Poly(7-deazaguanylic acid), the Homopolynucleotide of the Parent Nucleoside of Queuosine†

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ABSTRACT: Poly(7-deazaguanylic acid) was enzymatically synthesized by the polymerization of 7-deazaguanosine 5′-diphosphate with polynucleotide phosphorylase from *Micrococcus luteus* in high yield. The homopolymer showed a similar thermal and total hypochromicity to poly(G) at the long wavelength absorption maximum. No sigmoid melting profile was observed for poly(c^7G) as is found for poly(G), implying a single-stranded structure in aqueous solution. From the circular dichroism spectra it can be concluded that the 7-deazapurine nucleotide is much more flexible than the purine nucleotide. In analogy to poly(G), the homopolymer poly(c^7G) forms a 1:1 complex with poly(C) under neutral conditions, melting at a similar temperature to the poly(G) complex. However, at pH 2.5, where a poly(G)·2poly(C) complex is observed, poly(c^7G) still binds only one poly(C) strand. This is due to the lack of N-7 in poly(c^7G), not allowing Hoogsteen base pair formation, which occurs with poly(G). RNase T_1 cleaves poly(c^7G), indicating that N-7 of guanosine is not a requirement for nucleotide binding to the enzyme, as has been suggested. Because of the single-stranded structure of poly(c^7G), the polynucleotide chain is rapidly hydrolyzed by the single-strand-specific nuclease S_1, whereas multistranded poly(G) is completely resistant.

Pyrrolo[2,3-*d*]pyrimidine nucleosides are rare constituents of tRNAs and have been isolated as monomers in the form of 7-deazaadenosine (tubercidin)[1] and C-5-modified derivatives from the cultural filtrates of microorganisms (Suhadolnik, 1979). The nucleoside queuosine (Kasai et al., 1975) and related 7-deazaguanosine derivatives (Noguchi et al., 1978; Okada et al., 1978) have been detected in the wobble position of the anticodon of *Escherichia coli* tRNAs.

It is assumed that the nucleoside Q modulates codon/anticodon recognition. This idea is underlined by the fact that 7-deazanucleosides such as tubercidin when incorporated into nucleic acids are highly efficient in ribosomal polylysine synthesis (Seela et al., 1981). The novel properties of pyrrolo[2,3-*d*]pyrimidine nucleosides (7-deazapurine nucleosides) have prompted us to prepare the homopolynucleotide of 7-deazaguanosine.

The latter (Townsend et al., 1976), which is the parent compound of queuosine (Ohgi et al., 1979), has been recently synthesized by the method of phase transfer glycosylation (Seela & Hasselmann, 1980, 1981), which was also applied to the synthesis of other ribo- and arabinonucleosides (Seela & Winkeler, 1982).

A structural feature of 7-deazanucleosides is the stability of the N-glycosylic bond, which is much higher than in purine nucleosides. This extraordinary stability, resulting from the low nucleophilicity of the pyrrole nitrogen of 7-deazapurines, is the chemical basis for an enzymatic transglycosylation reaction (Farkas & Singh, 1973). The enzymatically catalyzed process results in the excision of guanine from the anticodon of tRNA and its replacement by queuine (Shindo-Okada et al., 1980). This process can be induced by queuine from exogenous sources (Reyniers et al., 1981) and is a factor in the development of organisms (Jacobson et al., 1981).

An important requirement for the study of the structural and functional parameters of 7-deazaguanosine-containing polynucleotides is their efficient synthesis. Large quantities of regular polynucleotides have been prepared by polymerization of nucleoside diphosphates with polynucleotide phosphorylase (Godefroy-Colburn & Grunberg-Manago, 1972). Whereas homopolymers of A, C, I, and U are easily available, the preparation of poly(G) remained difficult with the *Micrococcus luteus* enzyme. When the *E. coli* enzyme and modified incubation conditions are used, a high molecular weight poly(G) can be obtained (Thang & Grunberg-Manago, 1968).

In the following paper we describe the synthesis of poly(c^7G) by PNPase from *M. luteus* along with various structural parameters of this homopolynucleotide. Furthermore the interaction of poly(c^7G) with poly(C) and the enzymatic cleavage of the homopolynucleotide by RNase T_1 and nuclease S_1 have also been studied.

Experimental Procedures

UV spectra were measured with a Varian SuperScan 3 or a Shimadzu UV-210A spectrometer. CD spectra were run on a Mark V UV–VIS autodichrograph (Instruments SA, France). Temperature was controlled in the melting experiments by an R 40/2 digital thermometer connected with a platinum resistor (MGW Lauda, West Germany). NMR spectra were measured with a Bruker WM 250 spectrometer; δ values were relative to H_3PO_4; (−) corresponds to an upfield shift. Column chromatography was performed on DE-52 cellulose and Dowex 50 WX-4 resins and gel chromatography on Ultrogel AcA 34 (LKB, Sweden). Columns were connected

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[1] Abbreviations: queuine, 2-amino-5-[[(1*S*,4*R*,5*S*)-(4,5-dihydroxycyclopent-2-en-1-yl)amino]methyl]-3,7*H*-pyrrolo[2,3-*d*]pyrimidin-4-one; c^7G, 2-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one; c^7I, 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one; queuosine, 2-amino-5-[[(1*S*,4*R*,5*S*)-(4,5-dihydroxycyclopent-2-en-1-yl)amino]-methyl]-7-(β-D-ribofuranosyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-4-one; Tu, tubercidin, 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine; c^7GDP, 7-deazaguanosine 5′-diphosphate; 3′,5′-c^7GMP, 7-deazaguanosine 3′,5′-phosphate; PNPase, polynucleotide phosphorylase; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid disodium salt; TLC, thin-layer chromatography; TLE, thin-layer electrophoresis; CD, circular dichroism; *A*, absorbance; DMF, *N*,*N*-dimethylformamide; NMR, nuclear magnetic resonance; PEI, poly(ethylene imine).

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Table I: Chromatographic Mobilities of Guanosine and 7-Deazaguanosine Phosphates

compound	R_f (PEI-cellulose, solvent C)
GMP	0.56
c^7GMP	0.45
GDP	0.49
c^7GDP	0.40

with a Uvicord S as the detection unit and an UltroRac fraction collector (LKB, Sweden). Thin-layer chromatography (TLC) was performed on cellulose plates, Polygram CEL 300 PEI/UV_{254} (Macherey-Nagel, West Germany) and G 1440/LS 254 (Schleicher & Schüll, West Germany). Thin-layer electrophoresis (TLE) was carried out on precoated silica gel plates, SIL G-25 UV_{254} (Macherey-Nagel, West Germany), in a TLE double chamber (Desaga, West Germany). Solvent systems were the following: A, 1.0 M aqueous triethylammonium bicarbonate; B, 0.1 M sodium citrate, pH 6.5; C, 1 M LiCl; D, 0.04 M NH_4HCO_3. The phosphate reagent (Chen et al., 1956) was prepared as described (Seela et al., 1981).

Polynucleotide phosphorylase (EC 2.7.7.8, *M. luteus*) was a product of P-L Biochemicals (Milwaukee, WI). Nuclease S_1 (EC 3.1.4.*x*) and RNase T_1 (EC 3.1.27.3), both from *Aspergillus oryzae*, were purchased from Boehringer (Mannheim, West Germany). Poly(G) and poly(C) were products of Sigma Chemical Co. (St. Louis, MO).

7-Deazaguanosine 5′-Diphosphate Triethylammonium Salt. A total amount of 1850 A_{258} units (0.135 mmol) of triethylammonium 7-deazaguanosine 5′-phosphate (Tran-Thi et al., 1982) in 40 mL of water was applied to a 16 × 1.5 cm cation-exchange column (Dowex 50 WX-4, pyridinium form) and eluted with 500 mL of water. The solvent was evaporated, and 32 μL (0.135 mmol) of tri-*n*-butylamine was added. Water was removed by repeated evaporation with anhydrous DMF. The tributylammonium salt was dissolved in 1 mL of DMF, and the imidazolidate was generated by adding of 120 mg (0.75 mmol) of 1,1′-carbonyldiimidazole dissolved in 1.5 mL of DMF and by stirring 12 h at room temperature. The excess of 1,1′-carbonyldiimidazole was destroyed with 30 μL of methanol. After 30 min tri-*n*-butylammonium phosphate (0.8 mmol) in 4 mL of DMF was added, and the reaction mixture was kept for 1 day at room temperature. After removal of the solvent in high vacuum the residue was dissolved in water and chromatographed on a 45 × 3 cm ion-exchange column (DE-52 cellulose, HCO_3^- form). The triethylammonium salt of c^7GDP was eluted with a linear gradient of 1500 mL of solution A/1500 mL of water between 0.3 and 0.35 M. Evaporation of the solvent yielded 1260 A_{258} units (68%, with ϵ = 13 200 at 258 nm) of colorless material, which was obtained as a fluffy solid after lyophilization: TLE (silica gel, solvent B) R_f 1.7 (+) relative to c^7GMP (Table I); UV (water) λ_{max} 258 nm; phosphate 2 mol/mol of aglycon; ^{31}P NMR (D_2O, pD 12) δ −4.99 (d, J = 22 Hz), −9.68 (d, J = 22 Hz).

Preparation of Poly(c^7G). The reaction mixture (3 mL) contained c^7GDP (30 μmol), 1 M Tris-HCl (150 μL, pH 9.6), 0.1 M $MgCl_2$ (150 μL), 3 mg (15 phosphorolysis units) of *M. luteus* polynucleotide phosphorylase, and 2.7 mL of water. The mixture was incubated for 24 h at 37 °C, cooled to 0 °C, diluted with 2 mL of water, and extracted twice with $CHCl_3$–isoamyl alcohol (5:3) and once with ether to remove the protein on the phase boundary. After the residual ether had been removed under a stream of nitrogen, the deproteinized aqueous layer was applied to a 35 × 2.5 cm gel column (Ultrogel AcA 34) and eluted with solvent D. Poly(c^7G) appeared in the void volume, whereas the monomeric material was eluted in a slower migrating zone. After lyophilization, the polynucleotide was taken up in water and dialyzed successively against 2-L changes of 10 mM EDTA, 0.1 M NaCl, and water. The aqueous solution was then lyophilized. A total of 5.6 mg (49%) of colorless amorphous polynucleotide was obtained: UV (water) λ_{max} 258 nm. The extinction coefficient of poly(c^7G) was determined by ashing aliquots, followed by determination of inorganic phosphate, and found to be 10 300.

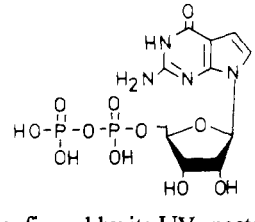
Melting Curves. The melting curves were measured in Teflon-stoppered quartz cuvettes with a 1-cm light path length in a thermostatically controlled cell holder in a Varian SuperScan 3 recording spectrophotometer. The increase of absorbance at the appropriate wavelength as a function of temperature was determined at a rate of 18 °C/h maintained by a Lauda temperature programmer and bath.

Hydrolysis by Nuclease S_1. The cleavage of poly(c^7G) and poly(G) by nuclease S_1 was determined spectrophotometrically. For this purpose approximately 1 OD of each polynucleotide dissolved in 1 mL of 0.03 M sodium acetate, pH 5.5 or 6.5, 0.29 M NaCl, and 0.001 M $ZnSO_4$ was treated with 2000 units of nuclease S_1 in a 1-cm quartz cuvette. The increase of absorbance was recorded as a function of time at 35 °C. The percentage of cleavage was calculated from the hypochromicities determined at that temperature and in the buffer solution used in each experiment.

Cleavage of Poly(c^7G) with Ribonuclease T_1. A total of 0.198 A_{258} unit of poly(c^7G) in 700 μL of water, 200 μL of 0.1 M Tris-HCl buffer (pH 7.5), and 100 μL of 0.02 M EDTA was incubated with 2 μL (200 units) of a suspension of RNase T_1 for 3 h at 37 °C. After that time an absorbance of 0.239 was determined which corresponds to a cleavage of 77%.

Results and Discussion

For the synthesis of poly(c^7G) with polynucleotide phosphorylase it was first necessary to prepare the diphosphate c^7GDP. The starting material was c^7G, which was prepared by phase-transfer glycosylation (Seela & Hasselmann, 1980, 1981). From the nucleoside the monophosphate c^7GMP was obtained by phosphorylation in trimethyl phosphate, as previously described in the synthesis of 3′,5′-c^7GMP (Tran-Thi et al., 1982). The conversion of the 5′-monophosphate into the 5′-diphosphate was accomplished according to the method of Hoard & Ott (1965). Activation of the tri-*n*-butylammonium salt of c^7GMP with 1,1′-carbonyldiimidazole yielded the imidazolidate, and condensation with tri-*n*-butylammonium phosphate gave c^7GDP



Its structure was confirmed by its UV spectrum coinciding with that of c^7G and doublets at −4.99 and −9.68 ppm with a coupling constant of 22 Hz in the ^{31}P NMR spectrum.

Homopolymer Poly(c^7G). Although polynucleotide phosphorylase from *M. luteus* catalyzes the polymerization of a number of ribonucleoside diphosphates, the synthesis of high molecular weight poly(G) only proceeds with difficulty under the usual conditions. The synthesis of this latter polymer can, however, be achieved by using polynucleotide phosphorylase

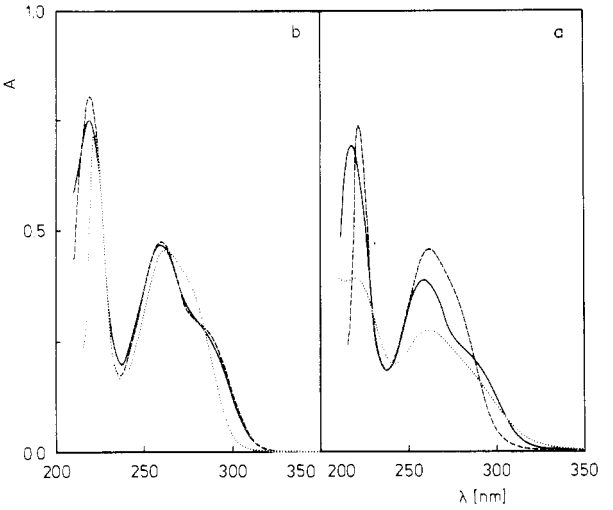


FIGURE 1: UV spectra of poly(c^7G) (a) and c^7G (b) in 0.07 M phosphate buffer, pH 7.0 (—), 0.1 M HCl (···), and 0.1 M NaOH (---) at 25 °C.

Table II: UV Data and Hypochromicities[a] of Poly(c^7G) and Poly(G)

	poly(c^7G)	poly(G)
UV_{max} (nm)		
pH 7.0	258, 278 (sh)	253
pH 1.0	260	256, 278
pH 13.0	261	260, 268
ϵ_{max} (pH 7.0)	10 300	10 100
hypochromicity by phosphate determination (%)	22	26
hypochromicity by nuclease S_1 treatment (%)	21	
thermal hypochromicity between 20 and 90 °C (%)	11	12

[a] Hypochromicities are measured at the long-wavelength maximum.

from *E. coli* at elevated temperatures (60 °C) and in the presence of Mn^{2+} instead of Mg^{2+}. In contrast, the polymerization of c^7GDP proceeds instantaneously even with polynucleotide phosphorylase from *M. luteus* in the absence of manganese ions under regular conditions, giving poly(c^7G) in about 50% yield. An explanation for this difference may be derived from the observation that c^7G and its phosphates do not form gels in aqueous solution. This implies that the lack of N-7 in the guanosine residue results in an altered electron distribution in the aglycon, preventing the self-aggregation that is characteristic of guanosine. Consequently there will be a more ready availability of the substrate at the active center of polynucleotide phosphorylase. Furthermore the polynucleotide poly(c^7G) may not form such strong aggregates as does poly(G). It should also be noted that the diphosphate of tubercidin—another 7-deazanucleotide—was even more readily incorporated into polymers by *M. luteus* PNPase than was ADP (Seela et al., 1981).

UV Spectra and Hypochromicity. The UV spectrum of poly(c^7G) at pH 7.0 shows a maximum at 258 nm with a shoulder around 278 nm (Figure 1a). The shape of the absorption band is similar to that of poly(G), but the maximum shows a bathochromic shift of 5 nm (Table II). The absorbance in 0.1 N HCl is decreased, whereas in 0.1 N NaOH it shows an increase. These changes are not observed for c^7GMP (Figure 1b). The increase of absorbance under alkaline conditions can be interpreted as an unstacking of the nucleobases by anion formation. This is underlined by p*K* measurements. From the difference in the absorbances at 275 nm between the neutral and the anionic form of c^7GMP the p*K* value of base deprotonation was determined to be 10.7.

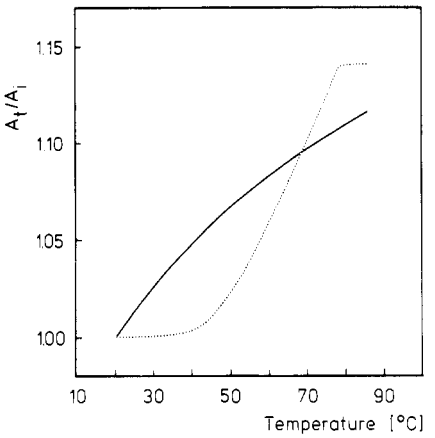


FIGURE 2: Denaturation profiles of poly(c^7G) (—) and poly(G) (···) in 0.07 M phosphate buffer–1 mM EDTA (pH 7.0). A_t/A_i is the ratio of the λ_{max} absorbances at a given temperature (t) to that at the initial temperature (i).

The hypochromicities of poly(c^7G) and poly(G) were determined by the molybdenum blue method (Chen et al., 1956). When extinction coefficients of 13 200 for c^7G and 13 600 for G were used, hypochromicities of 22% and 26% were found (Table II). The hypochromicity of poly(c^7G) was therefore very close to that of poly(tubercidylic acid), which exhibited 23% (Seela et al., 1981; Ikehara & Fukui, 1968; DeClercq et al., 1974).

Thermal Melting and CD Spectra of Poly(c^7G). Poly(c^7G) is similar to poly(G) in that it shows no hyperchromic change during heating under high-salt conditions and in the presence of magnesium ions, indicating a rigid structure. If divalent cations were removed by dialysis against EDTA and the change of absorbance was measured in 0.07 M phosphate–1 mM EDTA at pH 7.0, thermal melting was found. Figure 2 shows the melting profile of poly(c^7G) and poly(G) under identical salt concentrations and in the absence of magnesium. Whereas poly(G) showed a sigmoid denaturation curve indicating strong cooperativity of this process, the curve of poly(c^7G) shows no inflection point. The same has been found for poly(c^7I) (Torrence et al., 1974). While poly(G) forms a tetrastranded helix (Thiele & Guschlbauer, 1971) by Hoogsteen base pair formation, involving N-7, this is not possible for poly(c^7G) because of the lack of N-7. This implies that poly(c^7G) will only exist as a single-stranded polymer under these conditions with increased flexibility of the polynucleotide chain. A more flexible structure of poly(c^7G) compared to that of poly(G) is also indicated by the CD spectra. At pH 7.0 (Figure 3a) poly(G) exhibits a strong positive band (261 nm), a negative lobe at 241 nm, and a zero transition of 248 nm. The corresponding spectrum of poly(c^7G) looks very similar, except that it shows another positive band at 221 nm and an intensity of the strongest band of about only 10% of that of poly(G). Since it has been shown that the dipole moment of 7-deazaguanine is orientated in the same direction as that found for guanine (Liu, 1978), the base-stacking angles of both polynucleotides at neutral condition should be very similar. However, the strength of stacking should be different. While guanine possesses a dipole moment of 7.2 D, the moment for 7-deazaguanine was determined to be 3.0 D (Liu, 1978). If we assume that a decreased dipole moment reduces stacking, the data also imply a more flexible structure of poly(c^7G).

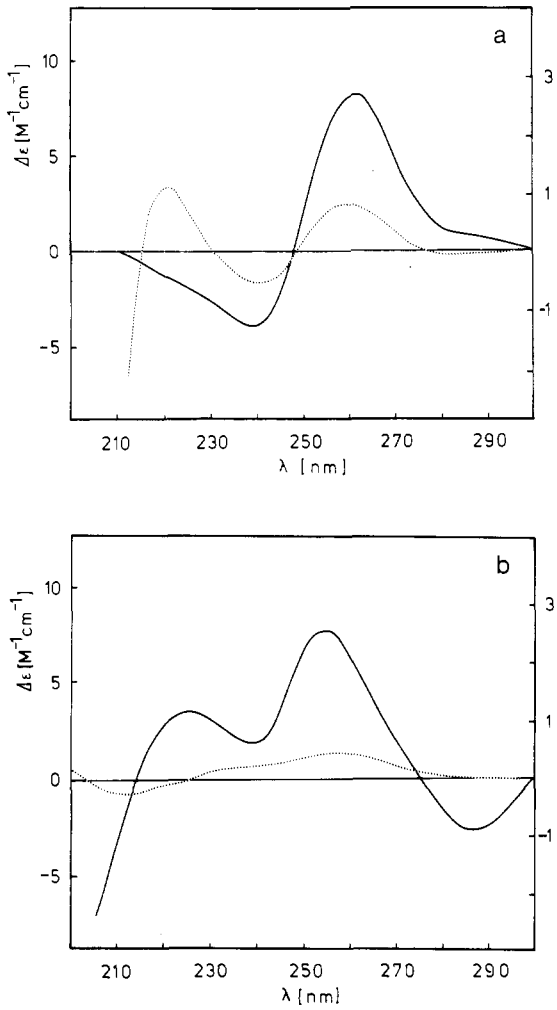


FIGURE 3: CD spectra of poly(c^7G) (···) (right scale) and poly(G) (—) (left scale) in 0.1 M NaCl–0.07 M phosphate buffer, pH 7.0 (a), or 0.1 M NaCl–0.1 M HCl, pH 1.0 (b). Measurements were made in a 1-cm cell at 25 °C and at a polynucleotide concentration of 100 μM. The instrument was calibrated with an aqueous solution of poly(U).

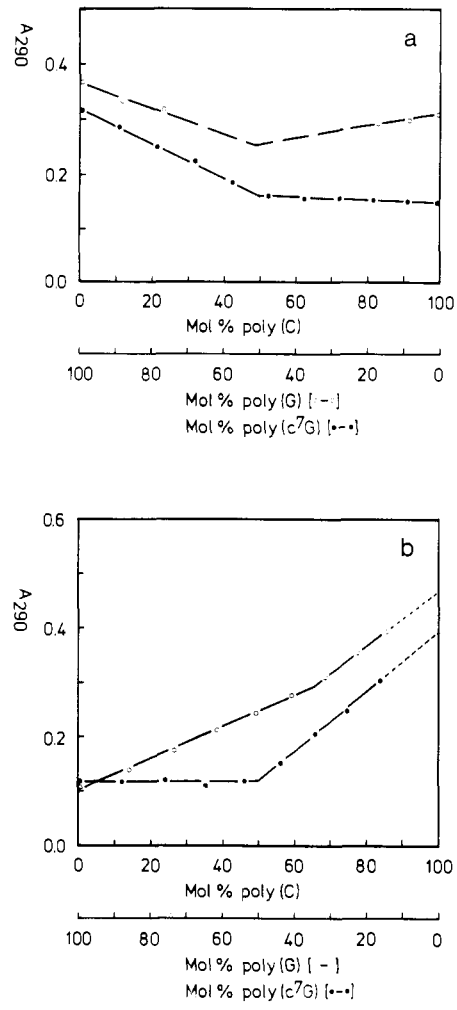


FIGURE 4: Mixing curves of poly(c^7G) and poly(G) with poly(C) at 290 nm in 0.1 M Tris-HCl buffer, pH 7.0, 0.2 M NaCl, and 1 mM $MgCl_2$ (a) and the same compounds at 290 nm in 0.1 M glycine–HCl buffer, pH 2.5, containing 0.2 M NaCl and 1 mM $MgCl_2$ (b).

It is well documented that guanosine is protonated at N-7 under acidic conditions. This protonation results in a conformational change from the anti to the syn form (Guschlbauer, 1972). Protonation at the polynucleotide level destroys the tetrahelix structure of poly(G). Consequently the CD spectrum of poly(G) (Wolfe et al., 1969; Zimmerman et al., 1975) is altered at pH 2.5, but the high intensities still imply a rigid structure. A conformational change of c^7G as found for guanosine is unlikely in the case of poly(c^7G). The indistinct CD spectrum of poly(c^7G) at pH 2.5 (Figure 3b) suggests a random structure at this pH value.

Base Pairing of Poly(c^7G) with Poly(C). The stoichiometry of the complex poly(c^7G)·poly(C) was determined by the method of continuous variation (Felsenfeld, 1958). A well-defined absorbance minimum was obtained at 290 nm. This minimum was reached at exactly 50 mol % poly(c^7G) (Figure 4a), clearly demonstrating a 1:1 complex at pH 7.0. The same stoichiometry is observed for the complex of poly(G) and poly(C) under identical conditions. A definite break in the mixing curves of poly(G)·poly(C) was only observed if the mixtures were allowed to come to equilibrium for 2 h. In contrast to poly(G), an immediate complex formation of poly(c^7G) with poly(C) was observed. The decreased rate of poly(G)·poly(C) formation can be explained by the necessary disaggregation of the tetrastranded poly(G); this does not occur in the case of poly(c^7G) because of its single-stranded structure.

The protonation of poly(G) at acidic pH is a requirement for Hoogsteen base pair formation with poly(C). In agreement with this formation a 1:2 stoichiometry was observed at pH 2.5 of poly(G)·poly(C) (Figure 4b). Complex formation was immediate, underlining the fact that protonation disaggregates the multistranded poly(G). At the same time the base is activated by protonation of N-7 for Hoogsteen base pairing with poly(C). In contrast to poly(G), triplex formation of poly(c^7G) with poly(C) did not occur even under acidic conditions. As Figure 4b shows, the minimum of the mixing profile was still located at 50 mol % poly(c^7G), showing that poly(c^7G) can only form Watson–Crick base pairs and the triplex formation of poly(G) definitely occurs via N-7.

It has yet to be proven whether poly(c^7G) is able to base pair with other polynucleotides. This is of interest with regard to the mispairing of the nucleoside Q in the wobble position. From protonation studies of 7-deazaguanine (Secrist & Liu, 1978) it is likely that protonation of this 7-deazapurine heterocycle mainly occurs at N-1. The strong basicity of that position could result in altered Watson–Crick base pairing of polynucleotides containing c^7G instead of G.

At pH 5.3 in 0.2 mM EDTA (sodium salt), the complex of poly(G)·poly(C) melts cooperatively (Figure 5) with a T_m of 75 °C. Surprisingly the complex of poly(c^7G)·poly(C) shows an almost identical temperature of T_m = 74 °C. These data imply that the stereochemical arrangement of c^7G in a

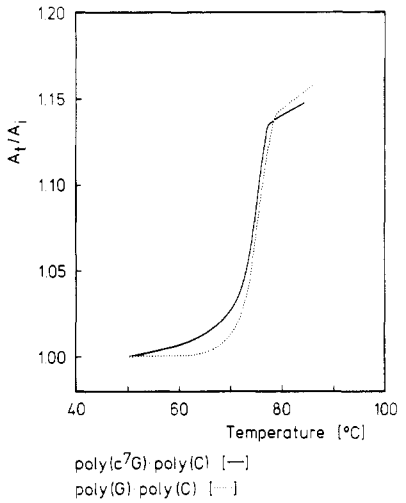


FIGURE 5: Melting curves of poly(c^7G)·poly(C) (—) at 265 nm and poly(G)·poly(C) (···) at 257 nm in 0.2 mM EDTA (pH 5.3). A_t/A_i is the ratio of absorbance at a given temperature (t) to that at the initial temperature (i).

base-paired Watson–Crick double helix is very similar to that of G, a fact that would allow us to consider whether 7-deazanucleotides are utilized to modulate polynucleotide recognition.

Hydrolysis of Poly(c^7G) by Ribonuclease T_1 and Nuclease S_1. The action of ribonuclease T_1 on polynucleotides has been subject to intensive study. The enzyme specifically hydrolyzes the internucleotide bonds of RNAs adjacent to the 3′-phosphate of GMP. It has been reported that N-7 of guanosine is involved in the binding of the substrate to the active site of the enzyme (Egami et al., 1980). On the basis of enzyme action on various substrates, a model of the enzyme–substrate complex has been proposed. Results on the protonation of N-7 of guanosine during enzymatic binding were controversial (Oshima & Imahori, 1971). Actually poly(c^7G) is the very best probe, allowing to prove the proposed model, which is based on a hydrogen bridge between N-7 of guanosine and the His-92 residue of the enzyme. We therefore applied RNase T_1 on poly(c^7G) and measured the cleavage of the polynucleotide by observing the change of absorbance. Rapid hydrolysis occurred within 3 h, yielding to an 80% cleavage calculated from hypochromicity (Table II). This result clearly indicates that N-7 of the guanosine residue is not a requirement for RNase T_1 action on polynucleotides. The proposed complex model has therefore to be revised. It is not possible to compare hydrolysis rates of poly(G) and poly(c^7G) by RNase T_1. According to X-ray fiber diffraction (Zimmerman et al., 1975) poly(G) has a highly ordered multiple-stranded structure, on which the enzyme is not able to act like on a monomer chain.

An even higher specificity for complexed polynucleotides is observed with nuclease S_1 (Ando, 1966; Vogt, 1973). The enzyme is single-strand specific and recognizes secondary structures of nucleic acids. Double-stranded 2′-deoxyribo- and ribopolynucleotides are not significantly cleaved by this enzyme; the same is valid for polynucleotides, forming multistrand structures. Consequently poly(G) is not cleaved by nuclease S_1 (Figure 6). However, as Figure 6 shows, this enzyme hydrolyzes poly(c^7G) immediately. These findings are in agreement with the results reported above that poly(c^7G) does not form multistranded structures, because of its inability to bind another strand via Hoogsteen base pairs resulting from the lack of N-7. We therefore suggest that poly(c^7G) is single stranded under neutral or weakly acidic conditions.

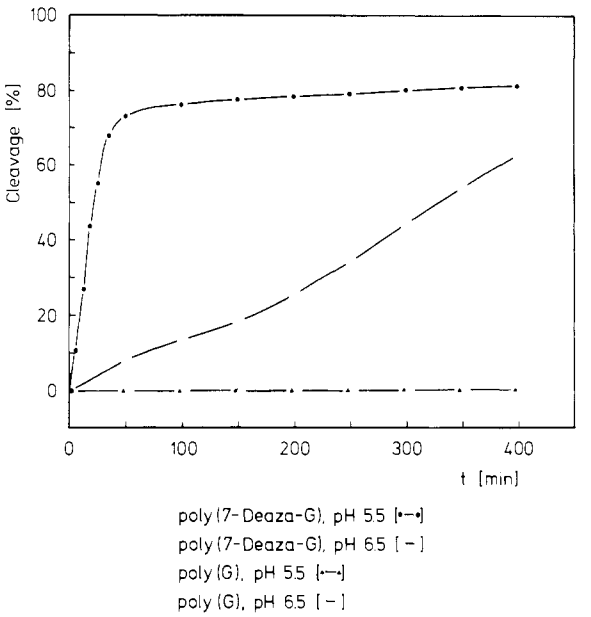


FIGURE 6: Cleavage of polynucleotides as indicated with nuclease S_1 at pH 5.5 and 6.5, at 25 °C. The reaction mixture contained 0.1 μmol/mL polynucleotides, 2000 units of the enzyme, 0.29 M NaCl, 1 mM $ZnSO_4$, and 0.03 M sodium acetate (pH 5.5 or 6.5).

However, the Watson–Crick base-paired complex of poly(c^7G)·poly(C) behaves differently. As shown from stoichiometric data (Figure 1), a 1:1 complex is formed under neutral conditions. This complex is not significantly cleaved during treatment with nuclease S_1. This result is in accordance with the resistance of the complex poly(Tu)·poly(U) or other polynucleotides base paired with a complementary strand. Our findings indicate that not only Watson–Crick but also Hoogsteen base pair formation prevents nuclease S_1 hydrolysis of polynucleotides. Since the rate of hydrolysis of poly(c^7G) is decreased compared to that of poly(Tu) (Seela et al., 1982), we assume a more ordered structure of poly(c^7G) compared with that of poly(Tu) but a much more flexible structure than those of the corresponding purine polynucleotides.

Combining all data into a model of the function of queuosine in the wobble position of tRNAs, we suggest that due to the lack of N-7 and the bulky substituent at that position the nucleoside exhibits an enhanced flexibility compared to guanosine during triplet recognition and translation of the genetic message.

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A Kinetic Study of the Binding of Carbon Monoxide to Ferrous Chloroperoxidase†

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ABSTRACT: The binding of carbon monoxide to ferrous chloroperoxidase in the pH range 4–6.5 is influenced by a titratable group on the enzyme having a pK_A of 5.5 ± 0.2 at 20 °C. The basic form of the enzyme reacts much faster with carbon monoxide than does the protonated form of the enzyme. The $\Delta H°$ for the ionization of the functional group in the enzyme involved in carbon monoxide binding is about 8 kcal mol^{-1}, and the $\Delta S°$ is approximately 1 cal mol^{-1} K^{-1}. These pK_A and $\Delta H°$ values suggest that this functional group is an imidazole ring associated with a histidine residue situated at the active site of the enzyme. The rates of the reaction for the formation and dissociation of the complex suggest that this histidine residue is not directly liganded to the iron atom of the heme prosthetic group. The relatively good agreement between the various kinetic approaches with several methods of experimentation, data collection, and data analysis lends strength to a proposed model in which the histidine occupies a distal site close to the sixth axial ligand position of the heme iron atom.

Chloroperoxidase, produced by *Caldariomyces fumago*, is a hemoprotein capable of catalyzing several different types of peroxidative reactions. These reactions include the halogenation of metabolic products, the oxidation of hydrogen donors, and the dismutation of hydrogen peroxide (Thomas et al., 1970). Chloroperoxidase also resembles cytochrome P-450$_{cam}$ in terms of its spectral and chemical properties. The close similarities between chloroperoxidase and cytochrome P-450$_{cam}$ in terms of optical absorption (Hollenberg & Hager, 1973), electron spin resonance (Chiang et al., 1975), Mössbauer properties (Champion et al., 1975), and resonance Raman spectra (Champion et al., 1976) suggest similar environments for the heme prosthetic groups in these two enzymes. A comparison of the ferrous enzyme–carbon monoxide complexes of the two enzymes is especially striking. Both enzymes form complexes with carbon monoxide that have the long-wavelength absorption band in the 446–450-nm range,

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