Volume 11 Number 17 1983 Nucleic Acids Research

Poly(2-methylthio-7-deazainosinic acid) – hydrophobic stabilization of polynucleotide secondary structure by the 2-methylthio group

Frank Seela*, Johann Ott and Doris Franzen

Laboratory of Bioorganic Chemistry, Department of Chemistry, University of Paderborn, D-4790 Paderborn, FRG

Received 6 May 1983; Revised 13 July 1983; Accepted 8 August 1983

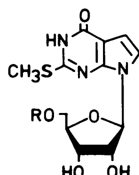
ABSTRACT

Poly(2-methylthio-7-deazainosinic acid) [poly(ms^2c^7I)] was enzymatically synthesized by polymerization of 2-methylthio-7-deazainosine 5'-diphosphate with polynucleotide phosphorylase from *Micrococcus luteus* in high yield. The homopolymer shows much higher thermal stability than its parent polynucleotides poly(7-deazainosinic acid) [poly(c^7I)] and poly(I). Its sigmoidal melting curve and pronounced hypochromicity imply a rigid, ordered structure. Poly(ms^2c^7I), like poly(2-methylthio-inosinic acid) [poly(ms^2I)], does not form a complex with poly(C) because of the bulky 2-methylthio substituent. On the other hand, two poly(ms^2c^7I) strands form very rigid triple strands with poly(A). Different from poly(I) and poly(c^7I) the homopolymer poly(ms^2c^7I) is very stable against cleavage by nuclease S_1 and ribonuclease T_2 as expected from its rigid secondary structure.

INTRODUCTION

Among the rare nucleosides which have been isolated as monomeric nucleoside antibiotics or as constituents of nucleic acids is a thionucleoside having a methylthio group attached to the purine moiety at C-2[1]. This N^2-(Δ^2-isopentenyl)-2-methylthio-adenosine has been isolated from tRNAs and is located adjacent to the 3'-site of the anticodon[2].

In order to obtain information on the chemical and biological consequences of the presence of a methylthio group homopolymers of 2-methylthio-adenosine and 2-methylthio-inosine have been prepared in vitro by polymerization of the corresponding 2-methylthio-purine 5'-diphosphates by polynucleotide phosphorylase from *Micrococcus luteus* and it has been shown that a 2-methylthio group can prevent Watson-Crick base pair formation[3,4]. From binding



1a : R = H

1b : R = PO_3H_2

1c : R = $P_2O_6H_3$

© IRL Press Limited, Oxford, England.

studies on 7-deazapurine nucleotides it is evident that Hoogsteen base pairing cannot occur[5,6]. Consequently a homopolymer of 7-deazapurine nucleotides having a methylthio group attached to the C-2 position should exhibit a very restricted tendency to form base pairs with complementary nucleotides[7]. To test this hypothesis we attempted to synthesize a poly(I) analogue being altered at C-2 and N-7 in this way.

We describe here the enzymatic synthesis of poly(ms^2c^7I) and characterization of the homopolymer with respect to formation of a secondary structure, base pairing, and hydrolysis by nucleases.

MATERIALS AND METHODS

UV spectra were measured on a Kontron Uvikon 810 spectrophotometer. Mixing curves and cleavage rates of polynucleotides were determined with a Shimadzu UV-210A spectrophotometer (Shimadzu, Japan) and the temperature was controlled by a R 40/2 digital thermometer connected with a Pt resistor (MGW Lauda, West Germany). NMR spectra were recorded with a Bruker WP 250 spectrometer. For measurements of ^{31}P nuclei samples were dissolved in 100 mM aqueous EDTA containing 25% D_2O and adjusted to pH 8.0 with triethylamine; δ-values are relative to external phosphoric acid, positive values denoting a downfield shift.

Column chromatography was performed on DE-52 cellulose. Columns were connected with a Uvicord S as detection unit and an UltroRac fraction collector (LKB Instruments, Sweden). The polynucleotides were dialyzed in Visking dialysis tubes, medium pore size 2.4 nm, (Serva, West Germany). Thin layer chromatography (Tlc) was performed on polyethylene imine impregnated cellulose plates, Polygram CEL 300 PEI/UV_{254} (Macherey & Nagel, West Germany), thin layer elektrophoresis (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 as follows: A (1.0 M aqueous triethylammonium bicarbonate); B (0.1 M sodium citrate, pH 6.5); C (1 M LiCl); D (0.04 M aqueous ammonium bicarbonate). The phosphate reagent was prepared according to Chen et al.[8].

Polynucleotide phosphorylase (EC 2.7.7.8, *Micrococcus luteus*) was a product of P-L Biochemicals (Milwaukee, USA). Nuclease S_1 (EC 3.1.30.1, *Aspergillus oryzae*), ribonuclease T_2 (EC 3.1.27.1, *Aspergillus oryzae*, and snake venom phosphodiesterase (EC 3.1.4.1) were purchased from Boehringer Mannheim (Mannheim, West Germany) Poly(C), poly(A), and poly(I) were products of

Sigma Chemical Co. (St. Louis, USA). Poly(c^7I) was prepared according to previously published procedures[9,10].

Determination of hypochromicity. The hypochromicities were calculated by the formula[11-13]: $H = (\varepsilon_{monomer} - \varepsilon_{polymer}) / \varepsilon_{monomer} \times 100$ via phosphate determination or enzymatic cleavage.

a) Phosphate determination: The extinction coefficient of the polymer was determined by ashing 1.0 OD (λ_{max}) of the polynucleotide followed by determination of inorganic phosphate[8]. Values are given in the table.

b) Enzymatic cleavage: The polynucleotides were digested by snake venom phosphodiesterase in TRIS-HCl buffer (0.1 M, pH 8.5). The increase of absorbance was observed at λ_{max}. Hypochromicities were calculated from the absorbances before and after cleavage according to the formula above.

Mixing curves. Ultraviolet spectroscopic mixing curves were performed by the method of continuous variation. Series of mixtures were made with varying ratios of poly(A), poly(c^7I), and poly(C) to poly(ms^2c^7I) and a constant total polynucleotide concentration. The absorbance of each mixture was measured at various wavelengths.

Melting profiles. The melting profiles were measured in Teflon-stoppered quartz cuvettes with 1-cm light path length in a thermostatically controlled cell holder in a Varian SuperScan 3 recording spectrophotometer (Varian, Australia). The increase of absorbance at the appropriate wavelength as a function of time was recorded while the temperature was increased linearly with time at a rate of 18 °C/h using a Lauda temperature programmer and bath.

Hydrolysis by nuclease S_1. The cleavage of polynucleotides 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, 0.29 M NaCl, and 1 mM $ZnSO_4$ 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 hypochromicties determined at 35 °C and in the buffer solution used in the experiments.

Hydrolysis by ribonuclease T_2. For cleavage of poly(I), poly(c^7I), and poly(ms^2c^7I) by ribonuclease T_2 the polymers were dissolved in 0.05 M sodium acetate, pH 4.7, treated with 0.01 units of ribonuclease T_2 at 37 °C and measured as above.

3,7-Dihydro-2-methylthio-7-(β-D-ribofuranosyl)-4*H*-pyrrolo[2,3-d]pyrimidin-4-one-O-5'-monophosphate, Triethylammonium Salt (1b). A total of 80 mg (0.26 mmol) of 3,7-dihydro-2-methylthio-7-(β-D-ribofuranosyl)-4*H*-pyrrolo-[2,3-d]pyrimidin-4-one[14] in 1 ml of triethyl phosphate was treated for 5 h

with 70.0 µl (0.77 mmol) of phosphorus oxychloride at 4 °C. The mixture was hydrolyzed with crushed ice and neutralized with 1 M aqueous triethylammonium bicarbonate (TBK) and the solvent was evaporated in vacuo. The residue was then dissolved in water, applied to a 45 × 3.5 cm ion exchange column (DE-52 cellulose, HCO_3^- form), chromatographed with 1000 ml of water and the product eluted with a linear gradient of 1500 ml 0.6 M TBK / 1500 ml of H_2O at 0.3 M salt concentration. Fractions were pooled yielding 2592 A_{272} units (81%) of amorphous colorless material after lyophilization. - Tlc (PEI-cellulose, C) R_f 0.3; Tle (silica gel, B) R_e 2.0 (+) relative to compound 1a (-1.0); UV (H_2O) λ_{max} 272 nm; phosphate determination: 1 mol phosphate / mol aglycon.

3,7-Dihydro-2-methylthio-7-(β-D-ribofuranosyl)-4*H*-pyrrolo[2,3-d]pyrimidin-4-one-O-5'-diphosphate, Triethylammonium Salt (1c). A total of 2500 A_{272} units (0.2 mmol) of compound 1b in 40 ml of water was applied to a 25 × 2 cm cation exchange column (Merck, type I, pyridinium form) and eluted with 1000 ml of water. The solvent was evaporated and 48 µl (0.2 mmol) of tri-n-butylamine was added. Water was removed by repeated evaporation with dry N,N-dimethyl formamide. The tributylammonium salt was then dissolved in 2 ml of N,N-dimethyl formamide, and the formation of the imidazolidate was accomplished by the addition of 160 mg (1 mmol) 1,1'-carbonyl diimidazole in 4 ml of N,N-dimethyl formamide and stirring for 5 h at room temperature. Excess 1,1'-carbonyl diimidazole was destroyed with 60 µl of methanol. Tri-n-butylammonium phosphate (1 mmol) in 10 ml of N,N-dimethyl formamide was added after 30 min, and the reaction mixture was kept for 1 d at room temperature. After removal of the solvent in high vacuo the residue was dissolved in water and applied onto a 45 × 3.5 cm ion exchange column (DE-52 cellulose, HCO_3^- form). The diphosphate was eluted with a linear gradient of 2000 ml 0.8 M TBK / 2000 ml of water between 0.35 M and 0.4 M. Evaporation of the solvent followed by lyophilization yielded 1900 A_{272} units (76%) of amorphous colorless material.- Tlc (PEI-cellulose, C) R_f 0.2; Tle (silica gel, B) R_e 5.9 (+) relative to compound 1a (-1.0); phosphate determination: 2 mol phosphate / mol aglycon; ^{31}P NMR (H_2O, pH 8.0) δ -7.91 (d, J=22Hz), -10.40 ppm (d, J=22Hz).

Preparation of Poly(2-methylthio-7-deazainosinic acid), Sodium Salt. The reaction mixture contained 360 µl (375 A_{272} units, 30 µmol) of compound 1c, 70 µl of 1 M Tris-HCl (pH 8.3), 70 µl of 0.1 M $MgCl_2$, and 0.8 mg (4 units) of polynucleotide phosphorylase in 200 µl of water. The mixture was incubated for 18 h at 37 °C, diluted with 1 ml of water, cooled to 0 °C, and extracted twice with chloroform/isoamyl alcohol (5:3, v/v) and once with ether. The polymer was dialyzed against 1 l changes of 1 mM EDTA/0.1 M NaCl (pH 7.0),

1 M NaCl and several times against distilled water. The aqueous solution was then lyophylized, yielding 6.17 mg (54%) of the colorless amorphous polynucleotide (data see table).

RESULTS AND DISCUSSION

Enzymatic Synthesis of Poly(ms^2c^7I) with Polynucleotide Phosphorylase. The synthesis of the homopolymer poly(ms^2c^7I) was accomplished by enzymic polymerization of the nucleoside diphosphate 1c by polynucleotide phosphorylase from *M. luteus*[15]. The starting material for polymer synthesis was compound 1a which has been recently prepared from benzyl protected halogenose and the protected nucleobase by a phase-transfer technique[14,16]. From the nucleoside the monophosphate 1b was obtained by phosphorylation with $POCl_3$ in triethyl phosphate following a procedure of Yoshikawa[17]. The conversion of the monophosphate into the final product 1c was accomplished by activation of 1b with 1,1'-carbonyl diimidazole by the method of Hoard and Ott[18]. The structure of the diphosphate 1c was confirmed by phosphate analysis and ^{31}P NMR spectroscopy. Compound 1c exhibited doublets at -7.91 and -10.40 ppm with coupling constants of 22 Hz.

In contrast to poly(c^7I) which has been described by Torrence et al.[9] and Ikehara et al.[10] poly(ms^2c^7I) aggregates strongly in aqueous solution and could not be separated from the monomeric material by gel permeation chromatography. However, separation from monomers was achieved by thorough dialysis. The total yield of polymer was 54% which is similar to that found in polymerization of poly(c^7I) (50%).

UV Spectra, Hypochromicities, and pK Values of Poly(ms^2c^7I), Poly(I), and Analogues. The homopolymer poly(ms^2c^7I) exhibits a UV spectrum with an absorption maximum at 273 nm under neutral conditions (pH 7.0), which is bathochromically shifted with respect to poly(c^7I) and the corresponding purine homopolymers (table). An additional bathochromic shift is observed in the spectrum recorded under alkaline conditions. This shift is almost undetectable in the case of poly(c^7I) and implies that deprotonation of N-3 in the methylthio polymer is much easier than in poly(c^7I). Therefore the pK value of poly(ms^2c^7I) was determined and compared with those of poly(c^7I), the corresponding purine compounds, and the nucleoside constituents (table). In order to get an idea of pk changes between the nucleoside constituents and of the polymers in which they are incorporated, we measured first the pK values of the monomers 1a and 7-deazainosine and compared them with literature values of corresponding purine compounds. As can be seen from the table c^7I shows

Table: Data Determined by UV Measurement of Poly(ms^2c^7I), Poly(c^7I), Poly(ms^2I), and Poly(I)

	poly(ms^2c^7I)	poly(c^7I)	poly(ms^2I) [3]	poly(I)
UV_{max} (nm)				
pH 7.0	271	260	260	248
pH 1.0	---	261	---	258
pH 13.0	279	262	---	253
ε_{max} (pH 7.0)	9300	8500	8700	8400
pK nucleoside	9.5	>11	8.7	8.9
homopolymer	10.5	>12	9.7	10.0
Hypochromicity by phosphate determination (%)	26 (271 nm)	21 (260 nm)	--	27 (248 nm)
Hypochromicity by enzymatic hydrolysis (%)	26 (271 nm)	19 (260 nm)	24 (280 nm)	29 (248 nm)
Cleavage (%) by nuclease S_1 treatment (after 300 min)	7.2	75.0	--	45.5
Cleavage (%) by ribonuclease T_2 treatment (after 300 min)	22.4	97.1	--	56.8
Thermal hypochromicity between 20 °C and 90 °C (Soerensen-buffer, pH 8.5) (%)	15 (271 nm)	0.05 (260 nm)	--	0.04 (248 nm)

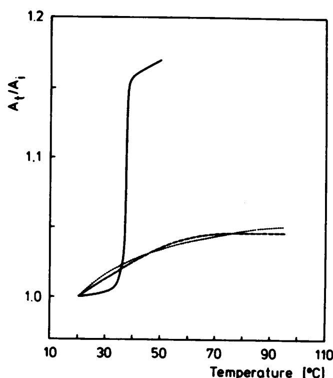


Fig. 1: *UV absorbance-temperature profiles of poly(ms^2c^7I) (271 nm, ——), poly(c^7I) (260 nm, •••), and poly(I) (248 nm, ---) in 0.07 M Soerensen phosphate buffer, pH 8.5, being 1mM in EDTA. A_t/A_i is the ratio of absorbance at a given temperature (t) to that at the initial temperature.*

the highest pK for deprotonation of N-1 (pK >11). The substitution at the C-2 position with a methylthio group decreases the pK value strongly (pK 9.5). Compared to literature values of the corresponding purine compounds this change is more pronounced in the pyrrolo[2,3-d]pyrimidine than in purine nucleosides.

A good indicator of the stability of a polynucleotide structure is its hypochromicity[11-13]. The hypochromicity of poly(ms^2c^7I) was determined by phosphate analysis using the molybdenum blue method according to Chen et al.[8] and by cleavage of the polynucleotide by phosphodiesterase. Both methods resulted a hypochromicity of 26% which is comparable to those of poly(I) (28%) and poly(ms^2I) (24%; ribonuclease M[3]). In comparison, poly(c^7I) showed a significantly lower hypochromicity (20%) indicating a less ordered structure. Thus, the presence of a methylthio group at the C-2 position seems to compensate the loss of stability caused by the lack of N-7.

Thermal Melting, CD Spectra, and Secondary Structure of Poly(ms^2c^7I). Under low salt conditions (0.15 M Na^+/K^+) at pH 7.0 the homopolymer poly(ms^2c^7I) was stable against thermal denaturation up to 99 °C. After increasing the pH to 8.5 - the nucleoside has a pK of 9.5 - cooperative melting of the polynucleotide was observed giving a T_m of 38 °C. Furthermore the thermal hypochromicity of poly(ms^2c^7I) is much higher than that of poly(c^7I) or even poly(I) (table). Neither poly(c^7I) nor poly(I) showed sigmoidal melting under these conditions (fig. 1). Poly(c^7I) did not exhibit cooperativity in

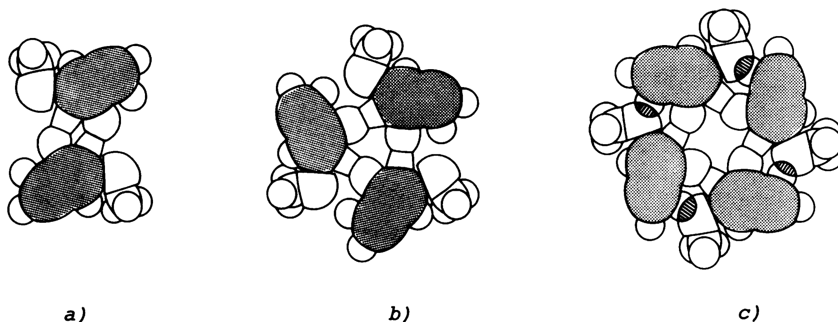


Fig. 2: Space-filling models of possible base arrangements in poly(ms^2c^7I) strands.

thermal denaturation even at high salt. However, the melting of poly(I) becomes cooperative at higher salt concentration (2.0 M Na^+, pH 7.0) indicating the presence of a highly ordered rigid structure now[19].

It is generally accepted that poly(I) forms a fourfold helix with parallel strands and only one hydrogen bond between adjacent bases[20,21]. Considering the melting behaviour of poly(ms^2c^7I) it should also have a very rigid, ordered structure. From CPK model building (fig. 2) it can be clearly shown that a planar base paired arrangement of four ms^2c^7I residues in a tetrahelical fashion is impossible for poly(ms^2c^7I) because of a steric repulsion of the 2-methylthio groups with the neighbouring base (fig. 2c). The highly ordered structure of poly(ms^2c^7I) cannot therefore be due to a fourfold helix as proposed for poly(I). Because of the bulkiness of the methylthio groups the formation of a triple, double, or a strongly stacked single helix is proposed for poly(ms^2c^7I).

From the small lobes in the CD spectra of 7-deazainosine compared to inosine[22] it can be concluded that this alteration is due to the following: Decreased UV absorbance of the 7-deazapurine nucleoside, an altered transition moment due to the lack of N-7, and a less hindered rotation of the nucleobase around the N-glycosylic bond. If c^7I is incorporated into a polynucleotide the molar ellipticity is still low (fig. 3), whereas the hypochromicity indicates (table) that the molecule has some order due to stacking interactions. The differences of the CD spectra of poly(c^7I) compared to poly(I), both under conditions where a regular helix is not formed imply that the overlapping of two neighboured bases (degree of winding) may be also different. The distinct CD spectrum of poly(ms^2c^7I) indicates that this molecule is highly ordered compared to poly(c^7I). Similar observations are reported for poly(ms^2I) and

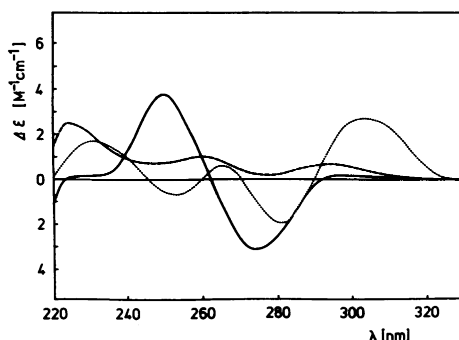


Fig. 3: *CD spectra of poly(ms^2c^7I) (•••), poly(c^7I) (---), and poly(I) (——) in 0.1 M TRIS-HCl, pH 7.5, containing 0.2 M NaCl. Measurements were made in an 1-cm cell at 25 °C and at a nucleoside residue concentration of 100 µM.*

poly(I)[3]. Due to the hydrophobic 2-methylthio groups the nucleobases are strongly stacked and the winding of base pairs generates the CD spectrum depicted in fig. 3. Moreover from enzymatic cleavage by single-strand specific nucleases (figs. 6,7) it is likely that poly(ms^2c^7I) forms a multistranded helix, which could influence the CD spectrum additionally. UV and CD data confirm that a 2-methylthio group in purines as well as in pyrrolo[2,3-d]-pyrimidines stablilizes internucleotide stacking and generates a more ordered helical secondary structure.

Complex Formation of Poly(ms^2c^7I) with Poly(A) and Poly(c^7A). Poly(I) is known to form a double stranded complex with poly(C) and a triple stranded with poly(A) under appropriate environmental conditions[23-26]. This is also true for its 7-deazapurine analogon, poly(c^7I)[10]. However, poly(ms^2c^7I) did not show complex formation with complementary poly(C). The same was found for poly(ms^2I)[3]. Therefore, a methylthio group introduced at the C-2 position of purines or 7-deazapurines hinders Watson-Crick base pairing due to the bulkiness of this group which interferes with the oxygen of the other heterocyclic base.

From the mixing curves (fig. 4) it is evident that poly(ms^2c^7I) is able to form a 2:1 complex with poly(A) and a 1:1 complex with poly(c^7A). In that case the bulky 2-methylthio group has space enough due to the small substituent (-H) at C-2 of the complementary bases. Furthermore, also Hoogsteen base pairing could take place via the N-7 of poly(A)[10,26]. The same situation has been observed with poly(ms^2I).

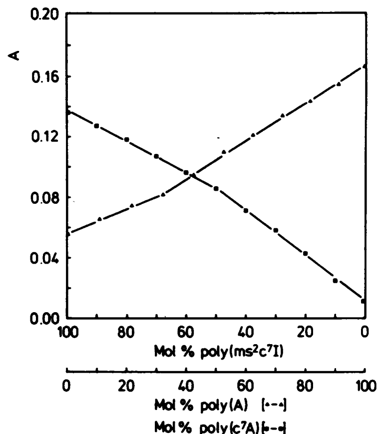


Fig. 4: *Mixing curves of poly(ms^2c^7I) with poly(A) (250 nm) and poly(c^7A) (300 nm) in 0.1 M TRIS-HCl, pH 7.5, containing 0.2 M NaCl and 1 mM $MgCl_2$.*

Information on the strength of the complexes can be obtained from the thermal melting profiles. In TRIS-HCl buffer, pH 7.5, and in the presence of EDTA 2poly(I)•poly(A) showed a monophasic melting[26] with a T_m of 44 °C whereas 2poly(c^7I)•poly(A) was denaturated stepwise showing T_ms of 30 °C and 49 °C (fig. 5a). The stepwise denaturation of the 2poly(c^7I)•poly(A) complex showed that in this case the formation of a double strand is possible although this

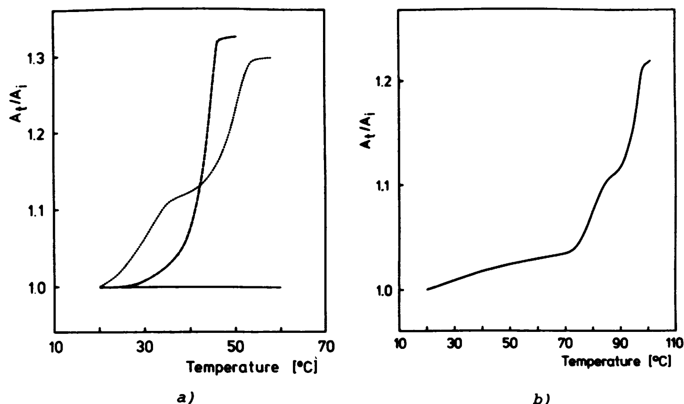


Fig. 5: *Melting profiles of (a) 2poly(I)•poly(A) (254 nm; ---), 2poly(c^7I)• poly(A) (261 nm; •••), and 2poly(ms^2c^7I)•poly(A) (264 nm; ——) in 0.1 M TRIS-HCl, pH 7.5, containing 0.2 M NaCl and 0.1 mM EDTA; (b) 2poly(ms^2c^7I)•poly(A) (264 nm; ——) in 0.1 mM EDTA. A_t/A_i see fig. 1.*

was not observed in the mixing curve.

It has been reported by Ikehara and Hattori that the 2poly(ms^2I)•poly(A) complex is more stable than 2poly(I)•poly(A)[3]. As expected from these results, the complex of 2poly(ms^2c^7I)•poly(A) did not melt at all under the conditions mentioned above (T_m >99 °C; fig. 5a). This clearly demonstrated that the methylthio group stabilizes the Watson-Crick as well as the Hoogsteen base pairs. We suggest that the water molecules are hindered by this bulky hydrophobic moiety to attack the hydrogen bonds of the base pairs and subsequently to solvate the bases. When the denaturation experiments were carried out in the presence of EDTA and in the absence of salt a biphasic melting profile for 2poly(ms^2c^7I)•poly(A) was obtained with T_m values of 78 °C and 94 °C (fig. 5b).

Enzymatic Hydrolysis of Poly(ms^2c^7I) and Related Polynucleotides. A basic requirement for the activity of synthetic polynucleotides in biological systems, e. g. for interferon induction, is their stability against premature cleavage by cellular nucleases[9,28]. The substitution of the common bases by modified ones is a promising approach to prevent the enzymatic hydrolysis of polynucleotides. On the one hand, the recognition of synthetic nucleosides by the enzymes may be reduced or even got be lost, or on the other hand, the structure itself of the new polynucleotide may inhibit digestion. Therefore, we investigated the enzymatic hydrolysis of poly(ms^2c^7I) and its parent analogues poly(c^7I) and poly(I) by nuclease S_1 and ribonuclease T_2.

Nuclease S_1 is an endonuclease specifically recognizing and hydrolyzing the phosphodiester internucleotide bonds of single-stranded nucleic acids and of unpaired regions in double-stranded DNA or RNA such as loops or nicks[29,30]. As we were able to show recently the N-7 in purine bases has nothing to do with their susceptibility to nuclease S_1[5,6]. Quite on the contrary the homopolynucleotides containing 7-deazapurine moieties are hydrolyzed on a much higher rate than their purine containing analogues. Thus, the structure of the nucleic acids gives rise to the pronounced differences in the digestion rates. As expected from the melting profile that indicated a highly ordered structure poly(ms^2c^7I) had a very low susceptibility to nuclease S_1. In contrast, the related compounds poly(c^7I) (fig. 6) and poly(c^7G)[6] which are singly stranded were cleaved very rapidly. The latter result with the polymer containing c^7G, the parent compound of the nucleoside Q, shows that binding of the polynucleotide to the enzyme is not hindered by steric repulsion of a substituent at C-2. Thus, the stability against digestion by the single-strand specific nuclease S_1 had to be caused by aggregation of poly(ms^2c^7I) to a

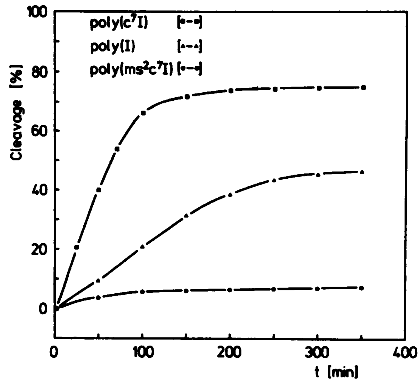


Fig. 6: *Cleavage of the polynucleotides indicated by nuclease S_1 at pH 5.5 at 37 °C. The reaction mixtures were 0.1 mM in nucleotide residue, 0.29 M in NaCl, 1 mM in $ZnSO_4$, 0.03 M in sodium acetate, and contained 2000 units of the enzyme.*

multistranded structure.

We also investigated the interaction of polynucleotides with ribonuclease T_2. This enzvme is said to split all phosphodiester bonds in RNA[31,32]. The purine N-7 is not necessary for the activity of this enzyme (fig. 7) and similar structure dependence as for nuclease S_1 is reported for this enzyme[31,33]. As fig. 7 demonstrates the cleavage rate of poly(ms^2c^7I) by ribonuclease T_2 was also very low as found similarly for nuclease S_1. Thus, both digestion

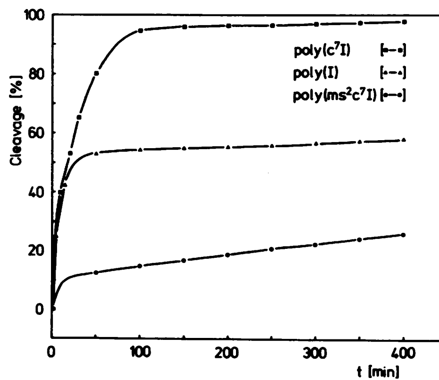


Fig. 7: *Cleavage of the polynucleotides indicated by ribonuclease T_2 at pH 4.7 at 37 °C. The reaction mixtures were 0.1 mM in nucleotide residue, 0.05 M in sodium acetate, and contained 0.01 units of the enzyme.*

experiments imply that poly(ms^2c^7I) forms a multistranded, presumably a double- or triple-stranded, structure.

SUMMARY

In conclusion our results show that a methylthio group located at position C-2 of a 7-deazapurine polynucleotide as poly(ms^2c^7I) stabilizes the secondary structure of the polynucleotide. The hydrophobicity of the methylthio group implies that this stabilization is mainly caused by hydrophobic forces. Due to steric repulsion a Watson-Crick complex between poly(ms^2c^7I) and poly(C) is not formed. However, poly(A) forms Watson-Crick and Hoogsteen complexes with poly(ms^2c^7I) which are again stabilized by the hydrophobicity of the methylthio group.

ACKNOWLEDGEMENT

We are indebted to Dr. B. V. L. Potter for critically reading the manuscript and we gratefully acknowledge the financial support by the Deutsche Forschungsgemeinschaft.

*To whom correspondence should be addressed

REFERENCES

1. McCloskey, J. A. and Nishimura, S. (1977) Acc. Chem. Res., 10, 403-410.
2. Harada, F., Gross, H. J., Kimura, F., Chang, S. H., Nishimura, S., and RajBhandary, U. L. (1968) Biochem. Biophys. Res. Commun., 33, 299-306.
3. Ikehara, M. and Hattori, M. (1972) Biochim. Biophys. Acta, 269, 27-36.
4. Fukui, T. and Ikehara, M. (1979) Biochim. Biophys. Acta, 562, 527-533.
5. Seela, F., Ott, J., and Franzen, D. (1982) Nucleic Acids Res., 10, 1389-1397.
6. Seela, F., Tran-Thi, Q.-H., and Franzen, D. (1982) Biochemistry, 21, 4338-4343.
7. Seela, F. and Bussmann, W. (1982) Nucleosides & Nucleotides, 1, 253-261.
8. Chen, Jr., P. S., Toribara, T. Y., and Warner, H. (1956) Anal. Chem., 28, 1756-1758.
9. Torrence, P. F., De Clercq, E., Waters, J. A., and Witkop, B. (1974) Biochemistry, 13, 4400-4408.
10. Ikehara, M., Fukui, T., Koide, T., and Inaba, J. (1974) Nucleic Acids Res., 1, 53-61.
11. Bush, A. C. (1974) in Basic Principles in Nucleic Acid Chemistry, Ts'o, P. O. P., Ed., pp. 91-169, Academic Press, New York.
12. Bloomfield, V. A., Crothers, D. M., and Tinoco, I., Jr. (1974) Physical Chemistry of Nucleic Acids, Harper & Row, New York.
13. Cantor, C. R. and Schimmel, P. R. (1980) Biophysical Chemistry, Freeman, San Francisco.
14. Seela, F. and Menkhoff, S. (1982) Liebigs Ann. Chem., 813-816.
15. Godefroy-Colburn, T. and Grunberg-Manago, M. (1972) in The Enzymes, 3rd ed., Boyer, P. D., Ed., Vol. 7, pp. 533-574, Academic Press, New York.

16. Seela, F. and Hasselmann, D. (1980) Chem. Ber., 113, 3389-3393.
17. Yoshikawa, M., Kato, T., and Takenishi, T. (1967) Tetrahedron Lett., 5065-5068.
18. Hoard, D. E. and Ott, D. G. (1965) J. Am. Chem. Soc., 87, 1785-1788.
19. Thiele, D. and Guschlbauer, W. (1973) Biophysik, 9, 261-271.
20. Chou, C. H., Thomas Jr., G. J., Arnott, S., and Smith, P. J. C. (1977) Nucleic Acid Res., 4, 2407-2419.
21. Howard. F. B. and Miles, H. T. (1982) Biochemistry, 21, 6736-6745.
22. Seela, F. and Hasselmann, D. unpublished results.
23. Davies, D. R. and Rich, A. (1958) J. Am. Chem. Soc., 80, 1003-1004.
24. Rich, A. (1958) Nature (London), 181, 521-525.
25. Arnott, S. and Bond, P. J. (1973) Science, 181, 68-69.
26. Howard, F. B. and Miles, H. T. (1977) Biochemistry, 16, 4647-4650.
27. Torrence, P. F. and Witkop, B. (1975) Biochim. Biophys. Acta, 395, 56-66.
28. Carter, W. A., Pitha, P. M., Marshall, L. W., Tazawa, I., Tazawa, S., and Ts'o, P. O. P. (1972) J. Mol. Biol., 70, 567-587.
29. Potter, B. V. L., Romaniuk, P. J., and Eckstein, F. (1983) J. Biol. Chem. 258, 1758-1760.
30. Shishido, K. and Ando, T. (1982) in Nucleases, Linn, S. M. and Roberts, R. J., Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, pp. 155-185.
31. Uchida, T. and Egami, F. (1967) J. Biochem. (Tokyo), 61,44-53.
32. Uchida, T. and Egami, F. (1971) in The Enzymes, 3rd ed., Boyer, P. D., Ed., Vol. 4, Academic Press, New York, pp. 205-250.
33. Hashimoto, J., Uchida, T., and Egami, F. (1970) Biochim. Biophys. Acta, 199, 535-536.