Base pairing-induced shift in tautomeric equilibrium of a promutagenic analogue, N^6-methoxyadenosine

Borys Kierdaszuk, Ryszard Stolarski and David Shugar*

Department of Biophysics, Institute of Experimental Physics, University of Warsaw, Zwirki and Wigury 93, 02-089 Warszawa and **Institute of Biochemistry and Biophysics, Academy of Sciences, Rakowiecka 36, 02-532 Warszawa, Poland*

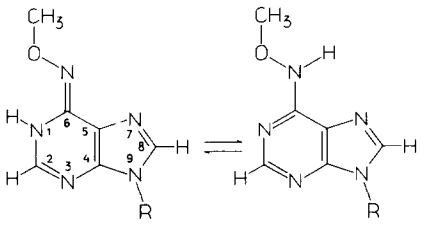
Received 12 May 1983

The nuclear magnetic resonance spectra of N^6-methoxyadenosine and of uridine, both methylated in the 2′-, 3′- and 5′-positions to obtain solution in deuterochloroform, reveal the formation of hetero-associates in which the amino–tautomeric equilibrium is shifted to the amino form. These results ar discussed in terms of the mutagenicity of *O*-methylhydroxylamine which converts adenosine to N^6-methoxyadenosine.

N^6-Methyoxyadenosine *Uridine* *NMR spectroscopy (^{14}C-, ^3H-)* *Amino–imino equilibrium* *Hydroxylamine mutagenesis* *Base-paring shift*

1. INTRODUCTION

N^6-Methoxyadenosine, the product of interaction of the mutagen *O*-methylhydroxylamine with adenosine [1–4] is a base analogue promutagen in the phage T4 [5,6] and *E. coli* [7] systems, giving rise to A → G transitions. In the in vitro transcription of a synthetic matrix containing N^6-methoxyadenosine residues by DNA-dependent RNA polymerase, the N^6-methoxyadenosine residues behave like adenosine and guanosine [8], consistent with the existence of N^6-methoxyadenosine in aqueous medium as an equilibrium mixture of two tautomers, imino and amino [9], as in scheme 1.



Scheme 1. Imino (left) and amino (right) forms of N^6-methoxyadenosine (R = ribose, actually 2′,3′,5′-tri-*O*-methylribose), with the N^6-methoxy group in the orientation *syn* with respect to the adenine ring N(1).

We have shown elsewhere, with the aid of optical and NMR spectroscopic methods, that the tautomeric equilibrium is markedly dependent on solvent polarity (in preparation). We report here observations demonstrating a shift in tautomeric equilibrium resulting from addition of a potentially complementary base, in this case uracil.

2. MATERIALS AND METHODS

N^6-Methoxy-2′,3′,5′-tri-*O*-methyladenosine was prepared by reacting 2′,3′,5′-tri-*O*-methyladenosine [10] with methoxyamine as reported for the preparation of N^6-methoxyadenosine [2]. The preparation of 2′,3′,5′-tri-*O*-methyluridine has been described in [11]. It should be noted that the sole purpose of methylation of the sugar hydroxyls was to enhance the solubility of the two nucleosides in chloroform and, for purposes of simplicity, we shall henceforth refer to them as N^6-methoxyadenosine and uridine.

C^2HCl_3 (99.5 mol% ^2H) was obtained from Merck (Darmstadt). ^1H NMR spectra were recorded on a Bruker 360 FT instrument, with TMS as internal standard.

Published by Elsevier Science Publishers B.V.
00145793/83/$ 3.00

3. RESULTS AND DISCUSSION

The ^1H spectrum of N^6-methoxyadenosine in C^2HCl_3 at 30°C exhibits two sets of signals, all with identical relative intensities (fig. 1), corresponding to the presence of two tautomeric species, amino and imino, in dynamic equilibrium with each other and with comparable energies, but separated by an energy barrier such that proton exchange between them is relatively slow on the NMR time scale at this temperature. The existence of the imino form, with the proton on the ring N(1), is testified to by the coupling constant of 3.5 Hz between H(2) and the N(1)–H. Fig. 1 shows the assignments of the various protons for the two tautomeric forms.

In solvents of low polarity, such as chloroform, N^6-methoxyadenosine forms planar autoassociates via hydrogen bonding, readily followed by the changes in chemical shifts of the protons directly involved in such hydrogen bonding with changes in concentration and temperature. An increase from 0.04–0.2 M is accompanied by marked deshielding of the N^6–H of the amino form by 1.37 ppm, and of the N(1)–H of the imino form by 0.72 ppm. At the higher concentration, a decrease in temperature from +30°C to −30°C leads to deshielding of the same protons by 1.48 ppm and 0.42 ppm, respectively. These results suggests that autoassociation occurs to a greater extent with the amino, than the imino, form. An increase in concentration, or decrease in temperature, leading to an increase in the extent of autoassociation, results in an increase in population of the amino form (table 1). Therefore, it is clear that planar autoassociation occurs preferentially with the amino form.

Addition to a chloroform solution of N^6-methoxyadenosine of an equimolar amount of the potentially complementary uridine results in analogous changes in chemical shifts of the N^6–H of the amino form by 0.68 ppm in a 0.04 M solution, and by 0.1 ppm in a 0.2 M solution. By contrast, the change in chemical shift of the N(1)–H of the imino form in a 0.04 M solution is only 0.02 ppm, and in a 0.2 M solution is even negative, −0.1 ppm. This points to preferential formation of heteroassociates between uridine and the amino form of N^6-methoxyadenosine, with the proviso that, at higher concentrations, autoassociation becomes a competing process. The formation of heteroassociates with uridine is further testified to by the large change in chemical shift of the uridine N(3)–H, about 1.5 ppm. These findings, together with the failure of the imino form to heteroassociate with uridine, are further supported by the results of ^{13}C NMR spectroscopy (in preparation).

The increase of population of the amino form is particularly marked (9% table 1) on formation of

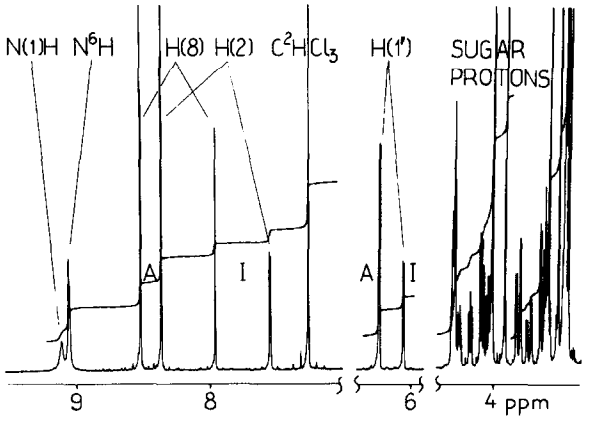


Fig. 1. ^1H NMR 360 MHz spectrum of N^6-methoxy-2′,3′,5′-tri-*O*-methyladenosine, 0.04 M in C^2HCl_3 at 30°C. Chemical shifts are in ppm *vs* internal TMS. Existence of an equilibrium mixture of amino (A) and imino (I) species is shown by the presence of two sets of signals for not only the adenine, but also the sugar, protons.

Table 1

Populations of the amino and imino tautomers of N^6-methoxy-2′,3′-5′-tri-*O*-methyladenosine in C^2HCl_3, at two concentrations and two temperatures, in the presence and absence of an equimolar concentration of the potentially complementary 2′,3′,5′-tri-*O*-methyluridine (tri-OMeU)

Solvent	Conc. (M)	Temp. (°C)	Population[a] (%)	
			Amino	Imino
C^2HCl_2	0.2	+30	70	30
	0.2	−30	78	22
	0.04	+30	67	33
	0.04	−30	76	24
+ tri-OMeU	0.2	+30	79	21
	0.04	+30	75	25

[a]Population were determined from relative signal intensities of both base and sugar (H1′) protons, and occasionally OCH_3 protons. Estimated accuracy ± 2%

heteroassociates with uridine. However, this effect is, also present with autoassociates (3–4%), due to the higher autoassociation of the amino, relative to the imino, form. Although the calculated values of the tautomer populations are subject to an error of about 2%, the reality of the shifts in tautomer populations, even when these are low, is testified to by the fact that an increase in population of one species is accompanied by a simultaneous decrease in the other.

Establishment of the types of base pairing involved in these shifts in tautomeric equilibrium is more difficult. In scheme 1 the exocyclic N^6-methoxy group is shown in the orientation *syn* with respect to the ring N(1). It may, however, also exist as the rotamer *anti*, or *syn* to N(7). For the imino species, the absence of association with uridine is consistent with the existence of the *syn* rotamer. With the amino form, on the other hand, Watson–Crick base pairing is possible with the *anti* rotamer, and Hoogsteen type base pairing with the *syn* rotamer. The change in ^{13}C chemical shift of C(5) is consistent with the latter (in preparation).

The experimental demonstration of the effect of base pairing on tautomeric equilibrium is of obvious relevance to interpretations of the mutagenicity of base analogues and, conceivably, of natural bases. The present findings are qualitatively consistent with the observation that, in transcription of a synthetic template consisting of a copolymer of dC with 17–33% N^6-methoxy-2′-deoxyadenosine residues, incorporation of uridine is 10-fold higher than cytidine [8], and suggests, furthermore, that at the polymer level the N^6-methoxy group is oriented *anti* to N(1), under the influence of the enzyme [12], thus permitting of Watson–Crick base pairing. There are no data available regarding the orientation of such exocyclic groups in polynucleotides, but at the monomer level the *syn* forms shown in scheme 1 are favoured, as detailed for such exocyclic *N*-methyl, *N*-hydroxy and *N*-methoxy groups in [13].

Furthermore, in contrast to demonstrations of possible formation of enol and imino tautomers of natural bases by exhange of a proton between the bases of a complementary pair at the level of monomers [14] and polymers such as tRNA [15], the system described here provides a model consisting of two well-defined tautomeric forms in equilibrium with each other. The corresponding N^6-hydroxyadenosine provides an additional such system, as will be described elsewhere. Further studies are under way to examine the behaviour of the potentially complementary cytidine in this system, and the types of base pairing involved.

ACKNOWLEDGEMENTS

We are deeply indebted to Dr Andre Debruyn of the University of Gent for the 360 MHz spectra. This investigation was supported by the Polish Academy of Sciences (09.7.1) and the Polish National Cancer Research Program (PR-6).

REFERENCES

[1] Budowsky, E.I. Sverdlov, E.D. and Monastyrskaya, G.S. (1969) J. Mol. Biol. 44, 205–207.
[2] Budowsky, E.I., Sverdlov, E.D. and Monastyrskaya, G.S. (1971) Biochim. Biophys. Acta 246, 320–328.
[3] Brown, D.M. and Osborne, M.R. (1971) Biochim. Biophys. Acta 247, 514–518.
[4] Budowsky, E.I. (1976) Prog. Nucleic Acid Res. Mol. Biol. 16, 125–188.
[5] Freese, E.B. (1968) Mutat. Res. 5, 299–301.
[6] Budowsky, E.I., Sverdlov, E.D. Spasokukotskaya, T.N. and Kudelka, Ja. (1975) Biochim. Biophys. Acta 390, 1–13.
[7] Poslovina, A.S., Basjunina, E.A., Andrejeva, I.S. and Salganik, R.I. (1976) in: Molecular Mechanisms of Genetic Phenomenon. Mutagenesis and Repair (Dubinin, N.P. ed.) pp. 142–145, Nauka Press, Moscow (in Russian).
[8] Singer, B. and Spengler, S. (1982) FEBS Lett. 139, 69–75.
[9] Morozov, Yu. V., Savin, F.A., Chekhov, V.O., Budowsky, E.I. and Yakovlev, D.Yu. (1982) J. Photochem. 20, 229–252.
[10] Kazimierczuk, Z., Darzynkiewicz, E. and Shugar, D. (1976) Biochemistry 15, 2735–2740.
[11] Kuśmierek, J.T., Giziewicz, J. and Shugar, D. (1973) Biochemistry 12, 194–199.
[12] Spengler, S. and Singer, B. (1981) Biochemistry 20, 7290–7294.
[13] Shugar, D., Huber, C.P. and Birnbaum, G.I. (1976) Biochim. Biophys. Acta 447, 274–284.
[14] Iwahashi, H. and Kyogoku, Y. (1980) J. Am. Chem. Soc. 102, 2913–2917.
[15] Rüterjans, H., Kaun, E., Hull, W.E. and Limbach, H.H. (1982) Nucleic Acids Res. 10, 7027–7039.