THE CONFORMATION OF 4-THIOURIDINE-5'-PHOSPHATE IN SINGLE AND DOUBLE STRANDED POLYNUCLEOTIDES

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1. Introduction

In the last two years some work was published concerning the physical, chemical and biological properties of polynucleotides and *E. coli* tRNA containing s^4UMP * [1–11]. There is still remaining hope that any of the properties of s^4UMP would explain its occurrence in tRNA. In this paper are described first attempts to determine the conformation of s^4UMP in single and double stranded polynucleotides. For the investigation of this conformational problem two techniques were combined: NMR-spectroscopy and ORD.

2. Materials and methods

The preparation and characterisation of s^4UMP [5], s^4Ups^{4}U [4], poly (U,s^4U) [8] and [poly(U,s^4U)] [poly A] [8] have been reported elsewhere. NMR-spectra were recorded on a Varian HA 100 instrument using D_2O containing *t*-butanol (1%, v/v) as solvent. Chemical shifts have been measured in cycles per second (cps) downfield from *t*-butanol with an accuracy of ± 2 cps relying on chart calibration. ORD spectra were recorded on a Cary 60 spectropolarimeter at low scan speed. The measurements were done at 25° in 0.1 M phosphate buffer pH 7.4. The cell path length was 1 cm and the optical density at λ_{max} of the samples was in the range of 0.30–0.80. The rotation is given by $[m] = \alpha/[s^4\mathrm{UMP}]$, where α is the rotation per centimeter and [s^4UMP] is given in Moles/l.

3. Results and discussion

Fig. 1 shows the NMR-spectra of s^4Urd and s^4UMP. It is clearly seen from this representation that the 5'-phosphoryl group in s^4UMP causes a downfield shift of the H_6-signal compared to the H_6-signal in s^4Urd. According to recent NMR studies [12] this means that s^4UMP in solution exhibits anti-conformation, since only in that case the phosphate residue is able to influence the H_6-atom. In table 1 the chemical shifts of the proton signals in question are listed. The differences of corresponding signals between s^4Urd and s^4UMP are significant. It is interesting to notice that no concentration dependence of the resonance signals neither in s^4Urd nor in s^4UMP could be observed.

The ORD-spectrum of s^4UMP displays a negative Cotton effect in the region of the UV-absorption maximum (330 mμ). The negative Cotton effect at 320 mμ seems to indicate anti conformation of s^4UMP since from NMR-spectroscopy it followed that s^4UMP exists in solution in anti-conformation. s^4Ups^{4}U and poly ($U_{2,5}$,s^4U) show likewise ORD-spectra with negative Cotton effects. The amplitude of the Cotton effect is much greater in s^4Ups^{4}U where two 4-thiouracil residues are adjacent to each other as compared to poly ($U_{2,5}$, s^4U) in which 4-thiouracil has uracil as next neighbours. The sign of

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* Abbreviations: s^4UMP, 4-thiouridine-5'-phosphate; s^4Urd, 4-thiouridine; s^4Ups^{4}U, 4-thiouridylyl-(3'5')-4-thiouridine; poly (U,s^4U), copolymer consisting of UMP and s^4UMP.

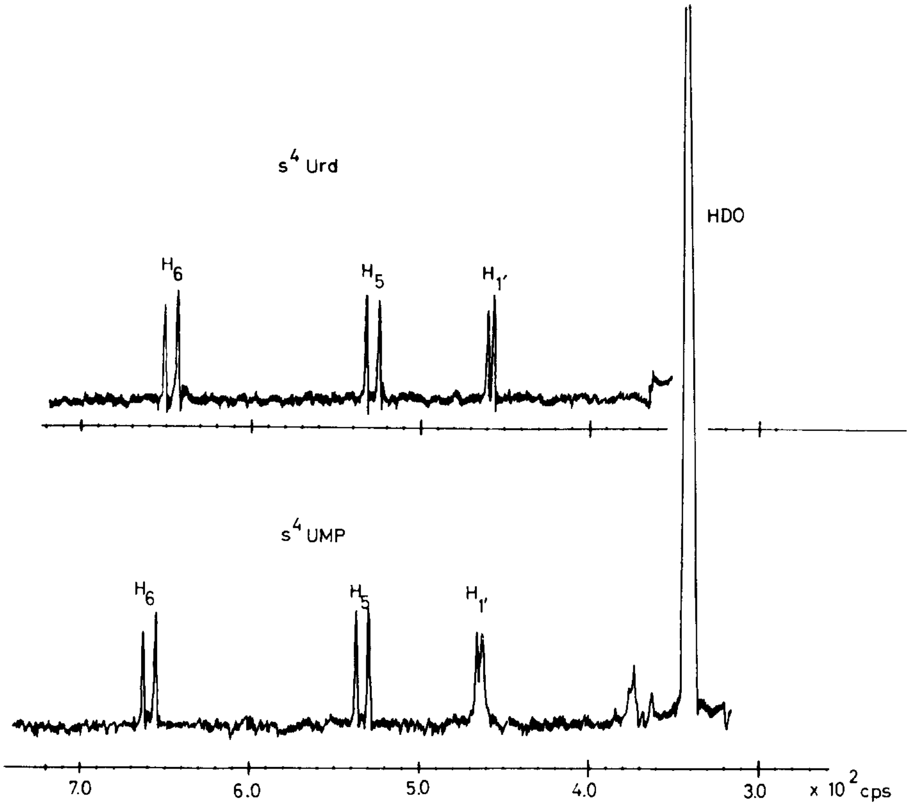

Fig. 1. HA 100 NMR-spectra of s^4Urd and s^4UMP. Details are described under materials and methods.

the Cotton effect obviously indicates that s^4UMP in oligonucleotides exists in anti conformation. The ORD curve of [poly ($U_{2,5}$, s^4U)] [poly A] turned out to be completely different exhibiting a positive Cotton effect in the region 370–335 mμ. This behaviour was also observed with [poly (U_5, s^4U)] [poly A] and [poly (U_8, s^4U)] [poly A]. It is known from a recent X-ray diffraction study on crystalline s^4Urd that this nucleoside has syn conformation with a torsion angle ϕ_{CN} = +83° [13]. Our experiments presented above, however, indicate the anti conformation (although we have not been able to estimate the torsion angle ϕ_{CN}) of s^4UMP in oligo- and polynucleotides in solution. The positive sign of the principal Cotton effect in the

Table 1
Chemical shifts of s^4Urd and s^4UMP protons in cps downfield from *t*-butanol.

	H_6	H_5	H_1	Concentration (Mol/l)
s^4Urd	651; 643	532; 524	460; 457	0.1
	652; 645	532; 524	461; 458	0.3
s^4UMP	664; 657	538; 531	467; 563	0.1
	662; 656	538; 530	467; 463	0.3
[s^4UMP-s^4URD]	13; 14	6; 7	7; 6	0.1

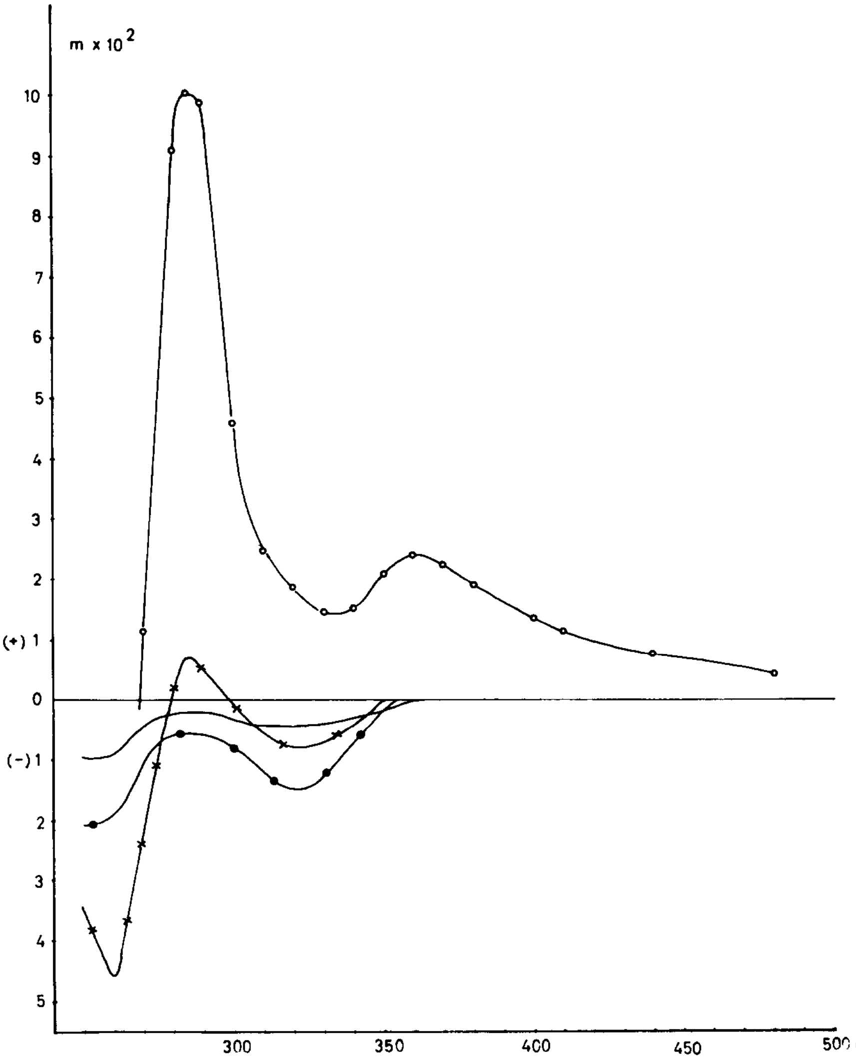

Fig. 2. ORD-spectra of s^4UMP (———), poly ($U_{2,5}$, s^4U) (x–x–x), s^4Ups^{4}U (●–●–●) and [poly ($U_{2,5}$, s^4U)] [poly A] (o–o–o). Details are described under materials and methods.

ORD-curve of [poly (U, s^4U)] [poly A] demonstrates that the conformation of s^4UMP has to be different from that of single stranded polynucleotides. The crystal structure of s^4Urd, with its unusual arrangement of syn s^4Urd molecules in form of somewhat displaced stacks forced us to assume that s^4UMP in double stranded structures prefers a similar, syn conformation. This assumption gains further support from recent ORD studies of nucleotides and nucleotides where several authors could show that a change in the sign of a Cotton effect corresponds to a conformational change [14–16].

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References

[1] M.N.Lipsett, J. Biol. Chem. 240 (1965) 3975.

[2] M.N.Lipsett and B.P.Doctor, J. Biol. Chem. 242 (1967) 737.

[3] M.N.Lipsett, J.S.Norton and A.Peterkofsky, Biochemistry 6 (1967) 855.

[4] K.H.Scheit, Biochim. Biophys. Acta 166 (1968) 285.

[5] K.H.Scheit, Chem. Ber. 101 (1968) 1141.

[6] K.H.Scheit, Angew. Chem. Intern. Ed. 6 (1967) 180.

[7] K.H.Scheit, Tetrahedron Letters (1967) 113.

[8] K.H.Scheit and E.Gaertner, Biochim. Biophys. Acta, in press.

[9] M.Uziel, Biochim. Biophys. Res. Commun. 25 (1966) 105.

[10] P.Cerutti, J.W.Holt and N.Miller, J. Mol. Biol. 34 (1968) 505.

[11] M.Pleiss, H.Ochiai and P.A.Cerutti, Biochim. Biophys. Res. Commun. 34 (1969) 70.

[12] M.P.Schweizer, H.P.Brown, P.O.P.Ts'o and S.P.Hollis, J. Am. Chem. Soc. 90 (1968) 1042.

[13] W.Saenger and K.H.Scheit, Angew. Chem. 81 (1969).

[14] T.R.Emerson, R.J.Swan and T.L.V.Ulbricht, Biochemistry 6 (1967) 843.

[15] W.A.Klee and S.H.Mudd, Biochemistry 6 (1967) 988.

[16] W.Guschlbauer and Y.Courtois, FEBS Letters 1 (1968) 183.