KINETICS OF THE IMIDAZOLIUM RING-OPENING OF mRNA 5′-*cap* ANALOGS IN AQUEOUS ALKALI

Alicja STACHELSKA[a1,*], Zbigniew J. WIECZOREK[a2], Janusz STĘPIŃSKI[b1], Marzena JANKOWSKA-ANYSZKA[c], Harri LÖNNBERG[d] and Edward DARŻYNKIEWICZ[b2]

[a] *Department of Physics and Biophysics, University of Warmia and Mazury in Olsztyn, Oczapowskiego 4, 10-719 Olsztyn, Poland; e-mail:* [1] *alicja.stachelska@uwm.edu.pl,* [2] *zbyszek@uwm.edu.pl*

[b] *Department of Biophysics, Institute of Experimental Physics, Warsaw University, Żwirki i Wigury 93, 02-089 Warsaw, Poland; e-mail:* [1] *jastep@biogeo.uw.edu.pl,* [2] *edek@biogeo.uw.edu.pl*

[c] *Department of Chemistry, Warsaw University, 1 Pasteura, 02-093 Warsaw, Poland; e-mail: marzena@chem.uw.edu.pl*

[d] *Department of Chemistry, University of Turku, 20014 Turku, Finland; e-mail: harlon@utu.fi*

Received October 28, 2005
Accepted December 14, 2005

Second-order rate constants for the hydroxide-ion-catalyzed imidazolium ring-opening of several mono- and dinucleosidic analogs of mRNA 5′-*cap* have been determined. Intramolecular stacking of the two nucleobases in the dinucleosidic analogs, m^7GpppN (m^7G = 7-methylguanosine, N = 5′-linked nucleoside), and electrostatic interaction between the *N*-alkylated imidazolium ring and phosphate moiety have been shown to shield the m^7G moiety against the nucleophilic attack of hydroxide ion. In addition, the effect of methylation of the nucleobase amino groups and replacement of the 7-methyl group with other alkyl groups have been studied. The influence of all the structural modifications studied turned out to be modest, the cleavage rates of the most and least reactive analogs (with the exception of non-phosphorylated nucleosides) differing only by a factor of 5.

Keywords: Kinetics; Rate constants; *cap* Analogs; Dinucleoside phosphates; Imidazolium ring; Ionic strength; Nucleotides; mRNA; Purines.

The 5′-terminus of eukaryotic mRNA displays a special structure, m^7GpppNp..., referred to as a *cap*. Here m^7G stands for 7-methylguanosine (**1**) linked via a 5′,5′-triphosphate bridge to the penultimate nucleoside (N) that is often 2′-*O*-methylated[1–3]. mRNA devoid of the *cap* exhibits a markedly reduced affinity to the 40S subunit of eukaryotic ribosomes[4,5]. The *cap* structure, hence, is a key element for stimulation of the translation process[6–8] in yeast[9,10] and mammalian[11] cells. In addition, it protects mRNA against depolymerization by exonucleases[12,13].

Collect. Czech. Chem. Commun. 2006, Vol. 71, No. 4, pp. 567–578

doi:10.1135/cccc20060567

N7-Methylation of guanosine markedly affects the protolytic equilibria of the parent nucleoside. The pK_a value of the N1-deprotonation is reduced from 9.4 to 7.07 for m^7Guo (**1**), to 7.24 for m^7GMP (**4**)[14] and to 7.5 for m^7GTP (**13**)[15]. In addition, quaternization of the imidazolium ring nitrogen makes the C8 atom highly susceptible to nucleophilic attack of a hydroxide ion, resulting in rapid imidazolium ring-opening at high pH [16–18].

The kinetics of this reaction have previously been studied spectrophotometrically[17,18] and the products formed have been characterized by HPLC and ^{1}H and ^{13}C NMR spectroscopy[18]. The other characteristic features of the *cap* structure include intramolecular stacking of 7-methylguanine with the neighboring nucleobase[19–22] and strong electrostatic attraction between the electron-deficient imidazolium ring and the negatively charged phosphate bridge[17,23]. These interactions largely determine the conformation of *cap*.

As mentioned above, the *cap* structure is highly susceptible to hydroxide ion catalyzed hydrolysis. The present study is aimed at clarifying the relationship between the hydrolytic stability and chemical structure of the *cap*. Such data is of considerable importance on choosing experimental conditions to which oligonucleotides bearing either naturally occurring or modified *cap* structures may be subjected. In addition, the effects of structure on hydrolytic stability lend additional information on molecular interactions, above all, intramolecular stacking, within the *cap* structure. For these purposes, the second-order rate constants for the hydroxide-ion-catalyzed imidazolium ring-opening of a number of *cap* analogs have been determined. The strength of intramolecular stacking has been varied by changing the structure of the base moieties. For comparative purposes, hydrolysis of several guanosines alkylated on base moiety and their mono-, di- and triphosphates has been studied. The effect of ionic strength on the observed differences in reaction rates has additionally been examined.

EXPERIMENTAL

Materials

7-Methylguanosine (**1**; m^7Guo) and its 5′-phosphate (**4**; m^7GMP) were products of Sigma. The preparation and characterization of the other *cap* analogs have been described earlier. The references for the synthesis of each compound are listed in Table I. The structures of the compounds are depicted in Charts 1 and 2. The inorganic salts and buffer constituents employed were of reagent grade. The solutions were made in redistilled water (Simplicity 185 UV Millipore). The kinetic measurements were carried out in a triethylamine/triethylammonium chloride buffer (9.30×10^{-4} M; $[Et_3N]/[Et_3NH^+Cl^-] = 1:1$) and in aqueous sodium hydroxide (5.00×10^{-3} and 1.00×10^{-2} M). The ionic strength of these solutions was adjusted with sodium chloride.

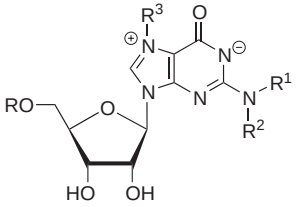
TABLE I
Second-order rate constants for the hydroxide-ion-catalyzed opening of the imidazolium ring of *cap* analogs at 298.2 ± 0.1 K (I = 0.10 M with NaCl)

Compound	Ref. for synthesis	k, $dm^3\ mol^{-1}\ s^{-1}$ [a]	k, $dm^3\ mol^{-1}\ s^{-1}$ [b]
1: m^7Guo		1.110 ± 0.010	1.10 ± 0.01 [17]
2: $m_2^{2,7}$Guo	14	0.500 ± 0.008	
3: $m_3^{2,2,7}$Guo	14	0.450 ± 0.009	
4: m^7GMP		0.112 ± 0.006	0.110 ± 0.002 [17]
5: $m_2^{2,7}$GMP	18	0.066 ± 0.003	0.064 ± 0.001 [18]
6: $m_3^{2,2,7}$GMP	18	0.039 ± 0.003	0.041 ± 0.002 [18]
7: et^{7}GMP	18	0.040 ± 0.004	0.039 ± 0.001 [18]
8: bn^{7}GMP	18	0.066 ± 0.002	
9: et^{7}m^{7}GMP	24	0.054 ± 0.006	
10: m^7GDP	15	0.104 ± 0.004	
11: $m_3^{2,2,7}$GDP	26	0.036 ± 0.001	
12: m^7GDP-(β-OMe)	23	0.148 ± 0.004	0.158 ± 0.004 [17]
13: m^7GTP	15	0.093 ± 0.001	0.102 ± 0.003 [17]
14: $m_3^{2,2,7}$GTP	14	0.030 ± 0.002	
15: et^{7}GTP	24	0.029 ± 0.003	
16: bn^{7}GTP	24	0.060 ± 0.009	
17: m^7GppG	25	0.095 ± 0.009	
18: m^7GpppG	18,25	0.094 ± 0.003	0.091 ± 0.001 [18]
19: et^{7}GpppG	18	0.029 ± 0.001	
20: $m_2^{2,7}$GpppG	18,25	0.046 ± 0.003	0.049 ± 0.001 [18]
21: $m_3^{2,2,7}$GpppG	18,25	0.036 ± 0.007	0.032 ± 0.001 [18]
22: m^7Gpp(m^7G)	25	0.094 ± 0.002 0.020 ± 0.004[c]	
23: m^7Gppp(m^7G)	25	0.107 ± 0.001 0.023 ± 0.001[c]	
24: m^7Gppp(m^6A)	7	0.051 ± 0.001	
25: m^7GpppA	7	0.048 ± 0.001	
26: m^7GpppC	7	0.120 ± 0.002	
27: m^7GpppU	7	0.114 ± 0.004	

[a] Based on pseudo-first-order rate constants obtained in 10.0 mM and/or 5.00 mM aqueous sodium hydroxide (I = 0.10 M with NaCl) or in a 1:1 triethylamine/triethylammonium chloride buffer (I = 0.10 M with NaCl). [b] Values reported previously. [c] The rate constants refer to Eq. (*3*).

Kinetic Measurements

The UV absorption spectra were measured on a Cary 300 UV-VIS spectrophotometer. For each compound, the spectrum from 230 to 330 nm was first recorded at appropriate intervals to identify the wavelength at which the change in absorbance due to imidazolium ring-opening was maximal. The progress of the ring-opening was then followed at this wavelength, keeping the temperature of the cell-housing block at 293.2 ± 0.1 or 298.2 ± 0.1 K. The initial concentration of the starting material ranged from 5×10^{-5} to 1×10^{-4} M. The hydroxide ion concentration of the triethylamine buffer was calculated with the aid of the dissociation constant reported for the triethylammonium ion under the experimental conditions[27]. The pseudo-first-order rate constants were obtained by fitting the absorbance at time t (A_t) to the equation of one-phase exponential decay (*1*) or association (*2*), depending on whether the absorbance experienced time-dependent decrease or increase at the wavelength employed. In these equations A_0 and A_∞ refer to the absorbance at time 0 and infinity, respectively. The second-order rate constants were then obtained by dividing the pseudo-first-order rate constant by the concentration of hydroxide ion.



1: m^7Guo: $R^1 = R^2 = R = H$, $R^3 = CH_3$
2: $m_2^{2,7}$Guo: $R^1 = R^3 = CH_3$, $R^2 = R = H$
3: $m_3^{2,2,7}$Guo: $R^1 = R^2 = R^3 = CH_3$, $R = H$
4: m^7GMP: $R^1 = R^2 = H$, $R^3 = CH_3$, $R = PO(O^-)_2$
5: $m_2^{2,7}$GMP: $R^1 = R^3 = CH_3$, $R^2 = H$, $R = PO(O^-)_2$
6: $m_3^{2,2,7}$GMP: $R^1 = R^2 = R^3 = CH_3$, $R = PO(O^-)_2$
7: et^7GMP: $R^1 = R^2 = H$, $R^3 = C_2H_5$, $R = PO(O^-)_2$
8: bn^7GMP: $R^1 = R^2 = H$, $R^3 = CH_2C_6H_5$, $R = PO(O^-)_2$
9: et^2m^7GMP: $R^1 = C_2H_5$, $R^2 = H$, $R^3 = CH_3$, $R = PO(O^-)_2$
10: m^7GDP: $R^1 = R^2 = H$, $R^3 = CH_3$, $R = PO(O^-)OPO(O^-)_2$
11: $m_3^{2,2,7}$GDP: $R^1 = R^2 = R^3 = CH_3$, $R = PO(O^-)OPO(O^-)_2$
12: m^7GDP-(β-OMe): $R^1 = R^2 = H$, $R^3 = CH_3$, $R = PO(O^-)OPO(O^-)OCH_3$
13: m^7GTP: $R^1 = R^2 = H$, $R^3 = CH_3$, $R = PO(O^-)OPO(O^-)OPO(O^-)_2$
14: $m_3^{2,2,7}$GTP: $R^1 = R^2 = R^3 = CH_3$, $R = PO(O^-)OPO(O^-)OPO(O^-)_2$
15: et^7GTP: $R^1 = R^2 = H$, $R^3 = C_2H_5$, $R = PO(O^-)OPO(O^-)OPO(O^-)_2$
16: bn^7GTP: $R^1 = R^2 = H$, $R^3 = CH_2C_6H_5$, $R = PO(O^-)OPO(O^-)OPO(O^-)_2$

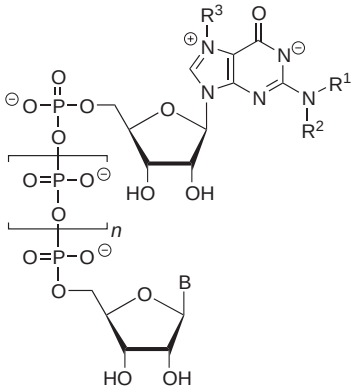
CHART 1
Structural formulae of the mononucleosidic *cap* analogs analyzed

$$A_t = (A_0 - A_\infty)e^{-kt} + A_\infty \quad (1)$$

$$A_t = A_\infty - (A_\infty - A_0)e^{-kt} \quad (2)$$

The imidazolium ring-opening of the symmetric dinucleosidic *cap* analogs, $m^7Gpp(m^7G)$ (**22**) and $m^7Gppp(m^7G)$ (**23**), did not, however, obey this simple first-order kinetics, since ring-opening of one m^7G moiety affects the cleavage rate of the remaining one. In this case, the time-dependent absorbance (A_t) was fitted to a biexponential equation (*3*), where b_3 stands for the absorbance at infinity and the sum of b_1, b_2 and b_3 for the absorbance at time 0. k_1 and k_2 are the pseudo-first-order rate constants for the two reactions.

$$A_t = b_1e^{-k_1t} + b_2e^{-k_2t} + b_3 \quad (3)$$



17: m^7GppG: $R^1 = R^2 = H$, $R^3 = CH_3$, $n = 0$, B = guanine
18: m^7GpppG: $R^1 = R^2 = H$, $R^3 = CH_3$, $n = 1$, B = guanine
19: et^7GpppG: $R^1 = R^2 = H$, $R^3 = C_2H_5$, $n = 1$, B = guanine
20: $m_2^{2,7}GpppG$: $R^1 = R^3 = CH_3$, $R^2 = H$, $n = 1$, B = guanine
21: $m_3^{2,2,7}GpppG$ $R^1 = R^2 = R^3 = CH_3$, $n = 1$, B = guanine
22: $m^7Gpp(m^7G)$: $R^1 = R^2 = H$, $R^3 = CH_3$, $n = 0$, B = 7-methylguanine
23: $m^7Gppp(m^7G)$: $R^1 = R^2 = H$, $R^3 = CH_3$, $n = 1$, B = 7-methylguanine
24: $m^7Gppp(m^6A)$: $R^1 = R^2 = H$, $R^3 = CH_3$, $n = 1$, B = N6-methyladenine
25: m^7GpppA: $R^1 = R^2 = H$, $R^3 = CH_3$, $n = 1$, B = adenine
26: m^7GpppC: $R^1 = R^2 = H$, $R^3 = CH_3$, $n = 1$, B = cytosine
27: m^7GpppU: $R^1 = R^2 = H$, $R^3 = CH_3$, $n = 1$, B = uracil

CHART 2
Structural formulae of the dinucleosidic *cap* analogs analyzed

RESULTS

The imidazolium ring-opening of the *cap* analogs was followed spectrophotometrically in a triethylamine buffer ([OH^-] = 9.3 × 10^{-4} M) and in aqueous sodium hydroxide ([OH^-] = 5 × 10^{-3} and 1 × 10^{-2} M), i.e. under conditions where the 7-methylguanine base occurs in a zwitterionic N1-deprotonated form[17].

Figure 1 shows as an illustrative example the time-dependent changes of the absorption spectrum of m^7GpppU (**27**) at the concentration 5 × 10^{-5} M in 0.01 M aqueous NaOH (the large figure). In the inset of the same figure, the absorbance changes at 265 and 290 nm, referring to an increase and decrease in absorbance, respectively, are plotted against the time. Table I summarizes the observed pseudo-first-order rate constants for the hydroxide-ion-catalyzed imidazolium ring-opening of the *cap* analogs at various hydroxide ion concentrations (298.2 K, I = 0.10 M with NaCl) and the second-order rate constants calculated from them.

The data in Table I allow the following conclusions concerning the relationship between the structure and hydrolytic stability of *cap* analogs.

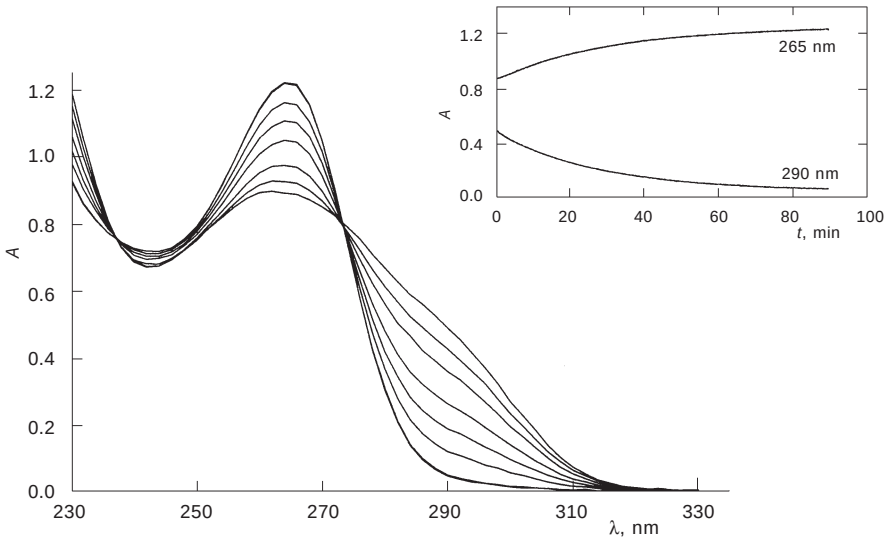


FIG. 1

Time-dependent absorption spectra of m^7GpppU (**27**) and the dependence of the absorbance at wavelengths λ = 265 and 290 nm on time (inset). The concentration of **27** in 0.01 M aqueous NaOH was 5 × 10^{-5} M, the ionic strength was adjusted to 0.1 M and the temperature was 298.2 ± 0.1 K. Absorption spectra from bottom to top obtained at 0, 3, 8, 16, 25, and 70 min

(i) 5′-phosphorylation of m^7Guo (**1**), $m_2^{2,7}$Guo (**2**) or $m_3^{2,2,7}$Guo (**3**) retards the imidazolium ring-opening by one order of magnitude. Conversion of 5′-monophosphates (**4**, **6**) to triphosphates (**13**, **14**), in turn, retards the cleavage by less than 30%. Conversion of m^7GDP (**10**) to its β-methyl ester (**12**) accelerates the ring-opening by 40%. (ii) m^7GpppG (**18**) and $m_3^{2,2,7}$GpppG (**21**) react approximately as readily as m^7GTP (**13**) and $m_3^{2,2,7}$GTP (**14**), respectively, and m^7GppG (**17**) by 36% less readily than m^7GDP methyl ester (**12**). Replacement of G in m^7GpppG (**18**) with a pyrimidine nucleoside, either cytidine (**26**) or uridine (**27**), results in an up to 30% rate acceleration, while replacement with adenosine (**25**) or N^6-methyladenosine (**24**) retards the reaction by 50%. (iii) Methylation of m^7GMP (**4**) to the corresponding N^2,7-dimethyl (**5**) derivative reduces the rate of imidazolium ring-opening by 40% and further methylation to $m_3^{2,2,7}$GMP (**6**) by another 40%. The respective methylations of m^7Guo (**1**) and m^7GpppG (**18**) show comparable increments of the hydrolytic stability. (iv) Replacement of 7-methyl group with 7-ethyl group retards the ring cleavage of m^7GMP (**4**→**7**), m^7GTP (**13**→**15**) and m^7GpppG (**18**→**19**) by 70%. By contrast, replacement of N^2-methyl group with ethyl group in $m_2^{2,7}$GMP (**5**→**9**) retards the reaction only by 20%. The rate-retarding effect of 7-benzyl substitution is smaller than that of 7-ethyl group (cf. **15** and **16** with **13**).

Figure 2 shows the effect of the ionic strength (adjusted with sodium chloride) on the pseudo-first-order rate constant for some mono- (**4**) and dinucleosidic (**18**, **24**–**26**) *cap* analogs in 10.0 mM aqueous NaOH at 293.2 K. As can be seen, the imidazolium ring-opening of m^7GMP (**4**) is continuously accelerated with increasing ionic strength, the rate constant at I = 1.0 M being two-fold compared with that at I = 0.1 M. By contrast, the ring-opening of all the dinucleosidic *cap* analogs studied is less sensitive to ionic strength, the rate constant passing through a broad maximum at $I \approx 0.6$ M.

DISCUSSION

It has been shown previously[18] that the alkaline cleavage of 7-methylguanosine (**1**) is initiated by an attack of hydroxide ion on the C8 atom, accompanied by rapid rupture of the C8–N9 bond and concomitant formation of a 5-formamido derivative (Scheme 1). The reaction is virtually irreversible and shows strict first-order dependence on the concentration of hydroxide ion. This ring-opening is followed by isomerization of the sugar moiety to α-furanoside and α/β-pyranoside forms and eventually by cleavage of the sugar moiety. The four anomeric forms of the 5-formamido-

pyrimidine derivative and N^5-formyl-N^5-methyl-2,5,6-triaminopyrimidin-4-one formed as the final product have previously been characterized by ^{1}H and ^{13}C NMR spectroscopy[18]. These consecutive reactions do not result in marked changes in the UV spectrum of the product of the ring-opening step. In addition, they are so much slower than the ring-opening that conversion of the starting material to the 5-formamidopyrimidine products may be easily followed spectrophotometrically without any interference of the consecutive steps. The reactions of 7-methylguanosine nucleotides and dinucleosidic *cap* analogs may be followed similarly, since 5′-mono-, di-

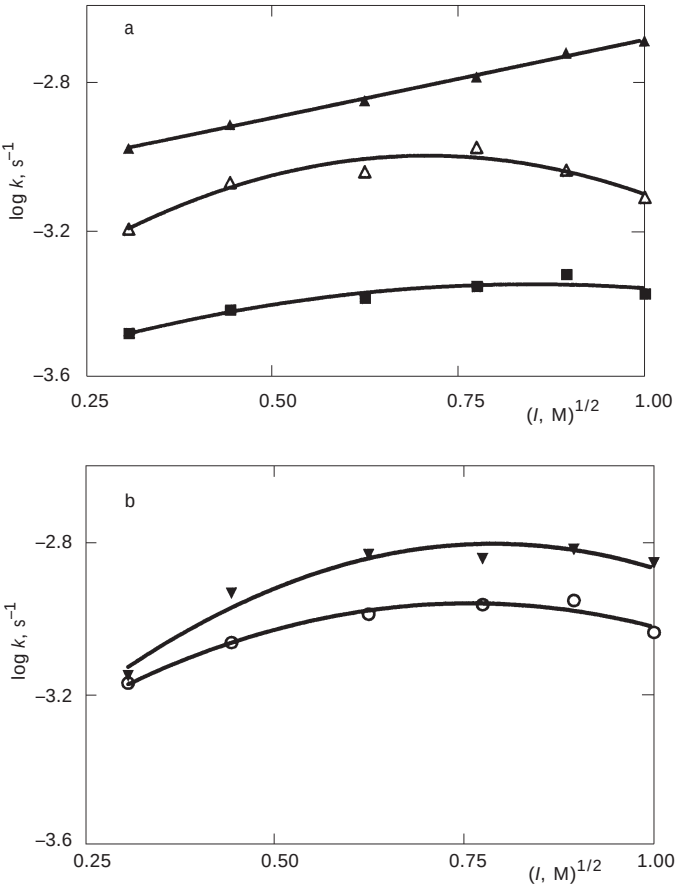
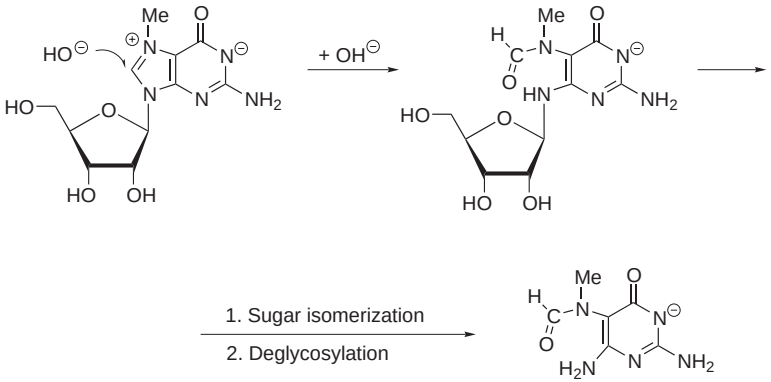


FIG. 2

The effect of ionic strength on the first-order rate constants for some mono- and dinucleosidic *cap* analogs in 10.0 mM aqueous NaOH at 293.2 K. ▲ m^7GMP, △ m^7GpppG, ■ m^7GpppA (a); ▼ m^7Gppp(m^6A), ○ m^7GpppC (b). The ionic strength was adjusted with sodium chloride

and triphosphate groups[28], as well as purine[29] and pyrimidine[30] bases (including m^6A), are hydrolytically much more stable under alkaline conditions than the 7-methylguanine moiety.



SCHEME 1

Derivatives of 7-methylguanine exhibit a marked tendency to associate in aqueous solution by vertical stacking[14,19,21,22,31]. Compared with other purine bases, the stacking interactions are exceptionally strong with heterocyclic compounds exhibiting high polarizability and low polarizing power[20]. Spectroscopic analyses carried out by Wieczorek et al.[14,22] show that the intramolecular stacking is more marked with purine-derived *cap* analogs (m^7GpppN, N = purine nucleoside) than with their pyrimidine counterparts (N = pyrimidine nucleoside). This has been shown to be the case both at pH 9.0, when the 7-methylguanine ring is in a zwitterionic form, and at pH 5.2, when it is monocationic. The results in Table I suggest that the intramolecular stacking protects the 7-methylguanine moiety from the attack of hydroxide ion. As indicated above, the imidazolium ring of m^7GpppC (**26**) or m^7GpppU (**27**) is cleaved two times as fast as that of $m^7Gppp(m^6A)$ (**24**) or m^7GpppA (**25**). A rate retardation of this magnitude is consistent with a previous observation, according to which base-stacking with a neutral heterocyclic molecule retards the nucleophilic attack of hydroxide ion on a purine base by 40% [32]. Guanine base undergoes N^1H deprotonation at pH 9.5. This in all likelihood weakens the intramolecular base-stacking of m^7GpppG (**18**) and, hence, the alkaline ring-opening is not retarded as with m^7GpppA (**25**) or $m^7Gppp(m^6A)$ (**24**). m^7GppG (**17**) reacts by 36% more slowly than m^7GDP β-methyl ester (**12**), which may possibly be attributed to stabilization by stacking. The symmetrical *cap* analogs, $m^7Gpp(m^7G)$ (**22**) and $m^7Gppp(m^7G)$ (**23**), are cleaved as readily as

m^7GppG (**17**) and m^7GpppG (**18**) in spite of the fact that they contain two sites susceptible to the nucleophilic attack and, hence, acceleration by a factor of two might be expected. Possibly two zwitterionic 7-methylguanine bases stack more efficiently than a zwitterionic and monoanionic base.

The data in Table I indicate that the additional methyl substituent(s) on the 2-amino group of the m^7G moiety of m^7GpppG lead to deceleration of the alkaline ring-opening, the relative rates obtained with m^7GpppG (**18**), $m_2^{2,7}$GpppG (**20**) and $m_3^{2,2,7}$GpppG (**21**) being 1, 0.49 and 0.38, respectively. These rate retardations, however, result rather from reduced electron density at the C8 atom than from enhanced stacking, since the influence of N^2-methylation on the hydrolytic stability of mononucleosidic model compounds, viz. m^7GMP (**4**→**5**→**6**), m^7GDP (**10**→**11**) and m^7GTP (**13**→**14**), is very similar. Consistent by with this argument, methylation of the 6-amino group of the adenosine moiety in m^7GpppA (**25**→**24**) has practically no influence on the hydrolytic stability.

Replacement of the 7-methyl group with an ethyl (**4**→**7**) or benzyl (**4**→**8**) group retards the imidazolium ring-opening by 65 and 41%, respectively. Evidently the ethyl group is both inductively and sterically rate-decreasing. Benzyl group, while also sterically rate-decreasing, inductively reduces the electron density at the C8 atom, resulting in a less marked rate decrease.

As seen in Fig. 2, the cleavage of dinucleosidic *cap* analogs (**18**, **24**–**26**) is less sensitive to an increase in the ionic strength than the corresponding reaction of m^7GMP. It has been previously suggested[15,17] that electrostatic attraction between the electron-deficient imidazolium ring and the negatively charged phosphate moiety retards the nucleophilic attack of hydroxide ion on the C8 atom. At high ionic strength, i.e. in a more polar environment, the intramolecular electrostatic attraction is less important and a rate acceleration is observed. Accordingly, one may speculate that electrostatic interactions exert a more marked effect on the hydrolytic stability of m^7GMP than on the stability of dinucleosidic *cap* analogs. The differences in reaction rate are, however, too small to make definite conclusions.

In summary, the susceptibility of *cap* structure to hydroxide-ion-induced imidazolium ring-opening depends on the number and identity of alkyl groups on the 7-alkylated guanine base, on the strength of intramolecular base-stacking and on the electrostatic interaction between the *N*-alkylated imidazolium ring and the phosphate moiety. All these influences are, however, quite modest. In fact, the difference in the cleavage rate of the most and least reactive dinucleosidic *cap* analog studied is only three-fold.

This work was supported by grants from University of Warmia and Mazury in Olsztyn, Poland (No. 0706-0207), from Polish Ministry of Scientific Research and Information Technology (No. 2 P04A 006 28) and from Polish Committee for Scientific Research (PBZ-KBN-059/T09/10).

REFERENCES

1. Rhoads R. E. in: *Progress in Molecular and Subcellular Biology* (F. E. Hahn, D. J. Kopecho and W. E. Muller, Eds), Vol. 9, p. 104. Springer-Verlag, Berlin 1985.
2. Shatkin A. J.: *Cell* **1976**, *9*, 645.
3. Shatkin A. J.: *Cell* **1985**, *40*, 223.
4. Kozak M.: *Gene* **1999**, 234, 187.
5. Rhoads R. E.: *Trends Biochem. Sci.* **1988**, *13*, 52.
6. Darżynkiewicz E., Ekiel I., Lassota P., Tahara S. M.: *Biochemistry* **1987**, *26*, 4372.
7. Jankowska M., Stępiński J., Stolarski R., Wieczorek Z., Temeriusz D., Haber E., Darżynkiewicz E.: *Collect. Czech. Chem. Commun.*, Special Issue **1996**, *61*, S197.
8. Wasserman D. A., Steitz J.: *Science* **1992**, *257*, 1918.
9. Kuge H., Brownlee G. G., Gershon P. D., Richter J. D.: *Nucleic Acids Res.* **1998**, *26*, 3208.
10. Lo H.-J., Huang H.-K., Donahue T. F.: *Mol. Biol.* **1988**, *18*, 665.
11. Horikami S. M., De Ferra F., Moyer S. A.: *Virology* **1984**, *138*, 1.
12. Sonenberg N.: *Prog. Nucleic Acid Res. Mol. Biol.* **1988**, *35*, 173.
13. Tahara S. M., Morgan M. A., Shatkin A. J.: *J. Biol. Chem.* **1981**, *256*, 7691.
14. Wieczorek Z., Stępiński J., Jankowska M., Lönnberg H.: *J. Photochem. Photobiol. B: Biol.* **1995**, *28*, 57.
15. Hendler S., Fürer E., Srinivasan P. R.: *Biochemistry* **1970**, *9*, 4141.
16. Townsend L. B., Robins R. K.: *J. Am. Chem. Soc.* **1963**, *85*, 242.
17. Darżynkiewicz E., Labadi I., Haber D., Burger K., Lönnberg H.: *Acta Chem. Scand., Ser. B* **1988**, *42*, 86.
18. Darżynkiewicz E., Stępiński J., Tahara S. M., Stolarski R., Ekiel I., Haber D., Neuvonen K., Lehikoinen P., Labadi I., Lönnberg H.: *Nucleosides Nucleotides* **1990**, *9*, 599.
19. Nishimura Y., Takahashi S.-I., Yamamoto T., Tsuboi M., Hattori M., Miura K.-I., Yamaguchi K., Ohtani S., Hata T.: *Nucleic Acids Res.* **1980**, *8*, 1107.
20. Darżynkiewicz E., Lönnberg H.: *Biophys. Chem.* **1989**, *33*, 289.
21. Stolarski R., Sitek A., Stępiński J., Jankowska M., Oksman P., Temeriusz A., Darżynkiewicz E., Lönnberg H., Shugar D.: *Biochim. Biophys. Acta* **1996**, *1293*, 97.
22. Wieczorek Z., Zdanowski K., Chlebicka L., Stępiński J., Jankowska M., Kierdaszuk B., Temeriusz A., Darżynkiewicz E., Stolarski R.: *Biochim. Biophys. Acta* **1997**, *1354*, 145.
23. Darżynkiewicz E., Ekiel I., Tahara S. M., Selinger L. S., Shatkin A. J.: *Biochemistry* **1985**, *24*, 1701.
24. Jankowska M., Stępiński J., Stolarski R., Temeriusz A., Darżynkiewicz E.: *Collect. Czech. Chem. Commun.*, Special Issue **1993**, *58*, 138.
25. Stępiński J., Bretner M., Jankowska M., Felczak K., Stolarski R., Wieczorek Z., Cai A., Rhoads R. E., Temeriusz A., Haber D., Darżynkiewicz E.: *Nucleosides Nucleotides* **1995**, *14*, 717.
26. Cohen L. S., Mikhli C., Friedman C., Jankowska-Anyszka M., Stepinski J., Darzynkiewicz E., Davis R. E.: *RNA* **2004**, *10*, 1609.
27. Cox M. C., Everett D. H., Landsman D. A., Munn R. J.: *J. Chem. Soc. B* **1968**, 1373.

28. Mikkola S., Salomäki S., Zhang Z., Mäki E., Lönnberg H.: *Curr. Org. Chem.* **2005**, *9*, 999.
29. Lehikoinen P., Mattinen J., Lönnberg H.: *J. Org. Chem.* **1986**, *51*, 3819.
30. Lönnberg H., Suokas P., Käppi R., Darżynkiewicz E.: *Acta Chem. Scand., Ser. B* **1986**, *40*, 798.
31. Stolarski R., Zdanowski K., Chlebicka L., Wieczorek Z., Sitek A., Stępiński J., Jankowska M., Mattinen J., Temeriusz A., Darżynkiewicz E.: *Collect. Czech. Chem. Commun.*, Special Issue **1996**, *61*, S217.
32. Lönnberg H., Ylikoski J.: *Acta Chem. Scand., Ser. B* **1985**, *39*, 41.