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Polyribonucleotide Inhibition of Murine Leukemia Virus Replication: Effect of Strandedness

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SUMMARY

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We have previously reported on the structure-activity relationships of polyribonucleotides as inhibitors of murine leukemia virus replication in cultured cells. These studies raised the possibility that the active form of otherwise single-stranded polyribonucleotides may be a multistranded structure. We have now compared the inhibitory potencies of double- and triple-stranded complexes of poly(adenylic acid) and poly(uridylic acid) with those of single-stranded poly(adenylic acid) and poly(uridylic acid). We find that poly(adenylic acid)·poly(uridylic acid) and poly(adenylic acid)·2 poly(uridylic acid) are considerably less potent than uncomplexed poly(adenylic acid) and poly(uridylic acid). These results suggest that the inhibitory potential of polyribonucleotides does not reside in a multistranded structure but in a single-stranded molecule. These observations are consistent with the notion that the inhibition of RNA tumor virus replication by single-stranded polyribonucleotides is more likely to be due to their effect on reverse transcription than on interferon induction.

We have previously reported the inhibition of murine leukemia virus synthesis in cultured cells by several polyribonucleotides (1-3). The results of these studies suggested that (a) purine polyribonucleotides may in general be more potent than pyrimidine polyribonucleotides and (b) polyribonucleotides containing a ketoamino function may be more potent than polyribonucleotides lacking this function. We also noted that polyribonucleotides which were more potent as virus inhibitors were those which display a greater tendency to form self-aggregated, multistranded complexes (3). For example, poly(inosinic acid) and poly(xanthylic acid) were among the most potent of the series of polyribonucleotides investigated thus far (2, 3). These polyribonucleotides reportedly can form multistranded complexes in solution (4, 5). This raised the possibility that the biologically active forms of polyribonucleotides may be the multistranded structures. This in turn raises the possibility that polyribonucleotides inhibit virus replication because of interferon induction rather than their effect on viral RNA-directed DNA synthesis (1-3). Since there is no assured way of controlling the strandedness of some of the otherwise single-stranded homopolymers in solution [e.g., poly(I) and poly(X)], we decided to investigate a set of polyribonucleotides which can exist as single strands in solution and can be treated to form well-defined double- and triple-stranded complexes (6, 7). In this communication we

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report the effect of double-stranded poly(A)·poly(U) and triple-stranded poly(A)·2 poly(U) on MuLV[1] synthesis in cultured JLS-V9 (mouse bone marrow) cells and compare their effects with those of single-stranded poly(A) and poly(U) under identical conditions.

Poly(A) (8.1 S) and poly(U) (7.4 S) were purchased from Miles Laboratories. The concentrations of polyribonucleotides in solution were determined spectrophotometrically (2, 3). The double- and triple-stranded complexes of these polyribonucleotides were obtained by mixing solutions of them in 0.02 M sodium phosphate–0.15 M NaCl (pH 7.2) in their respective molar ratios and heating them at 65° for 5 min, followed by slow cooling. Under these conditions the strandedness of helical complexes of poly(A) and poly(U) is governed solely by their respective molar ratios in the mixture (6, 7).

The effect of polyribonucleotide on MuLV infection of cultured cells was studied as described before (2, 3). Briefly, duplicate monolayers of JLS-V9 cells were treated for 30 min with DEAE-dextran (10 μg/ml) and incubated for 2 hr with RPMI-1640 culture medium containing polyribonucleotide. The control cultures received culture medium without polyribonucleotide. They were then infected with MuLV for 1 hr and incubated for 20 hr with RPMI-1640 medium plus 10% fetal calf serum, with or without polyribonucleotide. Subsequently the monolayers were incubated with fresh culture medium lacking polyribonucleotide. Twenty hours later, the culture medium was harvested and the titer of progeny virus in the medium was determined by measuring the DNA polymerase activity of the virions after partial purification as described before (2, 3). The monolayers were washed with 0.02 M sodium phosphate–0.15 M NaCl before every operation. The stock MuLV, containing 1.6×10^7 PFU/ml, was obtained from monolayer cultures of JLS-V9 cells chronically infected with this virus. The culture medium, fetal calf serum, and phosphate–NaCl buffer were purchased from Grand Island Biological Company.

[1] The abbreviations used are: MuLV, murine leukemia virus (Moloney); PFU, plaque-forming units.

To study the effect of polyribonucleotides on cell growth, monolayers were treated and infected as described above. At specified times after infection, the cells were detached from the surface by mild trypsinization and counted.

The effects of poly(A), poly(U), and their complexes on MuLV infection of JLS-V9 cells are presented in Table 1. As reported before (1, 2), poly(A) and poly(U) showed significant inhibition of progeny virus synthesis in JSL-V9 cells infected with MuLV. The degree of inhibition observed for these polyribonucleotides at the concentrations shown is in reasonable agreement with inhibition noted at other concentrations studied before (1, 2). It is also apparent from the data in Table 1 that double- and triple-stranded complexes of poly(A) and poly(U) were significantly less potent at equivalent molar nucleotide concentrations. Whereas poly(A) and poly(U) at a concentration of 50 μM gave about 66% and 54% inhibition, respectively, poly-

TABLE 1

Effect of multistranded complexes of poly(adenylic acid) and poly(uridylic acid) on murine leukemia virus infection of JLS-V9 cells

Duplicate monolayers of JLS-V9 cells were treated with DEAE-dextran (10 μg/ml, 30 min), washed with 0.02 M sodium phosphate–0.15 M NaCl, and incubated for 2 hr in culture medium with or without polyribonucleotide. They were washed with the same buffer and infected for 1 hr with MuLV (multiplicity of infection, 5 PFU/cell). The monolayers were then washed with buffer and incubated for 20 hr in culture medium plus 10% fetal calf serum, with or without polyribonucleotides. They were washed again with buffer and reincubated in fresh culture medium lacking polyribonucleotides. Twenty hours later the culture medium was harvested and the titer of progeny virus was estimated by measuring the DNA polymerase activity of the virions as described before (2, 3).

Polynucleotide	Concentration	DNA polymerase activity	
	μM	*pmoles/ml*	*% control*
None		36.50 ± 2.67	100
Poly(A)	50	12.30 ± 0.52	33.7
Poly(U)	50	16.30 ± 0.10	44.6
Poly(U)	100	11.17 ± 0.62	30.6
Poly(A)·poly(U)	50.50	28.20 ± 2.05	77.2
Poly(A)·2 poly(U)	50.100	22.35 ± 1.03	61.2

(A)·poly(U) at the corresponding concentration yielded only 23% inhibition. Similarly, poly(A)·2 poly(U) caused about 40% inhibition, while poly(A) and poly(U) yielded about 66% and 70% inhibition, respectively, at the corresponding molar nucleotide concentrations. If multistrandedness itself were a major factor, we would expect the double- and triple-stranded complexes to be more potent than the single-stranded polyribonucleotides. At the very least, they should be as potent as the sum of the potencies of the corresponding single-stranded polyribonucleotides at equivalent molar nucleotide concentrations. In addition, if interferon induction were a factor in the inhibition of virus replication in our system, we would expect double- and triple-stranded complexes to be more potent than single-stranded polyribonucleotides (see below). Our results thus suggest that the inhibition potential of polyribonucleotides does not reside in their multistranded structure and that the inhibition of virus replication by single-stranded polyribonucleotides is not due to interferon induction but more likely to their effect on viral RNA-directed DNA synthesis (1–3). On the other hand, a part of the inhibition observed for double- and triple-stranded complexes of poly(A) and poly(U), limited though it is, may be related to their interferon-inducing capacity. Poly(A)·poly(U) is a fairly potent inducer of the interferon response, and poly(A)·2 poly(U) also induces detectable amounts of interferon (8, 9).

The above results, however, do not rule out the possibility that self-aggregated multistranded complexes may be inhibitory. Although poly(A) and poly(U) are not known to form duplexes or triplexes in isotonic neutral solution at 37°, it may be argued that they do so in the culture medium or intracellularly. This seems unlikely, because of the following considerations. The induction of interferon by polyribonucleotides is an exquisite measure of their strandedness; double-stranded polyribonucleotides are potent inducers of the interferon response, triple-stranded polyribonucleotides induce some interferon but much less than the duplexes, and single-stranded polyribonucleotides fail to induce significant amounts of interferon (8–11). Several workers have reported that the single-stranded poly(A), as well as poly(U), does not induce interferon or provide protection against lytic virus infection in several cell culture systems (for example, see refs. 8 and 9). Our results are consistent with these observations. Thus it can be assumed that these polyribonucleotides do not adopt a multistranded conformation in the culture medium or intracellularly.

An additional factor related to the possible cytotoxicity of polyribonucleotides may also be considered. The inhibitors of cell division are known to interfere with the replication of RNA tumor viruses in cultured cells (12). We have noted previously that neither single-stranded poly(A) nor poly(U) is toxic for cultured JLS-V9 cells (1, 2). This was confirmed in the present study, in which parallel cultures treated with poly(A) and poly(U) were examined (data not shown). The effect of poly(A)·poly(U) and poly(A)·2 poly(U) on the growth rates of cultured JLS-V9 cells

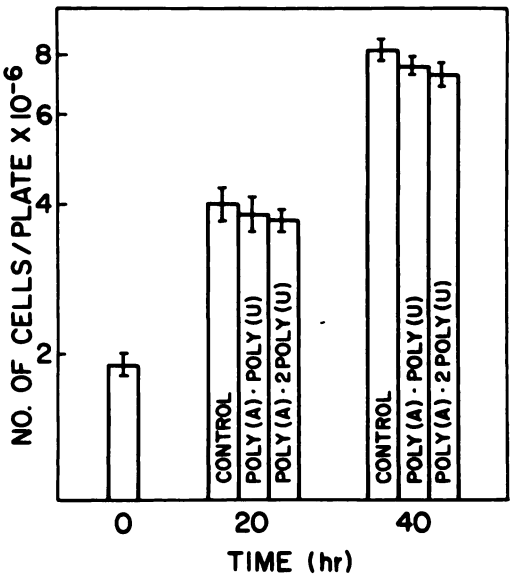

FIG. 1. *Effect of poly(A)·poly(U) and poly(A)·2 poly(U) on growth rates of cultured JLS-V9 cells*

Duplicate monolayers of JLS-V9 cells were treated with polyribonucleotides and infected with MuLV as described in Table 1. At specified times after infection, the monolayer was trypsinized gently and the number of cells in the culture was counted.

is depicted in Fig. 1. At the concentrations used for studying their effects on virus replication, these complexes appeared not to affect significantly the growth rates of the cultured cells. There is some indication, however, that poly(A)·2 poly(U) may be slightly toxic for these cells.

Regardless of these mechanistic considerations, the results presented in this communication demonstrate that double- and triple-stranded complexes of poly(A) and poly(U) are less potent than single-stranded poly(A) and poly(U) for inhibition of murine leukemia virus replication. These results support the notion that the active form of the polyribonucleotides is not a multistranded helical conformation but a single-stranded structure.

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