OLIGONUCLEOTIDE STUDIES
10. Structure and optical properties of N^1- and N^7-methylguanylyl-3′, 5′-uridine

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Received 1 June 1973

1. Introduction

Until recently, little has been known concerning the conformational properties of minor base-containing oligonucleotides, although it was anticipated that some basic information on the conformations and conformational stabilities of these molecules should be essential to a balanced picture of the factors involved in molecular properties of tRNA of known nucleotide sequence [1]. Much of the limited work that has been reported on oligonucleotides containing modified bases found in tRNA has been concerned with the optical properties of inosine-containing oligomers [2–4].

On the other hand, the weight of evidence in previous investigations suggests that the physico–chemical properties of Guo-containing region of nucleic acids frequently are different from the rest of the molecule: In oligonucleotides, Guo appears to have an 'unstacking' effect [5], and the protonation of Guo, which takes place on N-7, increases the contribution of the *syn* conformation both in the monomers and dimers [6,7].

In view of above remarks, it seemed of interest to examine the somewhat different situation encountered in the comparison between N^7-protonated and N^7-methylated guanylyl residues because of their structural similarities. Furthermore, a recent work by Guschlbauer et al. [7] concerning the effect of environmental factors on the GpU conformation prompts us to report our results on the conformational characteristics of GpU, m^1GpU, $m^7\overset{+}{G}$pU, and $m^7\overset{\pm}{G}$pU *.

2. Materials and methods

2.1. *Nucleosides and nucleotides used*

Guo, and 2′-, 3′- and 5′-GMP were purchased from C.F. Boehringer und Soehne. m^7Guo and m^7GMP's were prepared by a standard method [8], and purified by repeated chromatography. The physical data including absorption spectral characteristics and the values of basic ionization constant were almost unchanged from those previously recorded [8,9]. GpU was obtained from the enzymatic (RNase IA) digestions of RNA, followed by enzymatic dephosphorylation with alkaline phosphatase at 36°C, pH about 9. The product was checked on paper chromatography. The details of the preparation are the same as that reported in a previous paper [10].

2.2. *Instrumentations*

Light absorption spectra were measured on a Hitachi spectrophotometer Model 124. The CD spectra were measured on a JASCO J-10 spectrophotometer in 10 mm quartz cells. The pH measurements were made on a pH meter Model PHM 26 (Radiometer). For the CD measurements salt-free samples were dissolved in about 4 ml of appropriate buffer solutions to give the optical density values at λ_{max} of approximately 1–1.5.

* *Abbreviations:*
CD, circular dichroism; Guo, guanosine; $m^7\overset{+}{G}$MP and $m^7\overset{\pm}{G}$MP, N^7-methylguanylic acid in conjugate acid and zwitterionic form, respectively; in N^7-methylguanylyl-3′, 5′-uridine, (+)-$m^7\overset{+}{G}_p{}^{(syn)}U^{(anti)}$ (+) represents that the turn of the 3′–5′ backbone screw axis of the stack is right-handed, and (syn) and (anti) denote that m^7Guo and uridine residues are in *syn* and *anti* conformation with respect to the sugar-base torsion angle, respectively.

Table 1
Hypochromicity and molar extinction coefficients at λ_{max} and 260 nm (values at 25°C).

Substance	pH	(nm)	$\times 10^{-3}$	h (%)
$\overset{+}{G}pU$	0.4	258 (max)	20.3_2	9.4
	0.4	260	20.2	9.0
$m^1\overset{+}{G}pU$	0.4	258.5 (max)	19.2	9.9
	0.4	260	19.1_6	10.0
$m^7\overset{+}{G}pU$	4.51	258.5	20.1	13.7
	4.51	260	19.9	13.7

2.3. *Rate measurements on ring-opening of N^7-methylated guanosine derivatives*

Kinetic runs were carried out at pH 10.2 and 37°C. The optical densities at 270 nm for 2′-, 3′- and 5′-m^7GMP and at 290 nm for m^7Guo and m^7GpU were recorded continuously. Changes of optical density with time showed all the reactions to obey first-order kinetics, viz., the plot of log $(A_\infty - A_t)$ *vs.* time was linear.

3. Results

3.1. *Preparation of m^1GpU and m^7GpU*

GpU is known to undergo methylation by dimethylsulfate at pH 5.5 on N-7 of the guanine residue without effect on the corresponding uridine residue [11]. The structure of the product was proved by its ability to undergo ring-opening in moderately strong alkali to uridylyl-5′, 3′-[2-amino-4-hydroxy-5-N-methylformamido-6-ribosylamino pyrimidine] and by ribonuclease T_2 treatment; from m^7GpU, m^7Gp(3′) and uridine were obtained in equimolar quantities. On the other hand, GpU is methylated, when K_2CO_3 is present in the reaction mixture, mainly at N-1 of the guanine moiety, the structure of the product being based on spectral evidence.

3.2. *Optical properties of GpU and its methyl derivatives*

Table 1 summarizes results of measurements of hypochromicities and molar extinction coefficients at λ_{max} and 260 nm for GpU, and its methyl derivatives.

The CD spectra of GpU, m^1GpU, and m^7GpU at selective pH values are shown in fig. 1. The CD spectra of GpU at neutral and acidic media agreed qualitative-

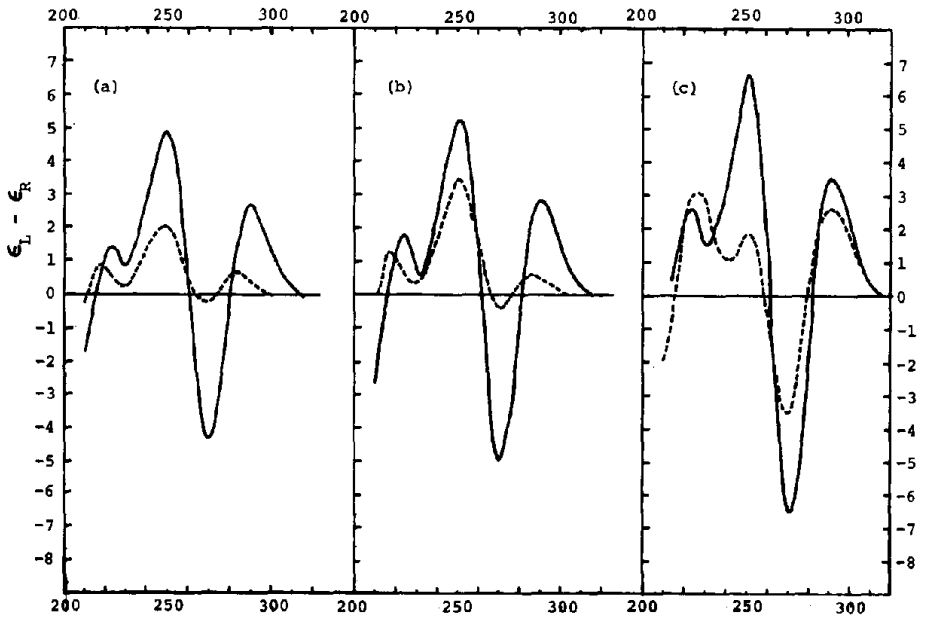


Fig. 1. CD spectra of: a) GpU ——, at pH 0.4, - - - -, at pH 6.57; b) m^1GpU ——, at pH 0.4, - - - -, at pH 6.97, and c) m^7GpU ——, pH 4.49; - - - -, at pH 8.5 (zwitterion).

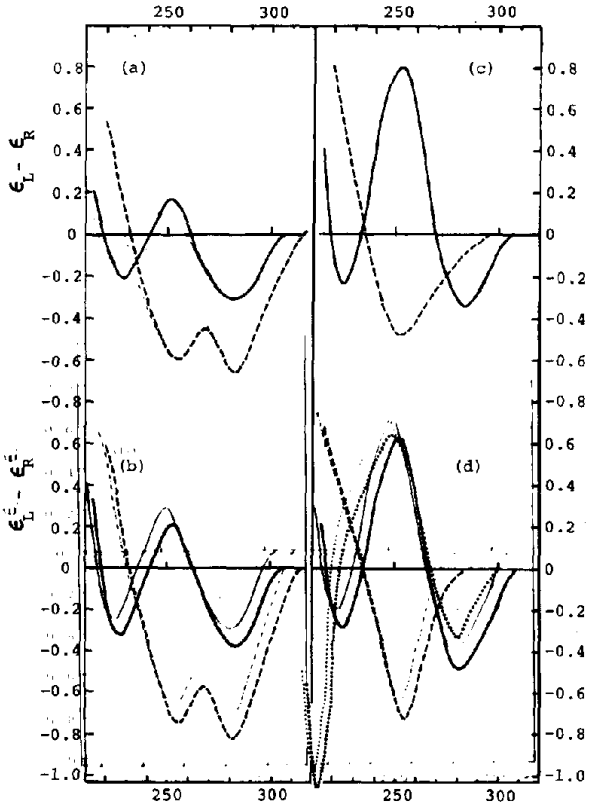


Fig. 2. CD spectra of: a) m^7Guo-2′, 3′-cyclic phosphate ——, at pH 4.5, -----, at pH 9.0; b) m^7Guo-2′-phosphate, ——, at pH 4.5; -----, at pH 9.0; c) m^7Guo-3′-phosphate, ——, at pH 4.5; -----, at pH 9.0, and d) m^7Guo-5′-phosphate, ——, pH 4.5; -----, pH 9.0, and Guo-5′-phosphate, · · · · ·, pH 0.4.

ly with those reported by Guschlbauer et al. [7]. The close similarity in shape and normal small bathochromic shift in the absorption spectra of these molecules in their monoprotonated form were noticed when compared with the parent compound in the corresponding form, $\overset{+}{G}$pU, but the spectra of $m^7\overset{+}{G}$pU at pH 8.31 differed from both the parent and N^1-methylated derivative in the neutral form of the bases [we were unable to prepare one reference compound, O^6-methylated GpU.]. The increased values in the CD band intensity of $m^1\overset{+}{G}$pU at pH 6.97 compared with that of GpU at pH 6.57 is ascribed to the enhanced intramolecular base–base stacking association partly by interference with solvation by the N-methyl group when a particular site of the guanine ring is methylated. The differentials in the increased magnitude of $E_L - E_R$ for

Table 2
First-order rate constants (sec^{-1}) for ring-opening at 37°C and pH = 10.2.

Substance	m^7Guo	2′-m^7GMP	3′-m^7GMP	5′-m^7GMP	m^7GpU
$k \times 10^{-4}$	22.3	12.7	8.46	3.99	23.6

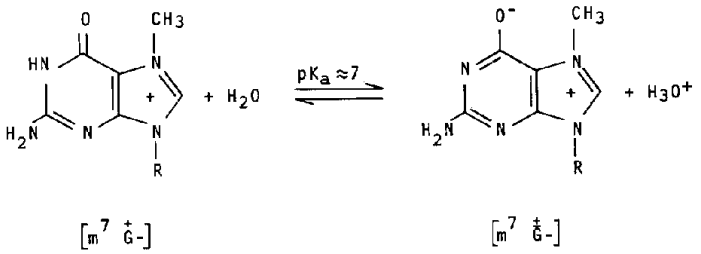
$m^1\overset{+}{G}$pU and $m^7\overset{+}{G}$pU relative to $\overset{+}{G}$pU should also be due to the average geometry of the stacks in solution. Apparently methylation at N-7 atom of GpU seems to cause a stacking effect to a greater extent than N-1 methylation as judged from the larger values of $\Delta(E_L - E_R)_+ = \Delta\Delta E$ and difference in hypochromicity for $m^7\overset{+}{G}$pU than those for $m^1\overset{+}{G}$pU when compared to those for $\overset{+}{G}$pU.

To supplement these results the CD spectra of 2′-3′-, and 5′-m^7GMP and m^7 Guo-2′, 3′-cyclic phosphate were measured at pH 9.0 immediately after the preparation of solutions and the results are reproduced in fig. 2.

3.3. *Kinetics of the ring-opening of m^7Guo and its nucleotides*

A kinetic study of the base-catalyzed ring-opening of m^7Guo and its derivatives have been reexamined at pH 10.2 where the m^7G-residue exists in almost completely ionized form, and extended to m^7GpU, all of which add hydroxyl ion at C-8, followed by N^9–C^8 bond fission. We have found that, at constant wavelength and constant pH, absorbance changes due to the ring-opening obey first-order rate equations. Table 2 summarizes rate constants obtained at 37°C and pH 10.2.

First of all, it was noted that phosphate substitutions have a specific and substantial effect on the rate of ring-opening reaction. A 5′- or a 3′-phosphate group decreases the rate to about 1/5.5 and 1/2.6, respectively, while the introduction of a 5′-uridylyl group into the 3′ position of m^7Guo makes only a small difference to the rate. From the foregoing results it is concluded that $m^7\overset{\pm}{G}$uo and $m^7\overset{\pm}{G}$pU exist in the *syn* conformation while 5′-p$m^7\overset{\pm}{G}$ must be in the *anti* conformation with respect to the sugar-base torsion angle. The CD data obtained seems to be consistent with this interpretation.



4. Discussion

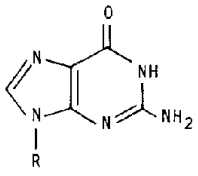
It is believed that the protonation of Guo increases the contribution of the *syn* conformation in the case of the nucleoside and mononucleotides [6]. The close similarity in shape in the CD spectra of m^7Guo and Guo confirmed the N-7 protonation of Guo in aqueous solution. Thus, from the structural similarities, it was expected that the conjugate acid of m^7GpU would be used as model compound for the monoprotonated GpU. (at pH where G is almost completely protonated, the U residue also undergoes partial protonation and neutralization of the primary phosphate group starts to occur). It seemed also of interest to us to examine the somewhat different situation encountered in the comparison between N^7-protonated and N^7-methylated guanylyl residues. Moreover, the conformational characteristics of oligonucleotides containing N-7 methylguanosine residue being unknown, an investigation has therefore been initiated by observing the optical properties of m^7GpU at different conditions.

m^7GpU (at pH pK_a-2, say 4.49) possesses a four-band CD spectrum with long wavelength positive band at 291 nm and the negative band at 270 nm (fig. 1c). This spectrum resembles that of GpU at pH about 0.3, although in the former each band is displaced to longer wavelengths by 2–5 nm its intensity is considerably enhanced (fig. 1). It is known that methylation at the base moiety usually enhances the association through hydrophobic-stacking interaction [12]. Thus, the CD and hypochromicity data suggest that m^7GpU is more strongly stacked than GpU. It is also known that, in the absence of steric complications, methyl groups normally produce bathochromic shifts of $\pi \rightarrow \pi^*$ bands since a methyl group constitutes an additional polarizable unit [13]. The *syn* conformation of m^7Guo residue in m^7GpU satisfies geometrical disposition for greater stacking interaction and appeared to coincide with enhanced intensity of the CD spectrum of m^7GpU compared with that of GpU. Examination of space-filling CPK models of m^7GpU indicates that an overlapped chromophore of right-handed chirality will be achieved when m^7GpU is in $m^7G_p^{(syn)}U^{(anti)}$ conformation.

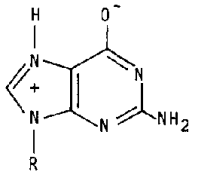
Because m^7Guo exists in a zwitterionic structure (as neutral molecule) for valency reasons, m^7Guo-containing dinucleotides, e.g. m^7GpU and m^7GpG, provide interesting examples on which to undertake some spectroscopic and thermodynamic studies.

As is usually the case with the 5′-mononucleotides [14], 5′-m^7GMP seems to be in an *anti* conformation as judged from the CD data (fig. 2). We prefer, however, at least in part the *syn* conformation for 2′-m^7GMP and m^7Guo-2′, 3′-cyclic phosphate on the admittedly intuitive grounds that their CD spectra exhibit an additional negative band at approximately 282 nm, the corresponding band being found for the cationic structure of m^7Guo derivatives regardless of the position of phosphate substitution. These results are in qualitative accord with a previously reported conclusion based on the stability of the N^7-methylguanine nucleotides against imidazole ring fission at pH 8.9 and 37°C [15]. We have measured rate constants for the ring-opening of m^7Guo, 2′-, 3′- and 5′-m^7GMP, and m^7GMP, and m^7GpU at pH above pK_a + 2, i.e. 10.2. In order to examine whether or not the zwitterionic m^7G-chromophore is constrained to be *syn* form with a fixed chirality [right-handed screw sense in the case of m^7GpU], the effect of successive additions of alkali on the CD spectrum of m^7GpU was examined. It was noted that the neutralization of the cation without the concomitant destruction of the highly stacked conformation results in essentially no change in the shape of the longer-wavelength CD bands excepting a significant reduction of the intensity of the lower-wavelength bands (fig. 1c). The large intensity of the CD bands observed at the higher

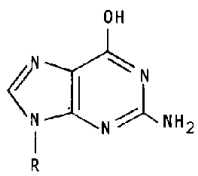
*For part 9, see [17].



(I), [H^1-G]



(II), [H^7-G]



(III), [H^6-G]

wavelength region seems to be characteristic of dissymmetric chromophoric interaction present in (+)-m$^7\overset{+}{G}_p^{(syn)}U^{(anti)}$ conformation and quite different from those observed for GpU and m^{1}GpU at neutral pH and low ionic strength. Conceivably, this particular conformational feature of the $-$m$^7\overset{+}{G}$pU$-$sequence, which is frequently found in the extra arm region of *E. coli* tRNA, plays a significant role in their molecular folding and function.

Guo could exist in any of a number of possible tautomeric structures, including generally accepted cyclic lactam form (I), zwitterionic tautomer (II), and lactim form (III).

In the present communication the first two of these possibilities are explored by comparing the CD spectra with those of methylated derivatives having fixed structures. It is conceivable that tautomeric equilibria might account for, at least, some of the unusual behavior of Guo residue reported in literature [16]. In view of this it is desirable to know where the tautomeric equilibrium lies especially when the Guo is incorporated in oligonucleotide chains. The CD spectra of GpU and m^{1}GpU (as neutral forms) resembled each other under the same environmental conditions, but differed from that of m$^7\overset{+}{G}$pU (at pH 9) confirming the cyclic lactam [as 2-amino-1,6-dihydro-6-oxo-9-D-ribosylpurine-(3′-5′)-uridine] rather than zwitterionic nature of the parent at least in aqueous solution of low ionic strength, 25°C. The situation might not be valid in high ionic strength and low temperature since, although not fully understood, it was found by Guschlbauer et al. that GpU at -20°C, pH 6 in 4.5 N LiCl exhibited the CD spectrum closely resembling that at pH 1, in water at 20°C [7]. Incidentally, the basic ionization constant of GpU in aqueous solutions was also found to vary markedly with temperature [*pK* 1.7 at 48°C; *pK* 2.4 at 24°C; *pK* 3.0 at 3°C; read directly from fig. 12 in ref. [7]. This trend is in qualitative agreement with a not unreasonable assumption that if polar zwitterion structure, H$^7\overset{+}{G}$pU, and possibly $\overset{+}{G}$pU are preferentially stabilized in such environmental factors, the value of $pK_{H^1\text{-}GpU}$ would be expected to become larger, and this results in an 'increase' in the tautomeric constant, [H^7-GpU]/[H^1-GpU], so that considerable amounts of GpU would exist in GpU at -20°C, pH 6. Thus at this stage the possibility of a preferred form, $\overset{+}{G}$pU, at low temperature and high ionic strength is not excluded unless further detailed work is made, particularly on a frozen reference compound, m^{1}GpU.

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