ANTIVIRAL ACTIVITY OF POLYNUCLEOTIDES: POLY 2'-O-ETHYLADENYLIC ACID AND POLY 2'-O-ETHYLURIDYLIC ACID

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1. Introduction

One of the most stringent structural requirements for the antiviral (interferon inducing) activity of double-stranded polynucleotides appears to be the presence of 2'-hydroxyl groups in both strands of the duplex. Substitution of this 2'-OH group by other radicals (−H [1−4], −F [5], −Cl [6], −O−CH_3 [7], −N=N^+=N^- [8,9], −O−CO−CH_3 [10]) in the pyrimidine (or purine) strand of either poly(A) · poly(U) or poly(I) · poly(C) invariably led to a significant decrease in antiviral activity. However, most of these modifications were introduced into the pyrimidine partner of the duplex [5−9]. The decrease in activity noted upon introduction of 2'-H and 2'-O−CO−CH_3 in the purine strand could largely be attributed to a fall in thermal stability of the corresponding duplex [1−3,10], except for poly(dI) · poly(cl^5C), poly(dI) · poly(br^5C) and poly(dI) · poly(i^5C) [4].

To further establish the role of the 2'-OH group in the antiviral activity of polynucleotides, complexes of poly(A) and poly(U) were examined in which either the poly(A) or poly(U) strand were substituted by their 2'-O-ethyl derivative.

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2. Materials and methods

The synthesis and properties of poly(Ae)[†] and poly(Ue) have been described recently [11,12]; sedimentation values (S_{20}) : > 14 and ∿ 20 respectively. Poly(A) and poly(U) were obtained from Miles Laboratories (Elkhart, Indiana); sedimentation values (S_{20}) : 7.0−11.5 and 3.3−6.4 respectively. The complexes poly(Ae) · poly(U) and poly (A) · poly(Ue) were formed by mixing equal volumes of homopolymer solutions, prepared at 50 μg/ml [poly(Ae) and poly(U)] or 30 μg/ml [poly(A) and poly(Ue)] in phosphate buffered saline (PBS) [0.15 M NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.05 M phosphate buffer (pH 7.0)] and incubating the mixtures for 1 hr at 37^0C. Evidence for annealing was based on hypochromicity after mixing : (at 260 nm) 30% for poly(Ae) · poly(U) and 22% for poly(A). poly (Ue), as compared to 26% for poly(A) · poly(U) when annealed under identical conditions.

Interferon production was assessed in primary rabbit kidney (PRK) cell cultures superinduced [13] with cycloheximide and actinomycin D, a highly sensitive assay system, particularly useful for determining the antiviral activity of polynucleotide materials of which only limited amounts are available [5,14]. PRK cell cultures in 60 mm Falcon or Nunc plastic petri dishes were exposed to 10 μg/ml of the polynucleotide in minimal Eagle's medium (MEM) (1 ml/Petri dish) for 1 hr at 37°C, washed (3 $\times$) with MEM, and then incubated with cycloheximide (2 μg/ml in MEM + 3% calf serum; 2 ml/Petri dish) for 3 hr at 37°C, washed again (3 $\times$) with MEM, further incubated with actinomycin D (3 μg/ml in MEM + 3% calf serum; 2 ml/Petri dish) for 30 min at 37°C, washed again (3 $\times$) with MEM and replenished with MEM + 3% calf serum (4 ml/Petri dish) for 20 hr. The supernatant fluids of the cell cultures were then withdrawn and titrated for interferon as described previously [5,14].

3. Results

Although poly(Ae) and poly(U) as well as poly(A) and poly(Ue) are capable of forming a stable double-stranded complex, as shown before [11,12] and evidenced by the hypochromicity obtained in this study, they failed to induce appreciable amounts of interferon in superinduced PRK cell cultures (table 1).

Did a previous exposure of the cells to the inactive complexes poly(Ae) · poly(U) or poly(A) · poly(Ue) alter the cells' responsiveness to the active complex poly(A) · poly(U)? As shown in table 2, PRK cells treated for 1 hr with either poly(Ae) · poly(U) or poly(A) · poly(Ue) and immediately thereafter exposed to poly(A) · poly(U) produced as much interferon as if they had not been pretreated with the 2'-O-ethyl substituted complexes.

Was the interferon inducing activity of poly(A) · poly(U) altered upon addition of the single homopolymers poly(Ae) and poly(Ue) to the cells either before or after or together with the poly(A) · poly(U) complex? As shown in table 3, poly(Ae) did not affect the activity of poly(A) · poly(U) whether it was applied before or after or together with the duplex. Poly(Ue) slightly reduced the activity of poly(A) · poly(U) when applied to the cells prior to the duplex. On the contrary, poly(A) enhanced the activity of

Table 1
Interferon induction by poly(Ae) · poly(U) and poly(A) · poly(Ue) in PRK cell cultures superinduced with cycloheximide and actinomycin D*

	Interferon titer (units/ml)	
	Exp. 1	Exp. 2
Poly(Ae)	3	8
Poly(A)	3	3
Poly(Ue)	3	3
Poly(U)	4	3
Poly(Ae) · poly(U)	4	8
Poly(A) · poly(Ue)	4	10
Poly(A) · poly(U) **	800	600
Poly(A) · poly(U)†	600	1200

* As described in 'Materials and methods'. All polymers tested at 10 μg/ml.

** Complex formed by mixing equal volumes of homopolymer solutions at 30 μg/ml in PBS.

† Complex formed by mixing equal volumes of homopolymer solutions at 1 mg/ml in PBS.

Table 2
Interferon production in PRK cell cultures successively exposed to the inactive complexes poly(Ae) · poly(U) or poly(A) · poly(Ue) and the active complex poly(A) · poly(U)*

Inactive complex	Active complex	Interferon titer (units/ml)
Poly(Ae) · poly(U)	MEM	10
Poly(A) · poly(Ue)	MEM	< 10
MEM	Poly(A) · poly(U)	1200
Poly(Ae) · poly(U)	Poly(A) · poly(U)	1000
Poly(A) · poly(Ue)	Poly(A) · poly(U)	2000

* PRK cell cultures were first exposed to 10 μg/ml of the inactive complex in MEM (1 ml/petri dish) for 1 hr at 37°C washed (3 $\times$) with MEM, and immediatly thereafter exposed to 10 μg/ml of the active complex in MEM (1 ml/petri dish) for 1 hr at 37°C, washed again (3 $\times$) with MEM, and further processed as described in Materials and methods.

Table 3
Interactions among the homopolymers poly(A), poly(Ae), poly(Ue) and their duplexes as monitored by interferon production in PRK cell cultures

			Interferon titer (units/ml)

Homopolymers and homopolymer duplexes mixed in vitro and then added to the cells*[†]

	Mixture		
Poly(A) · poly(U)	+	MEM	1200
Poly(A) · poly(U)	+	Poly(Ae)	1500
Poly(Ae) · poly(U)	+	MEM	10
Poly(Ae) · poly(U)	+	Poly(A)	80
Poly(A) · poly(Ue)	+	MEM	< 10
Poly(A) · poly(Ue)	+	Poly(U)	< 10

Homopolymers and homopolymer duplexes added to the cells in sequential order*[††]

Sequence of addition

First	Second	
MEM	Poly(A) · poly(U)	1200
Poly(Ae)	Poly(A) · poly(U)	800
Poly(Ue)	Poly(A) · poly(U)	300
Poly(A) · poly(U)	MEM	1200
Poly(A) · poly(U)	Poly(Ae)	1000
MEM	Poly(Ae) · poly(U)	< 10
Poly(A)	Poly(Ae) · poly(U)	< 10
Poly(Ae) · poly(U)	MEM	< 10
Poly(Ae) · poly(U)	Poly(A)	< 10
Poly(A) · poly(Ue)	MEM	< 10
Poly(A) · poly(Ue)	Poly(U)	< 10

* The results obtained with the homopolymers alone are not presented in this table. They were invariably inactive (interferon titer ⩽ 10 units/ml).

† The homopolymers (final concentration: 5 μg/ml in MEM) and homopolymer duplexes (final concentration: 10 μg/ml in MEM) were mixed and incubated for 1 hr at 37°C before addition to the cell cultures. The cells were then incubated with the mixture (1ml/petri dish) for 1 hr at 37°C, washed (3 ×) with MEM, and further incubated as described in Materials and methods.

poly(Ae) · poly(U) when mixed with the complex before addition to the cells; poly(U) failed to increase the activity of poly(A) · poly(Ue) when added to the cells together with or after the complex (table 3).

4. Discussion

As may have been expected from previous findings [1–10], substitution of the ribose 2'-OH by $2'-O-CH_2-CH_3$ in the pyrimidine strand of poly(A) · poly (U) led to a drastic decrease of the interferon inducing activity of the duplex. A similar decrease in activity was noted upon introduction of $2'-O-CH_2-CH_3$ groups in the purine strand (table 1), suggesting that, for interferon induction by double-stranded duplexes such as poly(A) · poly(U), the presence of free 2'-OH groups is equally important in the purine strand as in the pyrimidine strand.

In contrast with the triple-stranded complex poly (A) · 2 poly(U) and the poly(c^7A) · poly(U) duplex [poly(c^7A) being poly(7-deaza adenylic acid)] [14], neither poly(Ae) · poly(U) nor poly(A) · poly(Ue) competed with the interferon inducing activity of poly(A) · poly(U) (table 2). Similarly, duplexes of poly(A) with poly(U) analogs in which the 2'-OH was replaced by a fluoro ($-F$) or azido ($-N=N^+=N^-$) group failed to reverse the activity of poly(A) · poly(U) [15]. In as far as the inhibitory effects of poly(A) · 2 poly(U) and poly(c^7A) · poly(U) on the interferon inducing capacity of poly(A) · poly(U) can be ascribed to a competitive binding with the postulated receptor sites for interferon induction [14], the inability of poly(Ae) · poly(U) and poly(A) · poly(Ue) to reverse the activity of poly(A) · poly(U) suggests that the 2' -OH substituted complexes do *not* interact with these receptor sites. This is in agreement with the hypothe-

++ The first polymer (homopolymer or homopolymer duplex) was added to the cell cultures at 10 μg/ml in MEM (1 ml/Petri dish). The cells were incubated for 1 hr at 37°C, washed (3 ×) with MEM, and immediately thereafter exposed to the second polymer (homopolymer or homopolymer duplex) at 10 μg/ml in MEM (1 ml/Petri dish) for another hour at 37°C, washed again (3 ×) with MEM, and further processed as described in Materials and methods.

sis proffered by Colby and Chamberlin [2] that only duplexes with an intact 2'-OH group are recognized by the receptor molecule(s).

Minor shifts in activity were noted if poly(A) · poly(U) was tested in combination with poly(Ae) or poly (Ue), or if poly(Ae) · poly(U) and poly(A) · poly (Ue) were tested in combination with either poly(A) or poly(U) (table 3). Poly(Ue) slightly decreased the activity of poly(A) · poly(U) when applied to the cells prior to the complex and poly(A) increased the activity of poly(Ae) · poly(U) when mixed with the complex beforehand. Similar, although quantitatively more pronounced shifts in activity have been noted in other systems: e.g. poly(I)—poly(A) · poly(U), poly (C)—poly(A) · 2 poly(I) [16]. Poly(I) markedly reduced the activity of poly(A) · poly(U) when mixed with poly(A) · poly(U) or applied to the cells before poly(A) · poly(U). This is most probably due to the formation of an hitherto unrecognized triple-stranded complex poly(I) · poly(A) · poly(U) [16]. Alternatively, poly(C) significantly enhanced the activity of poly(A) · 2 poly(I) when mixed with poly(A) · 2 poly(I) or applied to the cells before poly(A) · 2 poly (I) most probably because of a displacement reaction to poly(I) · poly(C) and free poly(A) [17]. Similar interactions (triple-strand formation and strand displacement) may underlie the shifts in activity noted above with the systems poly(Ue)—poly(A) · poly(U) and poly(A)—poly(Ae) · poly(U).

Acknowledgements

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