ANTIVIRAL AND INTERFERONOGENIC ACTION OF COMPLEXES OF POLYADENYLIC AND POLYURIDYLIC ACIDS

Yu. G. Atanasova, N. L. Pushkarskaya, G. A. Galegov, and S. S. Debov

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The antiviral and interferonogenic action of polymers of adenylic and uridylic acid uridylic acid (poly-A, poly-U) was studied. Double-helical complexes of poly-A:U in the ratio of 1:1 were obtained for this purpose, and their physicochemical parameters were studied. In concentrations nontoxic to cells, the stimulator was shown to inhibit reproduction of vesicular stomatitis virus and to stimulate the formation of interferon in vitro. Activity of the poly-A:U complexes in tissue cultures of chick fibroblasts was considerably increased in the presence of neomycin.

Synthetic polymers of adenylic (poly-A) and uridylic (poly-U) acids in dilute salt solutions are bonded together to firm double-helical structures in which the adenylic residues of one chain are hydrogen-bonded with uridylic residues of the other chain (poly-A:U). The resulting complexes can stimulate interferon production both in tissue culture and in vivo in laboratory animals [2, 5].

The object of the investigation described below was to study the antiviral and interferonogenic action of poly-A:U complexes in tissue cultures of chick fibroblasts.

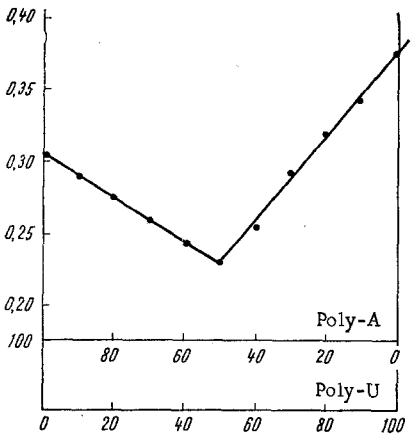

Fig. 1. Formation of poly-A:U complexes. Abscissa, quantity of poly-A and poly-U (in percent of molar content); ordinate, extinction of solutions at 259 nm.

EXPERIMENTAL METHOD

Poly-A and poly-U (Serva, West Germany), and the polyamines spermin (Serva, West Germany) and neomycin (generously provided by Professor S. M. Navashin) were used in the investigation. A primary trypsinized culture of chick fibroblasts was grown in 0.5% lactalbumin hydrolysate with 10% bovine serum. Vesicular stomatitis virus (VSV), maintained by subculture in chick fibroblasts, was used as the test virus.

Sedimentation analysis was carried out on the Spinco E analytical ultracentrifuge with ultraviolet optical system at 59,000 rpm. The sedimentation constants were determined in 0.15 M NaCl–0.006 M Na_2HPO_4 solution, pH 7.0. The optical density of the solutions was 0.5–0.6 at 259 nm. Photographs were taken at intervals of 10 min after the rotor had reached the above speed.

The melting temperature of the complexes was determined in hermetically sealed quartz cells placed in the thermostatically controlled cell of a Hiliger (England) spectrophotometer. The ultraviolet absorption of the solutions was recorded at 259 nm; the melting temperature was measured with a thermocouple.

D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow. Department of Biochemistry, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician V. V. Parin.)
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TABLE 1. Melting Temperature of Poly-A:U Complexes in the Presence of the Polyamines Neomycin and Spermin

Preparation	Concentration (in μg/ml)	T_m (in deg)
Poly-A:U	30	60
Poly-A:U + neomycin	30 100	77
Poly-A:U + spermin	30 3	73

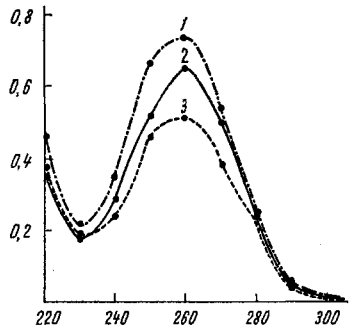

Fig. 2. Spectrophotometric characteristics of polynucleotides poly-A (1) and poly-U (2) and their complex poly-A:U (3). Abscissa, wavelength (in nm); ordinate, extinction of solutions.

The solutions of the polynucleotides were made up in 0.15 M NaCl–0.006 M Na_2HPO_4 solution, pH 7.0. The concentration was measured spectrophotometrically and calculated from the corresponding molar extinction coefficients: for poly-A $E_{257} = 9.9 \cdot 10^3$, for poly-U $E_{260} = 9.4 \cdot 10^3$ [1, 6]. The poly-A:U complexes were obtained immediately before the experiment by mixing equal volumes of solutions of the corresponding polynucleotides. The stoichiometric characteristics of the complexes were determined by preparing a series of dilutions of poly-A and poly-U in which the total volumes of the polynucleotides remained constant, but the volumes of the individual polynucleotides were constantly varied. The samples were left for 10-15 min at room temperature, after which their ultraviolet absorption was measured with an SF-4 spectrophotometer at 269 nm.

Activity of the stimulator (poly-A:U) was tested by the method of direct inhibition of VSV reproduction in cultures of chick fibroblasts. The specimens were added to flasks containing a monolayer cell culture and left for 1-1.5 h at 37°C. The stimulator was then removed, the cells were washed with medium No. 199, and VSV (0.01-0.001 p.f.u./cell) was added. After incubation for 1 h at 37°C, the virus was removed, the monolayer was washed twice with medium No. 199, 5 ml of the same medium was added, and the sample was again incubated for 18 or 24 h at 37°C. The virus in the control cultures and in the cultures treated with stimulator was titrated with respect to plaque formation under the agar.

The presence of interferon in cultures of chick fibroblasts treated for 1-1.5 h by various concentrations of stimulator, in presence or absence of polyamines, was determined by titration of double dilutions of the interferon-containing culture fluid. The degree of inhibition of virus reproduction by interferon was estimated from the decrease in the number of plaque-forming units per monolayer compared with the control.

EXPERIMENTAL RESULTS

In view of personal experience, and also of the work of Scruggs and Ross [6], and Huang et al. [3], showing that a large excess of poly-A is required in order to form poly-A:U complexes of the 1:1 type and to give them greater stability, the poly-A solutions were made up in a concentration approximately 30% higher than the poly-U solutions. When equal volumes of the polynucleotides were mixed, 30-35% hypochromism was observed, indicating the formation of complexes of the 1:1 type (Fig. 1). These results were confirmed by the spectrophotometric characteristics of the individual polynucleotides and of their complex measured at different wavelengths on the SF-4 instrument (Fig. 2), and also by sedimentation analysis; the following sedimentation constants were obtained: for poly-A $S_{20} = 7.8$, for poly-U $S_{20} = 4.5$, and for poly-A:U $S_{20} = 10.85$. The resulting complexes had a comparatively high melting temperature (60°C); addition of the polyamines spermin and neomycin to the poly-A:U solutions, even in concentrations as low as 3 μg/ml, considerably increased the melting temperature of a complex, indicating the stabilizing action of the polyamines (Table 1).

To determine the antiviral and interferonogenic action of the stimulator, sterile solutions were used initially (the poly-A:U specimens were sterilized by filtration through a G = 5 glass filter). Sedimentation analysis showed that under these circumstances no appreciable injury took place to the complex, and only its concentration was reduced, by about 30%. Later the solutions were not sterilized, but this had no effect on the tissue culture.

TABLE 2. Direct Inhibition of VSV Reproduction by Poly-A:U Preparation in Tissue Cultures of Chick Fibroblasts

Preparation	Concentration (in μg/ml)	Multiplicity of infection (in p.f.u./ml)	Infectious titer (in p.f.u./ml after 18 and 24 h	Inhibition of VSV reproduction (in % of control)
Poly-A:U	50	0,01	$1 \cdot 10^4$(a)	86,7
Control (b)	—	0,01	$7,5 \cdot 10^4$(a)	—
Poly-A:U Poly-A:U	50 100	0,001 0,001	$9,5 \cdot 10^3$(a) $2,5 \cdot 10^3$(a)	85,4 96,4
Control	—	0,001	$6,5 \cdot 10^4$(a)	—
Poly-A:U Poly-A:U + neomycin	25 25 100	0,001 0,001	$1,6 \cdot 10^5$ $3,6 \cdot 10^4$	56,8 90,3
Control	—	0,001	$3,7 \cdot 10^5$	—
Poly-A:U Poly-A:U + spermin (c)	50 50 2.5	0,001 0,001	$2,5 \cdot 10^6$ $5,4 \cdot 10^6$	93.4 95,8
Control	—	0,001	$3,8 \cdot 10^7$	—

Legend: a) infectious titer 18 h after infection with virus; b) buffer (0.15 M NaCl–0.006 M Na_2HPO_4 solution, pH 7.0); c) spermin in concentrations higher than 5 μg/ml is toxic.

TABLE 3. Interferon Formation under the Influence of Poly-A:U Stimulator in Tissue Cultures on Chick Fibroblasts

Preparation	Concentration (in μg/ml)	Dilution of interferon-containing culture fluid	Number of plaques per monolayer
Poly-A:U	25	16	100
Poly-A:U + neomycin	25 100	16	34
Poly-A:U	50	16	60
Poly-A:U + spermin	50 100	16	63
Control (a)		–	103

Legend: a) Buffer (0.15 M NaCl – 0.006 M Na_2HPO_4 solution, pH 7.0).

The study of the toxic action of the poly-A:U preparations in experiments in vitro demonstrated their complete harmlessness in concentrations of between 1 and 300 μg/ml during incubation for 24–48 h. No visible morphological changes were found in uninfected cells in the presence of these doses of the stimulator, in agreement with data in the literature [2]. The stimulator was maximally effective in a concentration of 50–100 μg/ml, and no further increase in its effects were obtained when its concentration was increased up to 300 g/ml. As Table 2 shows, poly-A:U in a concentration of more than 25 g/ml depressed reproduction of the virus by comparison with the control. After the combined administration of the stimulator and neomycin, inhibition of virus reproduction was increased from 56.8 to 90.3%; neomycin alone, without poly-A:U, did not reduce the infectious titer of the virus. The presence of spermin had no appreciable effect on the activity of the poly-A:U preparations.

To determine the specific nature of the action of the stimulator, i.e., its ability to induce interferon production, a series of experiments was carried out and their results are given in Table 3. These results show that poly-A:U complexes have marked interferonogenic activity although, however, it is lower than that of other inducers, notably poly-I:C [2, 4, 5, 7, 8].

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