POLY 2′-DEOXY-2′-CHLOROURIDYLIC AND -CYTIDYLIC ACIDS

J. HOBBS, H. STERNBACH and F. ECKSTEIN
Max-Planck-Institut für experimentelle Medizin, Abteilung Chemie, Göttingen, Germany

Received 30 April 1971

1. Introduction

A large number of polynucleotides with modified purine and pyrimidine bases has been prepared and the influence of such modifications on the formation and stability of double helices has been studied [1].

Although it is well documented that the thermal stability of double helices is also determined by the nature of the sugar residue of the nucleotide [2], so far very few studies have been undertaken to examine this effect by modification of the sugar. The properties of poly 2′-*O*-methyladenylic acid were investigated by Bobst et al. [3] and those of poly 2′-*O*-methylcytidylic and poly 2′-*O*-methyluridylic acids respectively by Zmudzka et al. [4, 5]. In an attempt to widen our understanding of the factors determining thermal stability of double helices we have investigated some properties of poly 2′-deoxy-2′-chlorouridylic acid and poly 2′-deoxy-2′-chlorocytidylic acid.

2. Materials and methods

2′-Deoxy-2′-chlorouridine and 2′-deoxy-2′-chlorocytidine were prepared following the method of Fox et al. [6] with slight modifications. Phosphorylation was carried out with $POCl_3$ in triethyl phosphate [7], and synthesis of the diphosphates via activation of the nucleoside 5′-phosphates with carbonyl diimidazole [8]. Polynucleotide phosphorylase from *Micrococcus lysodeikticus* (specific activity 30 U/mg), poly rA, poly rI and GpU as primer were purchased from Boehringer, Mannheim (Germany). Mn^{2+} was used as cation for the polymerisations [9]. A typical polymerisation experiment consisted of 100 mM tris-HCl pH 8.5, 5 mM substrate, 8 mM $MnCl_2$, 0.05 mM GpU and 45 units of enzyme/ml incubation solution. Polymers were isolated by deproteinisation with chloroform–isoamyl alcohol (5:2, v/v) and gel filtration over Sephadex G200. Details of the chemical synthesis of the substrates as well as of the preparation of the polymers will be described in more detail shortly.

UV absorption temperature profiles were recorded in a Gilford Mod. 2000 recorder connected with a Beckman Mod. DUR spectrophotometer. Unless stated otherwise all melting temperature profiles were taken in sodium citrate buffer pH 7.5 (0.1 M Na^+) [10]. Sedimentation coefficients were determined by analytical ultracentrifugation in a Spinco Model E ultracentrifuge equipped with UV absorption optics in sodium citrate buffer pH 7.5 (0.05 M Na^+). Spectrophotometric titrations were performed in a Zeiss PMQII spectrophotometer using a flow cell and an Orion Research digital pH-meter (model 201).

3. Results

The yield of polymer in 1 ml incubation experiments was 2.7 A_{260nm} units of poly 2′-deoxy-2′chlorouridylic acid (poly Ucl) ($s_{20,w}$ = 7.1 S) and 7.0 A_{269nm} units of poly 2′-deoxy-2′-chlorocytidylic acid (poly Ccl) ($s_{20,w}$ = 10.5 S).

Upon annealing poly Ucl with poly rA a double stranded polymer, poly rA–poly Ucl, with $T_m = 56°$ (fig. 1) was formed. Poly Ucl alone showed no hypochromicity upon heating from 25° to 75° in sodium citrate buffer, or from 4° to 50° in 0.1 M Mg^{2+} and 0.1 M Na^+ where poly rU had $T_m = 6°$.

Poly Ccl formed a double stranded polymer with poly rI, poly rI–poly Ccl, with $T_m = 65°$ (fig. 2).

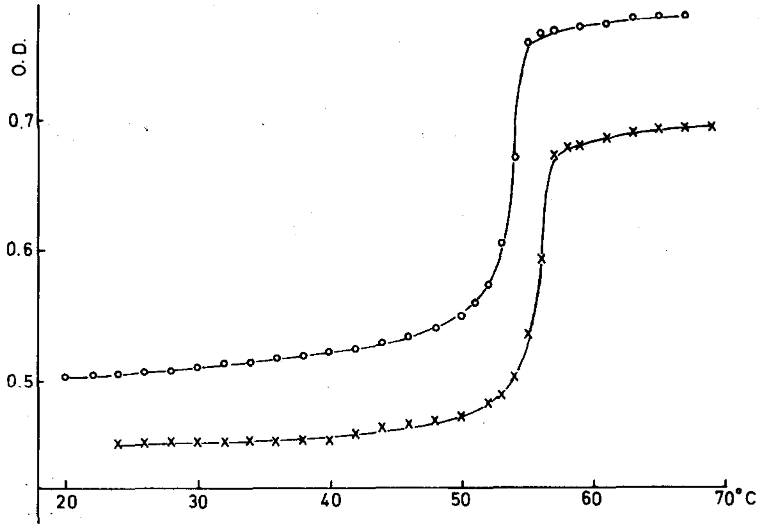

Fig. 1. UV absorption temperature profile of poly rA–poly rU (o——o) and poly rA–poly Ucl (x——x) at 260 nm in sodium citrate buffer pH 7.5 (0.1 M Na^+).

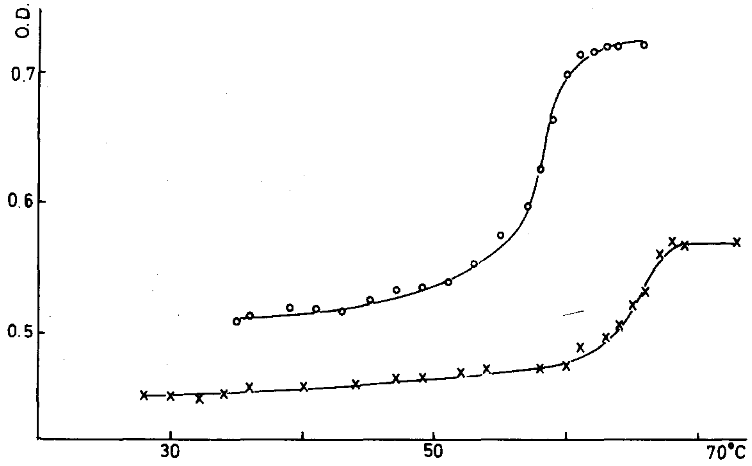

Fig. 2. UV absorption temperature profile of poly rI–poly rC (o——o) and poly rI–poly Ccl (x——x) at 252 nm in sodium citrate buffer pH 7.5 (0.1 M Na^+).

Poly Ccl has a $pK_a = 5.5$ similar to poly rC ($pK_a = 5.7$) [11]. At pH 5.0 poly Ccl formed a twin stranded structure which melted at 67°. Poly Ccl showed a linear increase in absorbance with increasing temperature in sodium citrate buffer pH 7.5 (0.1 M Na^+) very much like poly rC [11].

4. Discussion

Polynucleotide phosphorylase is known to accept 2′-deoxy- as well as 2′-*O*-methylribonucleoside 5′-diphosphates as substrates in the presence of Mn^{2+} [4, 9, 12]. As this report shows 2′-deoxy-2′-chlorouridine and -cytidine 5′-diphosphates can also be polymerized under such conditions.

Table 1
Thermal stability of polymers involving poly rU and its analogs.

	T_m (°C)			T_m (°C)	
	Sodium citrate pH 7.5 (0.1 M Na^+) + 0.1 M Mg^{2+}	0.1 M Mg^{2+} [5]		Sodium citrate pH 7.5 (0.1 M Na^+)	0.1 M Na^+ 0.01 M phosphate buffer pH 7.5 [5]
Poly rU	6	8[b]	Poly rA–poly rU	54	57
Poly Um	–	27[b]	Poly rA–poly Um	–	68.5
Poly dU	–	ns[a]	Poly rA–poly dU	–	54
Poly Ucl	ns[a]	–	Poly rA–poly Ucl	56	–

[a] ns = no structure discernible under these conditions between 4° and 50°.
[b] Values taken from fig. 1 in [5].

Under identical conditions neither 2′-ara UDP nor 2′-ara CDP, which could possibly have been formed from the 2′-chloro compounds by hydrolysis during the incubation period, could be polymerized.

The thermal stabilities of structures involving poly rU and its analogs are summarized in table 1.

Since neither poly dU nor poly Ucl form helical structures under these conditions, in contrast to poly rU and poly Um, it seems that formation of such a structure requires the presence, not necessarily of a hydroxyl group, but at least of an oxygen in the 2′-position. Whether this stabilisation is caused by conformational or solvation effects cannot be decided at the present time with certainty. Further information concerning the influence of chlorine in the 2′-position on the conformation of the ribose should become available from the three-dimensional structure of 2′-deoxy-2′-chlorouridine, which is under investigation [13].

Three of the four double stranded polymers formed with the complementary polynucleotide poly rA (table 1), as well as poly Am–poly rU [3] have their thermal transitions around 55°, but poly rA–poly Um is considerably more stable. In this series the contribution of the 2′-substituent to the thermal stability of the polymers is less pronounced than in the poly rI–poly rC series.

A comparison of the thermal stabilities of the double stranded polymers formed between poly rC and its analogs and poly rI (table 2) reveals no easy pattern by which the 2′-substituent influences this stability.

Table 2
Thermal stability of double-stranded polymers of poly rI and poly rC and its analogs.

	T_m (°C)	
	Sodium citrate pH 7.5 (0.1 M Na^+)	0.05 M phosphate buffer pH 7.8 0.01 M Na^+ [4]
Poly rI–poly rC	57	52
Poly rI–poly Cm	–	43
Poly rI–poly dC	52 [2]	35 [2]
Poly rI–poly Ccl	65	–

Table 3
pK_a values and thermal stabilities of twin-stranded structures of poly rC and analogs.

	pK_a	T_m (°C) sodium citrate pH 5.0 (0.1 M Na^+)
Poly rC	5.5	66
Poly Cm	4.6	–
Poly dC	7.4 [14]	>90 [14]
Poly Ccl	5.7	67

In contrast to those for the corresponding nucleosides the pK_a values for poly rC and its analogs vary widely (table 3). They seem to decrease with the bulkiness of the 2′-substituent. Oxygen and chlorine have similar van der Waals radii and the pK_a values for poly rC and poly Ccl are similar. The T_m values for such twin-stranded structures are dependent on the pH [11] and it is therefore difficult to compare thermal stabilities of polymers with different pK_a values. However, poly rC and poly Ccl with similar pK_a have similar T_m values (table 3).

From the data presented it is quite clear that more information is needed to evaluate the effects of different substitutions in the 2′-position on the thermal stability. Studies along these lines as well as on the stability of such polymers against nucleases are under way.

Acknowledgements

Sedimentation coefficients were kindly determined by Dr. E. Gottschalk (Mrs.) and Miss E. Kopp. We thank Dr. K.H. Scheit for valuable discussions. This work was partially supported by the Deutsche Forschungsgemeinschaft. One of us (J.H.) thanks the Royal Society, London, for a European Programme award.

References

[1] F. Eckstein and K.H. Scheit, in: Procedures in nucleic acid research, Vol. II, eds. G.L. Cantoni and D.R. Davies (Harper and Row, New York and London, 1971) in press, and references cited therein.
[2] M.J. Chamberlin and D.L. Patterson, J. Mol. Biol. 12 (1965) 410.
[3] A.M. Bobst, F. Rottman and P.A. Cerutti, J. Mol. Biol. 46 (1969) 221.
[4] B. Zmudzka, C. Janion and D. Shugar, Biochem. Biophys. Res. Commun. 37 (1969) 895.
[5] B. Zmudzka and D. Shugar, FEBS Letters 8 (1970) 52.
[6] I.L. Doerr and J.J. Fox, J. Org. Chem. 32 (1967) 1462.
[7] M. Yoshikawa, T. Kato and T. Takeneishi, Tetrahedron Letters (1967) 5065.
[8] D.E. Hoard and D.G. Ott, J. Am. Chem. Soc. 87 (1965) 1785.
[9] F. Rottman and K. Henlein, Biochemistry 7 (1968) 263.
[10] M. Chamberlin, R.L. Baldwin and P. Berg, J. Mol. Biol. 7 (1963) 334.
[11] K.H. Hartman and A. Rich, J. Am. Chem. Soc. 87 (1965) 2033.
[12] J.Y. Chou and M.F. Singer, Biochem. Biophys. Res. Commun. 42 (1971) 306.
[13] J. Hobbs and W. Saenger, in preparation.
[14] R.B. Inman, J. Mol. Biol. 9 (1964) 624.