INTERFERON-INDUCING ACTIVITY OF POLY(I)·POLY(C) AND ITS COMPLEXES WITH POLYCATIONS AND THEIR RESISTANCE TO ENZYMATIC DESTRUCTION

V. D. Kol'tsov, L. V. Krasnova,
I. G. Balandin, and E. N. Meshkova

UDC 578.245.2.04:577.152

KEY WORDS: interferon; interferon inducers.

It is now generally accepted that only those nucleic acids and synthetic polyribonucleotides which are resistant to destruction by the corresponding cell and blood enzymes are capable of inducing interferon synthesis, and that double-stranded polyribonucleotides and RNA, which have greater resistance, are better inducers [4, 11]. Increasing the resistance of inducers by chemical modifications [7] or by complex formation with polycations [1, 9] leads to an increase in their interferonogenicity. However, the possibility cannot be ruled out that chemical modifications of inducers, besides increasing resistance to enzymic destruction, may also alter some of their other properties, and the increase in inducing activity may take place not entirely on account of the increase in resistance.

In the present writers' opinion, to answer the question whether the increase in resistance of inducers to enzymic destruction is responsible for the increase in their interferon-inducing capacity, it is essential to obtain and make a comparative study of complexes of the same inducer with polycations, in which all known parameters required of substances as interferon inducers, other than resistance, were left unchanged.

In the investigation described below insoluble complexes of poly(I)·poly(C) with histones from calf thymus and with poly-L-lysine, containing different quantities of the latter and free from contamination with the free polycations mentioned above, were obtained and their melting point, resistance to nucleases and serum enzymes, and their ability to undergo adsorption on cells were studied and compared with their interferon-inducing activity.

EXPERIMENTAL METHOD

Complexes of poly(I)·poly(C) with histones from calf thymus and with poly-L-lysine were prepared by the method described previously for nucleohistones [3]. The insoluble complexes thus formed were repeatedly washed with 0.02 M Tris-HCl buffer, pH 7.4, with 0.15 M NaCl, and their protein [10] and RNA [5] content was determined, using standard curves appropriate for the components to be determined.

Interferon-inducing activity of the insoluble complexes was tested on noninbred mice after intravenous and intraperitoneal injection of the preparations in a dose of 100 μg/mouse and in a volume of 0.2 ml. Blood was collected from the subclavian vein at the time necessitated by the experimental conditions. Interferon was titrated on 3-day cultures of L_{929} cells, using encephalomyocarditis virus. Adsorption of poly(I)·poly(C) and its insoluble complexes was determined on sandwich cultures of L_{929} cells, repeatedly washed with Hanks' solution. The materials for testing were added at the rate of 20 μg poly(I)·poly(C), alone or in complex form, to 5×10^6 cells. Adsorption continued for 1 h at 37°C, after which the unadsorbed material was collected (by washing off with Hanks' solution) and the content of poly(I)·poly(C) and protein was determined in it. Liquid with control cells after appropriate treatment, was used as the control.

The action of pancreatic ribonuclease and blood serum enzymes on poly(I)·poly(C) and its insoluble complexes was determined as follows: 200 μg poly(I)·poly(C), free or in complex form, in 0.05 M Tris-HCl buffer, pH 7.5, containing 0.15 M NaCl was mixed in an ice bath either with human or mouse serum (0.2 ml per sample) or with a solution (in the same buffer)

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. D. Solov'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 9, pp. 74-77, September, 1983. Original article submitted April 23, 1982.

0007-4888/83/9609-1279$07.50

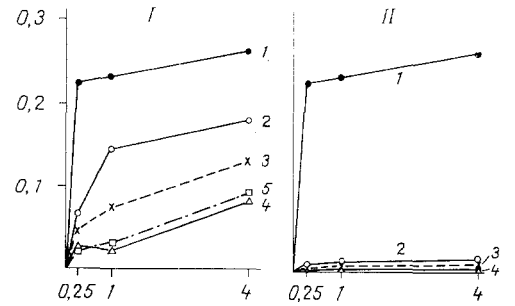

Fig. 1. Action of pancreatic ribonuclease on poly(I)·poly(C) and its complexes with histones (I) and poly-L-lysine (II). Abscissa, incubation time (in h); ordinate, E_{260} of material not precipitated by uranyl acetate. 1) Poly(I)·poly(C); 2-5) complexes with the polycation and polyribonucleotide in the ratio of 0.75/1-1.5/1 and 2.5/1 and 3/1 respectively.

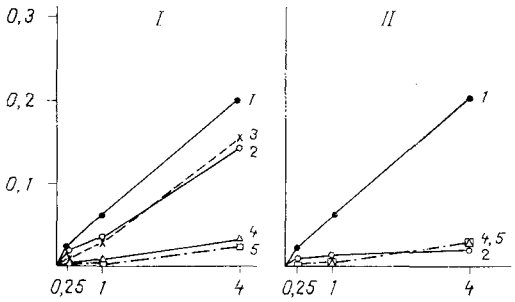

Fig. 2. Action of human serum enzymes on poly(I)·poly(C) and its complexes with histones (I) and with poly-L-lysine (II). Legend as to Fig. 1.

of pancreatic ribonuclease (20 μg per sample), the samples were made up with the same buffer to a volume of 2 ml, and incubated at 37°C for the time required by the experimental conditions, after which they were transferred back to the ice bath and to each sample 0.4 ml of 0.25% solution of uranyl acetate in 25% $HClO_4$ was added. After 15 min the samples were centrifuged, 1-ml samples of the transparent supernatant were taken and made up with distilled water to 3 ml, and the optical density of the solution was measured at a wavelength of 260 nm. A correction was made to the results, taking into account the results obtained with control samples to determine spontaneous breakdown of the preparations (+ all the components except the enzyme, which was added after the reaction had been stopped with uranyl acetate). The melting point (T_m) of poly(I)·poly(C) and of its complexes with histones was determined in 0.01 M cacodylate buffer, pH 7.0, with 0.15 M NaCl.

EXPERIMENTAL RESULTS

The study of the resistance of the insoluble complexes of poly(I)·poly(C) with histones from calf thymus and with poly-L-lysine thus obtained to the action of pancreatic ribonuclease and human blood serum hydrolases showed that the polyribonucleotide was more resistant in complex form than native poly(I)·poly(C) (Figs. 1 and 2). The experiments showed than an increase in the content of polycations in the complexes led to an increase in their stability. It was also found that complexes with poly-L-lysine were more resistant than complexes containing various quantities of histones. Neither poly(I)·poly(C) nor its complexes were hydrolyzed in the presence of mouse serum. The study of the action of pancreatic ribonuclease on poly(I)·poly(C); 2-5) complexes of mouse serum revealed no inhibitory factors in the latter: In this variant of the experiment the degree of destruction of the polyribonucleotide likewise did not differ from that in samples containing pancreatic ribonuclease alone.

T_m was studied by the use of soluble complexes of poly(I)·poly(C) with histones obtained with ratios of histones to poly(I)·poly(C) of 1/1 and 20/1 in the original incubation mixture. Insoluble complexes could not be used to determine T_m because of their rapid sedimentation. T_m for poly(I)·poly(C) was found to be 63-64°C, T_m of the complex obtained with the ratio 1/1 was 75°C, and T_m of the complex obrained with the ratio 20/1 was 88-89°C.

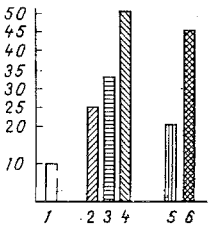

Fig. 3. Adsorbability of poly(I)·poly(C) and its insoluble complexes with histones and with poly-L-lysine on L_{929} cells. Abscissa: 1) poly(I)·poly(C); 2-4) histone—poly(I) poly(C) complexes with ratio of 0.75/1, 1.5/1, and 3/1 respectively; 5, 6) poly-L-lysine—poly-(I)·poly(C) complexes with ratio of 0.75/1 and 3/1 respectively. Ordinate, quantity of adsorbed poly(I)·poly(C) (in % of quantity added).

TABLE 1. Dynamics of Serum Interferon Synthesis after Intravenous Injection of Poly-(I)·Poly(C) and Its Complexes with Poly-L-lysine and Histones from Calf Thymus into Mice

Inducer	Ratio between components	Interferon titer (in Units/ml) in mouse serum at different times after injection of inducer				
		2 h	4 h	8 h	24 h	48 h
Poly(I)·poly(C)		4096	8192	2048	128	<4
Histone—poly(I)·poly(C) complex	0,75/1	3072	4096	2048	192	<4
	3/1	512	1024	128	64	<4
Poly-L-lysine—poly(I)·poly(C) complex	0,75/1	4096	4096	4096	256	<4
	3/1	256	1024	2048	128	<4

Legend. Here and in Table 2 inducers were injected in a volume of 0.2 ml at the ratio of 100 μg poly(I)·poly(C) (free or in complex form) per mouse; w/w ratios of protein to polyribonucleotide in complexes are shown.

TABLE 2. Dynamics of Serum Interferon Synthesis after Intraperitoneal Injection of Poly(I)·Poly(C) and its Complexes with Poly-L-lysine and Histones from Calf Thymus into Mice

Inducer	Ratio between components	Interferon titer (in Units/ml) in mouse serum at different times after injection of inducer					
		4 h	8 h	16 h	24 h	48 h	72 h
Poly(I)·poly(C)	—	1280	4608	640	80	<4	<4
Histone—poly(I)·poly(C) complex	0,5/1	2304	4350	768	80	<4	<4
	0,75/1	2304	4352	768	144	<4	<4
	1,5/1	576	2162	192	40	<4	<4
	3/1	96	576	192	72	48	<4
	6/1	48	288	544	144	10	<4
Poly-L-lysine—poly(I)·poly(C) complex	0,5/1	2304	9024	1280	1152	<4	<4
	0,75/1	4608	9024	5120	2176	<4	<4
	1,5/1	288	2304	640	288	8	<4
	3/1	48	384	192	192	68	<4

The study of adsorption of poly(I)·poly(C) and its insoluble complexes with histones and poly-L-lysine on L_{929} cells showed that under the experimental conditions up to 10% of the poly(I)·poly(C) was adsorbed (Fig. 3). The adsorbability of the complexes was greater than that of the poly(I)·poly(C) alone and it increased with an increase in the polycation content in the complexes.

Investigation of interferon-inducing activity of the substances studied in experiments on mice showed that histone—poly(I)·poly(C) complexes, with ratios of 0.5/1 and 0.75/1 between their components, were just as effective as poly(I)·poly(C) alone both by intravenous and by intraperitoneal injection. An increase in the histone content in the complexes led to a decrease in their interferon-inducing ability. Similar results also were obtained in experiments with poly-L-lysine—poly(I)·poly(C) complexes, except that, when injected intraperitoneally, complexes with a ratio of 0.5/1 or 0.75/1 between their components were more effective than native poly(I)·poly(C) (Tables 1 and 2).

In the experiments with poly(I)·poly(C) and its insoluble complexes with histones and with poly-L-lysine described above, of the conditions required by synthetic polyribonucleotides as inducers of interferon synthesis [2, 4, 11], resistance to nucleases and T_m were changed (increased), whereas all the other parameters remained unchanged. According to data in the literature, these changes in the inducers ought to have increased its interferon-inducing activity. However, only complexes characterized by a low poly-L-lysine content possessed, besides increased resistance to nuclease action, higher interferon-inducing ability when injected intraperitoneally.

Together with data in the literature on the necessity for resistance of an inducer of polyanionic character to enzymic destruction in the cells and in the body as a whole for manifestations of interferon-inducing ability [2, 4, 11], and also that not every increase in resistance of the inducer is accompanied by an increase in interferon-inducing ability [6-8], leads to the conclusion that resistance to destruction is, although essential, not a property which determines the effectiveness of the polyanions as an inducer of interferon synthesis. The decisive factor which renders a polyribonucleotide (or polyanion) resistant to destruction by enzyme systems of the cells and of the body as a whole is a porperty which is modified through binding of the inducer with the polycations. It can be tentatively suggested that one such property, determining the interferon-inducing ability of the polycation, is its "specific capacity," taking the term "capacity" to mean the total quantity of proteins which is bound by the inducer under the given conditions. Preliminary loading of poly(I)·poly(C) with polycations reduces its charge and, consequently, its "capacity," and this reduces its interferon-inducing effectiveness, despite the increase in its resistance to destruction by the corresponding enzymes, the increase of T_m, and increased adsorbability on cells.

LITERATURE CITED

1. T. A. Bektemirov, M. P. Burgasova, and O. G. Andzhaparidze, Vopr. Virusol., No. 5, 536 (1976).
2. F. I. Ershov and A. S. Novokhatskii, Interferon and Its Inducers [in Russian], Moscow (1980).
3. A. M. Kolchinskii, D. F. San'ko, V. V. Shik, et al., Mol. Biol., No. 2, 365 (1978).
4. V. D. Solov'ev and T. A. Bektemirov, Interferons in the Theory and Practice of Medicine [in Russian], Moscow (1981).
5. A. Shatkin, in: Methods in Virology and Molecular Biology [in Russian], Moscow (1972), pp. 184-189.
6. C. Colby and M. J. Chamberlin, Proc. Natl. Acad. Sci. USA, 63, 160 (1969).
7. E. De Clercq, F. Eckstein, H. Sternbach, et al., Virology, 42, 421 (1970).
8. E. De Clercq, P. F. Torrence, B. D. Stollar, et al., Eur. J. Biochem., 88, 341 (1978).
9. H. B. Levy, G. Baer, S. Baron, et al., J. Infect. Dis., 132, 434 (1975).
10. O. H. Lowry, N. J. Rosebrough, et al., J. Biol. Chem., 193, 265 (1951).
11. W. E. Stewart, in: The Interferon System, Vienna (1979).