ANTIVIRAL ACTIVITY OF POLYNUCLEOTIDES: ROLE OF THE 2'-HYDROXYL AND A PYRIMIDINE 5-METHYL

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Received 7 April 1972

1. Introduction

Studies with a variety of synthetic double-stranded polynucleotides have shown that induction of interferon and resistance to viral infection depends on a) a sufficiently high molecular weight [1–3], b) thermal stability (T_m) [4], c) resistance to nuclease degradation [5, 6] and d) the presence of ribose as the carbohydrate moiety [7, 8], testified to by the relative inactivity of double-stranded DNA and RNA–DNA hybrids as compared to double-stranded RNA [7–9]. Studies on the antiviral activity of 2'-*O*-methylated and 5-methylated polynucleotides are presented in this report. These studies were aimed at delineating the effects of methylation of the heterocyclic ring and the ribose 2'-OH, and of the associated changes in thermal stability, on antiviral activity.

2. Materials and methods

Poly (rI), S_{20} 9.1 and poly (rC), S_{20} ~ 6.0 were products of P.-L. Biochemicals (Milwaukee, Wisc., USA); poly (rA), S_{20} 9.2 and poly (rU), S_{20} 3.5 were obtained from Miles Laboratories (Elkhart, Indiana); and poly (dC), S_{20} ~ 6.0 from Biopolymers (Pinebrook, New Jersey). The remaining polymers were synthesized according to published procedures: poly (2'-OMeC)‡, S_{20} 9.0 [10]; poly (2'-OMeU), S_{20} 9.0 [11]; poly (5-MerC), S_{20} 3.4 [12]; poly (5-MedC), S_{20} 7.6 [13]. Sedimentation constants, and melting profiles and T_m values (see also fig. 1) were obtained as described elsewhere [13].

Antiviral activity of polynucleotides was assessed by measurement of VSV plaque inhibition in PRK cell cultures in 50-mm Falcon plastic Petri dishes. Serial (1 to 10) dilutions of the polymers were prepared in MEM (minimal Eagle's medium) and incubated on the cells (4 ml/Petri dish) for 20 hr at 37°. The supernatant was then removed and the cells challenged with VSV. Virus plaques were counted 2 days later. Since previous trials demonstrated that the full activity of poly (rI)–poly (rC) may be attained, and even surpassed, when the homopolymer components are added sequentially [17, 18], experiments were conducted both with preformed complexes and by sequential administration of the two somponents. Complexes were tested at concentrations from 10^{-5} to 10 μg/ml. When tested sequentially, the first polynucleotide was applied to the cells at 10 μg/ml for 1 hr; the cells were then washed 3 times and further

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‡ Abbreviations: Poly (2'-OMeC), poly 2'-O-methylcytidylic acid; poly (2'-OMeU), poly 2'-O-methyluridylic acid; poly (5-MerC), poly 5-methylribocytidylic acid; poly (5-MedC), poly 5-methyldeoxycytidylic acid; VSV, vesicular stomatitis virus; PRK, primary rabbit kidney.

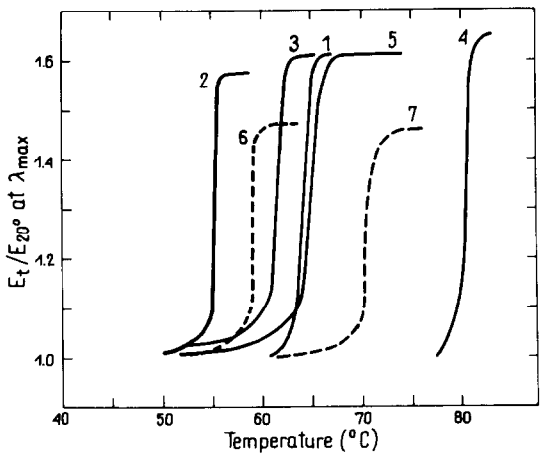

Fig. 1. Melting profiles, in 0.15 M NaCl at pH 7.8, of complexes of poly (rI) (——) with: 1) poly (rC), T_m 64° [12], 2) poly (dC), T_m 55° [14], 3) poly (2′-OMeC), T_m 61.5° [15], 4) poly (5-MerC), T_m 80.5° [12], 5) poly (5-MedC), T_m 65°; and of complexes of poly (rA) (- - - -) with: 6) poly (rU), T_m 59° [11, 16], 7) poly (2′-OMeU), T_m 70.5 [11]. Melting profiles 2), 6) and 7) have been recalculated from the original papers for a salt concentration of 0.15 M.

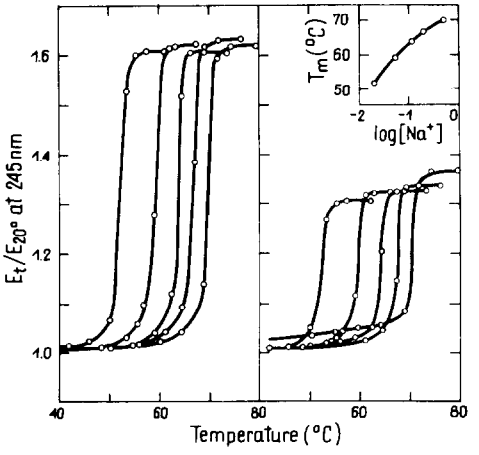

Fig. 2. Melting profiles, in 0.01 M phosphate buffer pH 7, 8, for mixtures of poly (rI) and poly (5-MedC) in molar ratios of 1 : 1 (left) and 2 : 1 (right). Individual profiles in each figure are for NaCl concentrations of 0.02, 0.05, 0.11, 0.20 and 0.50 M (from left to right). *Insert* shows dependence of T_m on Na^+ concentration for poly (rI)–poly (5-MedC).

incubated with concentrations of 10^{-5} to 10 μg/ml of the second polymer prior to virus challenge.

3. Results

It was previously shown [10–12] that, under the conditions herein employed for antiviral activity tests, double-stranded complexes are formed by poly (2′-OMeC) or poly (5-MerC) with poly (rI), and by poly (2′-OMeU) with poly (rA). The melting profiles for these complexes in 0.15 M NaCl are shown in fig. 1.

To establish the nature of the complex between poly (rI) and poly (5-MedC), 1 : 1 and 2 : 1 mixtures were prepared in various NaCl concentrations from 0.02 to 0.5 M. Observed hyperchromicities during melting were 62% and 32%, respectively (fig. 2), testifying [13] to formation of the double-stranded hybrid poly (rI)–poly (5-MedC), irrespective of the ratio of the two components. In 2 : 1 mixtures of poly (rI) and poly (5-MedC), the lower hyperchromicity during melting is due to the fact that 50% of the poly (rI) component is free. At higher salt concentrations (0.5 M), the free poly (rI) forms its own helical structure, the melting of which is responsible for a small increase in absorption up to 60°, as well as for an appreciable change in profile in 0.5 M NaCl (fig. 2, right-hand diagram). The insert to fig. 2 exhibits the T_m dependence of poly (rI)–poly (5-MedC) on salt concentration.

Antiviral activities are presented in table 1; the data obtained with poly (rI)–poly (rC), poly (rA)–poly (rU) and poly (rI)–poly (dC) are in accord with data reported in earlier studies [7, 8, 17–19].

It will be noted that methylation of the 2′-OH in poly (rC) and poly (rU) led to a drastic decrease in activity of the respective complexes with poly (rI) and poly (rA). Analogous results were obtained when the homopolymers were adminstered sequentially.

In contrast, replacement of poly (rC) by poly (5-MerC) did not markedly affect the activity of the complex with poly (rI). Sequential administration in the order poly (rI), poly (5-MerC) also yielded the same activity as the preformed complex, whereas administration in the reverse order led to considerably lower activity, a finding only in qualitative agreement with results for sequential adminstration of poly (rI) and poly (rC) [17]. This failure of poly (5-MerC) to effectively "prime" the cells for antiviral activity of poly (rI) may conceivably be due to the lower molecular weight of the former (S_{20} 3.4) as compared to

Table 1
Antiviral activity of different polynucleotide complexes in PRK cell cultures.

Polynucleotide no. 1	Polynucleotide no. 2	Minimum inhibitory concentration* (μg/ml)		
		Poly no. 1 complexed to poly no. 2	Poly no. 1, then poly no. 2 in 1 hr	Poly no. 2, then poly no. 1 in 1 hr
Poly rI	Poly rC	0.0004	0.00004	0.0004
Poly rA	Poly rU	0.04	> 10	> 10
Poly rI	Poly 2'-O-MeC	4	> 10	4–10
Poly rA	Poly 2'-O-MeU	> 10	> 10	> 10
Poly rI	Poly 5-MerC	0.001	0.0004	1
Poly rI	Poly dC	⩾ 10	⩾ 10	> 10
Poly rI	Poly 5-MedC	> 10	> 10	10

* Concentration of polymer (either the polynucleotide complex, or polynucleotide no. 2, if polynucleotide no. 2 is added second, or polynucleotide no. 1, if polynucleotide no. 1 is added second) required to reduce VSV plaque formation by 50%.

poly (rC) (S_{20} ~ 6); it should be recalled that a minimum S_{20} value of 3.2 established as necessary for maximum antivital activity applies only to preformed complexes [1–3].

Finally, it will be observed that neither poly (rI)–poly (dC), nor poly (rI)–poly (5-MedC), exhibited significant activity even at 10 μg/ml. Only slight activity was noted on administration of poly (5-MedC), followed by poly (rI), but not when they were given in the reverse order (table 1).

4. Discussion

The inactivity of poly (dC) as compared to poly (rC), and of poly (5-MedC) relative to poly (5-MerC), could be due to differences in conformation between the ribose and deoxyribose moieties, or to the presence of 2'-OH in the ribose residues. The negligible activities of poly (2'-OMeC) and poly (2'-OMeU) are rather surprising since their complexes with poly (rI) and poly (rA), respectively, exhibit physico-chemical properties similar to those of the correspondence ribo, but not deoxyribo, polymers [15, 16, 20]. The 2'-*O*-methyl complexes fulfil the general requirements [1–6] for antiviral activity and interferon induction, viz. a) sufficiently high molecular weight (cf. Materials and methods); b) high thermal stability (figs. 1 and 2); c) absolute resistance to pancreatic ribonuclease and low susceptibility to other nucleases [10, 11]. It therefore appears that the presence of the 2'-OH is a prerequisite for interferon induction activity, an inference further supported by the loss of activity of poly (rC) on replacement by poly (2'-O-acetyl C) [21] or by poly (2'-deoxy-2'-chloro C) [22, 23]. Furthermore, although poly (rI)–poly (rC) resembles poly (rI)–poly (dC) physico-chemically, as shown by similarity of X-ray diffraction patterns [24] and lack of formation of triple-stranded complexes with poly (rI) [14], they differ considerably in antiviral activity (table 1 and [7, 8]). Finally, it may not be without relevance that both poly (2'-OMeC) and poly (2'-OMeU) are inactive as in vitro messengers [25].

It is of additional interest that, notwithstanding the higher thermostability (16.5°) of poly (rI)–poly (5-MerC) relative to poly (rI)–poly (rC) (fig. 1), (although both complexes exhibit similar conformations [12]), the former does not exhibit an increased antiviral activity (table 1). Fig. 1 also shows that poly (rI)–poly (5-MedC) melts at a T_m 10° higher than poly (rI)–poly (dC); both complexes exhibit similar conformations, but the former is as inactive as the latter (table 1). From these results it can be concluded that neither the presence of the 5-methyl substituent, nor the associated increased thermal stability, influence the biological activity.

Acknowledgements

We are indebted to Prof. P. De Somer for continuous interest and support, to Dr. M. Joniau and

Dr. K. Heremans for help with the spectral absorption and thermal stability measurements, and to Mrs. A. Van Lierde and Mrs. E. Poddany for excellent technical assistance. This work was supported, in part, by the Belgian "Fonds voor Geneeskundig Wetenschappelijk Onderzoek" (F.G.W.O.), the Polish Academy of Sciences (Project 09.3.1.) and also profited from the support of the Wellcome Trust and the World Health Organization.

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