-3}.

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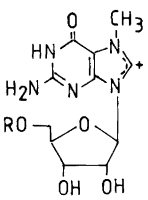
Table 1. First-order rate constants for the hydrolysis of some purine nucleosides and their 5′-monophosphates in aqueous acid and buffer solutions.

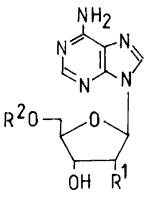
Compound	T/K	$k/10^{-4}$ s^{-1}		
		A	*B*	*C*
Adenosine (**2a**)	363.2	4.50(5)[d]		
Adenosine 5′-monophosphate (**2b**)	363.2	1.82(3)		
2′-Deoxyadenosine (**2c**)	323.2	32.0(4)[e]	3.43(3)[e]	
2′-Deoxyadenosine 5′-monophosphate (**2d**)	363.2	15.4(3)	2.17(3)	
Guanosine (**3a**)	363.2	6.9[f]		
Guanosine 5′-monophosphate (**3b**)	363.2	1.66(4)		
7-Methylguanosine (**1a**)	363.2	6.98(8)		1.47(3)
7′-Methylguanosine 5′-monophosphate (**1b**)	363.2	2.78(6)		0.29(1)
7-Methylguanosine 5′-monophosphate methyl ester (**1c**)	363.2	2.35(9)		0.36(2)
7-Methyl-2′,3′-*seco*-guanosine 5′-monophosphate (**4**)	363.2	13.7(4)		1.90(5)

[a]In 0.10 mol dm^{-3} aqueous hydrogen chloride. [b]In 0.010 mol dm^{-3} aqueous hydrogen chloride. Ionic strength adjusted to 0.10 mol dm^{-3} with sodium chloride. [c]In an acetic acid/sodium acetate buffer (0.020/0.020 mol dm^{-3}). Ionic strength adjusted to 0.10 mol dm^{-3} with sodium chloride. [d]From Ref. 12. [e]From Ref. 9. [f]From Ref. 11.

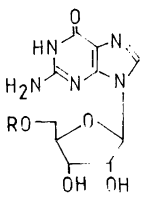
contrast, the rate profiles for purine nucleoside 5′-monophosphates pass through an inflection point at a pH approximately equal to the pK_1 of the 5′-phosphate group, as illustrated in Fig. 1 for adenosine 5′-monophosphate (**2b**) and its 2′-deoxy derivative (**2d**). Though the deviation from linear dependence is small, it is outside the limits of the experimental error and indicates that the neutral 5′-monophosphate group and its monoanion exert slightly different effects on the hydrolysis of the *N*-glycosidic bond.



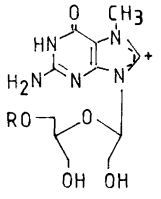
1a: R = H
1b: R = PO(OH)$_2$
1c: R = PO(OH)OCH$_3$



2a: R^1 = OH, R^2 = H
2b: R^1 = OH, R^2 = PO(OH)$_2$
2c: R^1 = H, R^2 = H
2d: R^1 = H, R^2 = PO(OH)$_2$



3a: R = H
3b: R = PO(OH)$_2$



4: R = PO(OH)$_2$

The kinetic data collected in Table 1 show that the rate constants for the hydrolysis of the 5′-monophosphates of adenosine (**2b**), 2′-deoxyadenosine (**2d**) and guanosine (**3b**) in aqueous hydrogen chloride (0.10 mol dm^{-3}) are 40, 48 and 24%, respectively, of those for the parent nucleosides. Under these conditions the 5′-phosphate group is predominantly in undissociated form.[13] On going to less acidic solutions, in which the 5′-phosphate group is mainly present as a monoanion, the relative rate constant obtained with **2d** increases from 0.48 to 0.63.

As shown by Zoltewicz *et al.*,[11] 7-methylguanosine (**1a**) undergoes in acidic solution both acid-catalyzed and spontaneous cleavage to 7-methylguanine and a ribofuranosyl oxocarbenium ion, the former reaction prevailing at pH < 2 and the latter at 3 < pH < 6. The data in Table 1 show that the 5′-phosphate group retards both of these reactions, the effects on reactivity being rather similar to those observed for the non-alkylated purine nucleosides (**2b**, **2d**, **3b**). The rate con-

stants obtained with **1b** and its methyl ester (**1c**) in aqueous hydrogen chloride (0.10 mol dm^{-3}) are 40 and 33%, respectively, of those of the parent nucleosides. In acetic acid buffers, in which the phosphate group bears one negative charge and the spontaneous hydrolysis of the *N*-glycosidic bond prevails, the rate-retarding effects are slightly larger, the relative rate constants being 0.20 for **1b** and 0.24 for **1c**. Accordingly, the influence of the anionic phosphate group on the stability of the *N*-glycosidic bond is slightly larger than that of the neutral form, in contrast to the behaviour observed in the hydrolysis of **2d**. One might speculate that electrostatic interactions between the anionic phosphate group and the cationic imidazole ring slightly stabilize the initial state in the hydrolysis of **1b**. Accordingly, the rupture of the *N*-glycosidic bond of **1b** is retarded more markedly in solutions in which the phosphate group is negatively charged. **2b** may be expected to be protonated at N1,[14] and hence the imidazole ring is not as electron deficient as with **1b**. It should be noted, however, that the differences in rate-retarding influences are too small to allow firm conclusions to be drawn.

Comparison of the reactivities of **1b** and its 2′,3′-*seco* counterpart (**4**) also shows that intramolecular electrostatic interaction does not play an important role in the acidic hydrolysis of 7-methylguanine nucleotides. **4** is hydrolyzed in aqueous hydrogen chloride (0.10 mol dm^{-3}) 4.9 times more rapidly than **1b**. This reactivity ratio is similar to that reported[15] for benzimidazole nucleosides and their *seco* derivatives (4.0–4.5), and may be accounted for by greater stability of an acyclic oxocarbenium ion than a cyclic intermediate. On going to an acetic acid buffer, in which the phosphate group is ionized, the reactivity ratio for **4** relative to **1b** is increased to a value of 6.5. Since the glycone moiety of a *seco* nucleoside is conformationally more flexible than that of the corresponding nucleoside, the influences that intramolecular electrostatic interactions have on the stability of the *N*-glycosidic bond may be expected to be smaller for **4** than for **1b**. In other words, ionization of the phosphate group should retard the hydrolysis of **1b** more markedly than that of **4**. Although this appears to be the case, the observed difference is too small to be interpreted as evidence of a reasonably strong intramolecular interaction.

In summary, the influence of intramolecular electrostatic interactions on the stability of the *N*-glycosidic bond of 7-methylguanosine 5′-monophosphate is hardly detectable. Accordingly, the data lend additional support to our previous[8] suggestion, according to which the importance of these interactions should not be overestimated in attempting to explain the chemical behaviour of *cap* analogues.

Experimental

The preparation of **1b**,[8] **1c**[4] and **4**[6] has been described previously. All the other nucleosides and nucleotides employed were commercial products from Sigma Chemical Company. The first-order rate constants were determined by the HPLC method described previously,[9] using a commercial Hypersil ODS column (4 × 250 mm, 5 μm) and a water-rich mixture of acetonitrile and acetic acid buffer (pH 4.3, acetonitrile less than 10% v/v) as eluent.

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