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Antiviral Activity of Partially Thiolated Polynucleotides

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SUMMARY

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Structural modification of poly(I)·poly(C) (thiolation of position 5 of some of the cytosine bases in polycytidylate) produces compounds which induce antiviral activity in both human and murine cells. Since increasing degrees of thiolation increase nuclease resistance, this study reaffirms the notion that enhanced nucleolytic resistance of double-stranded RNAs is not a critical property for interferon induction. Depletion of the full expression of the antiviral state in human cells occurred after washing the cells shortly after poly(I)·poly(C) exposure; addition of fresh poly(I)·poly(C) did not restore full antiviral function to the washed cells. However, prior treatment with thiolated complexes abolished this depletion. The thiolated polymers may prove to be useful in circumventing the hyporesponsiveness usually seen after repeated stimulation with the interferon inducer poly(I)·poly(C), in experimental viral infections.

INTRODUCTION

Inhibitory effects of partially thiolated polynucleotides on nucleic acid polymerases from *Micrococcus lysodeikticus* (1, 2), RNA tumor viruses (3, 4), cultured Burkitt lymphoma cells (5), and regenerating rat liver (6) have been previously noted. Polycytidylic acid, one of the components of a known antiviral agent, polyinosinic acid ·polycytidylic acid [poly(I)·poly(C)], after partial thiolation, strongly inhibits the DNA polymerases of Moloney murine sarcoma virus and Friend leukemia virus. Since polynucleotides with various structural modifications have proven useful in delineating the structural requirements for interferon induction (7-9), we have now evaluated the impact of thiolation of position 5 of some of the cytosine bases in polycytidylate on antiviral activity and interferon induction by poly(I)·poly(C). In addition, we determined the ability, if any, of poly(I)·mpC[1] to enhance, or compete with, the function of unmodified poly(I) ·poly(C). Since thiolated polymers contain a potentially reactive sulfhydryl group (1,

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[1] The abbreviations used are: mpC, thiolated polycytidylate; VSV, vesicular stomatitis virus; EMCV, encephalomyocarditis virus.

2), the opportunity for formation of a mixed disulfide bond with the postulated cell receptor protein (10) is evident: such a stable complex might result either in enhanced interferon induction or in actual blocking of the induction response if the unmodified 5-position of poly(C) were critical for interferon production.

MATERIALS AND METHODS

Preparation of partially thiolated polycytidylic acid. Poly(C) was modified according to the previously described general method for the thiolation of polynucleotides (2). Potassium polycytidylate was converted to the cetyltrimethylammonium bromide salt, which was then treated with methyl hypobromite in methanol at 0° for 1 hr, followed by reaction with sodium hydrosulfide in dimethylacetamide–methanol (1:1) at 0° for 1 hr under a nitrogen atmosphere. A 3 M NaCl solution was added to the reaction mixture, and the precipitated sodium polycytidylate was collected by centrifugation, washed, and dissolved in water. The thiolated product was further purified by gel filtration through both Sephadex G-25 and Bio-Gel A, 1.5 M. For enzymatic degradation studies, radioactive mpC was prepared using sodium [^{35}S]hydrosulfide in the thiolation step.

Structural characterization of partially thiolated polycytidylic acid. The products obtained by this procedure are high molecular weight (of the order of 1×10^6) polycytidylates containing 1–10% modified bases, i.e., 5-mercaptocytosines. The extent of modification can be controlled between the 1 and 10% limits by the amount of CH_3OBr used in the first reaction step. We determined the percentage of thiolated base either by radioactivity measurements of ^{35}S-labeled preparations or by the method of neutron activation analysis (2).

In order to determine the distribution of the 5-mercaptocytidylate units in a partially thiolated polymer, a ^{35}S-labeled mpC preparation was subjected to exhaustive digestion with micrococcal nuclease[2] (Worthington) according to Sulkowski *et al.* (11), followed by column chromatography of the hydrolysate on DEAE-cellulose using a linear gradient of NaCl (pH 7.5 buffer containing 0.001 M mercaptoethanol).

Annealing of poly(C) and mpC with poly(I). Poly(C) and mpC were annealed with poly(I) under the same conditions: 10 ml of poly(C) [1 mM in buffer A (10 mM phosphate, pH 7.2 at 25°, 150 mM NaCl, and 1 mM $MgCl_2$] and 10 ml of poly(I) (1 mM in the same buffer) were incubated at room temperature for 3 hr according to a method described previously (8). Melting profiles were recorded with a model 2400-S Gilford ultraviolet spectrophotometer.

Antiviral studies. Colorimetric assays for intracellular resistance to viral challenge and for extracellular interferon titers, as described previously (8), were carried out in human neonatal foreskin fibroblasts (passages 5–17), murine L-929 cells, or mouse peritoneal exudates. Human and L-929 cells were grown as monolayers in Eagle's minimal essential medium (12) supplemented with 5–10% fetal calf serum. Mouse peritoneal exudates were obtained by washing the peritoneum with phosphate-buffered NaCl and were collected by low-speed centrifugation; cells were resuspended at 2×10^6/ml in minimal essential medium.

Cells (all in monolayers except for mouse peritoneal cells) were exposed to the polynucleotide complexes in buffer A for 1 hr, washed, and reincubated in fresh medium at 37°. In experiments using polynucleotides applied sequentially, each incubation was also performed for 1 hr. To determine intracellular viral protection, vesicular stomatitis virus or encephalomyocarditis virus was used as the challenge virus. Subsequent cellular uptake of a vital dye, neutral red, was then used to determine the extent of viral cytopathogenicity (8).

RESULTS

The results of the degradation of the mpC sample are shown in Table 1. About 63% of the mpC was hydrolyzed to mono- and dinucleotides, but they contained only trace amounts of the modified base. Essen-

[2] We wish to thank Dr. Eugene Sulkowski for his thoughtful suggestions concerning the use of micrococcal nuclease for determination of distribution of thiolated bases in the modified polymers.

tially all the ^{35}S label was present in tri-, tetra-, and higher oligonucleotide fractions. In contrast, in parallel experiments in which unmodified poly(C) was the substrate, 96% of the polymer was hydrolyzed to mono- and dinucleotides; these were obtained in the characteristic 6:1 molar ratio as described previously (11). The remaining 4% was recovered in the tri- and tetracytidylate fractions. From this study we drew two conclusions: (a) the results are consistent with a random distribution of 5-mercaptocytosine bases in the modified homopolymer, and (b) thiolation of cytosine renders the phosphodiester bonds of the trinucleotide resistant to hydrolysis by micrococcal nuclease. If the mercapto group had been inserted nonrandomly (i.e., in "clusters"), large oligonucleotides would have been obtained, since thiolation of cytosine renders the phosphodiester bond resistant to hydrolysis by micrococcal nuclease.

The partially thiolated poly(C) preparations annealed with poly(I) to form stable double-helical complexes (Table 2), with T_m values substantially higher than the incubation temperature (37°) used in the antiviral study. Highly cooperative melting profiles were noted. However, some perturbation of double-helical structure, by introduction of the sulfhydryl group, was suggested, since the T_m progressively fell as the percentage of cytosine residues thiolated was increased.

Antiviral studies. Human neonatal fibroblasts, treated with unmodified or thiolated poly(I)·poly(C), were challenged with VSV. Poly(I)·poly(C) containing 1.7% and 3.5% 5-mercaptocytidylate units showed antiviral activity equal to, and in some cases (e.g., 5 μM) greater than, poly(I)·poly(C) (Table 3). However, further thiolation resulted in a decrease of intracel-

TABLE 1

Analysis of peak fractions from column chromatography of hydrolysate of mpC-16 with micrococcal nuclease

mpC-16 was digested by micrococcal nuclease as described under MATERIALS AND METHODS. Total radioactivity used for digestion and applied on the column was 9.675×10^6 cpm. Total radioactivity recovered was 9.34×10^6 cpm (97% recovery).

Peak	Fractions	No. of nucleotide units	Absorbance at 270 nm	Radioactivity	5-Mercaptocytidine content
			% total	%	%
mpC-16			100	100	10.1
1	22–30	1	49.7	1.99	0.828
2	40–50	2	12.9	2.14	1.28
3	53–62	3	4.52	4.32	7.49
4	63–72	4	4.74	5.21	8.54
5–11	73–160	≥5	29.2	79.53	≧10.1

TABLE 2

Melting characteristics of different samples of mpC annealed with poly(I)

Annealing of poly(I) with mpC was carried out as described under MATERIALS AND METHODS.

Sample (5-mercaptocytidylate content)	T_m	Hyperchromicity
		%
Poly(C)	65.3°	71
mpC (1.2%)	64.4°	55
mpC (1.7%)	65.5°	56
mpC (3.5%)	57.6°	41
mpC (5.8%)	50.4°	34
mpC (8.1%)	54.2°	33
mpC (9.3%)	50.0°	39

TABLE 3

Antiviral activity of thiolated polynucleotides in human fibroblasts

Inducer (5-mercaptocytidylate content)	Concentration	Protection
	μM	%
Poly(I)·poly(C) (0%)	5	52
	50	95
	500	95
Poly(I)·mpC (1.7%)	5	100
	50	100
	500	100
Poly(I)·mpC (3.5%)	5	100
	50	100
	500	100
Poly(I)·mpC (5.8%)	5	15
	50	27
	500	61
Poly(I)·mpC (8.1%)	5	42
	50	65
	500	76

TABLE 4

Effects of prior incubation with thiolated polymers on poly(I)·poly(C) antiviral activity in human fibroblasts

Group	Inducer I (5-mercapto-cytidylate content)		Inducer II (5-mercapto-cytidylate content)	Concentration of each	Protection		Interferon (Expt. A)
					Expt. A	Expt. B	
				μM	%	%	*units/ml*
1	Poly(I)·poly(C) (0%)			0.1	0		
				1	31	14	
				10	46	44	
				100	80	89	11
2	Poly(I)·poly(C) (0%)	→	Poly(I)·poly(C) (0%)	1	6	0	
				10	17	33	
				100	39	80	0
3	Poly(I)·poly(C) (0%)			2	35		
				20	42		
				200	70		38
4	Poly(I)·mpC (1.2%)			0.1	0		
				1	40		
				10	71		
				100	75		2
5	Poly(I)·mpC (1.2%)	→	Poly(I)·mpC (1.2%)	1	21		
				10	39		
				100	59		0
6	Poly(I)·mpC (1.2%)			2	60		
				20	80		
				200	75		0
7	Poly(I)·mpC (9.3%)			0.1	0		
				1	33		
				10	39		
				100	45		0
8	Poly(I)·mpC (9.3%)	→	Poly(I)·mpC (9.3%)	1	19		
				10	32		
				100	32		0
9	Poly(I)·mpC (9.3%)			2	38		
				20	51		
				200	44		0
10	Poly(I)·mpC (1.2%)	→	Poly(I)·poly(C) (0%)	1	17	4	
				10	44	62	
				100	75	100	8.5
11	Poly(I)·mpC (9.3%)	→	Poly(I)·poly(C) (0%)	1	0	0	
				10	61	37	
				100	61	98	19
12	Poly(I)·mpC (1.2%) + poly(I)·poly(C) (0%)			1	45		
				10	83		
				100	85		39

TABLE 4—*Continued*

Group	Inducer I (5-mercapto-cytidylate content)	Inducer II (5-mercapto-cytidylate content)	Concentration of each	Protection		Interferon (expt. A)
				Expt. A	Expt. B	
			μM	%	%	*units/ml*
13	Poly(I)·mpC (9.3%) + poly(I)·poly C (0%)		1	32		
			10	83		
			100	90		14
14	Poly(C) (0%)		1	0	0	
			10	0	0	
			100	0	0	0
15	mpC (1.2%)		1	0	0	
			10	0	0	
			100	0	0	0
16	mpC (9.3%)		1	0	0	
			10	0	0	
			100	0	0	0
17	Poly(C) (0%)	→ Poly(I)·poly(C) (0%)	1	0	4	
			10	2	34	
			100	68	100	13
18	mpC (1.2%)	→ Poly(I)·poly(C) (0%)	1	0	4	
			10	3	57	
			100	60	100	19
19	mpC (9.3%)	→ Poly(I)·poly(C) (0%)	1	0	3	
			10	4	58	
			100	78	98	11

lular antiviral activity, presumably because of a reduction in intracellular interferon.

To determine whether thiolated polymers might augment or compete with the triggering of an interferon response by poly(I)·poly(C), human fibroblasts were treated with single- or double-stranded polymers (0, 1.2, or 9.3% thiolation) and incubated for 1 hr at 37°. After washing, cells were then exposed to poly(I)·poly(C) and subsequently challenged with VSV. We reasoned that any loss of antiviral activity, normally elicitable by unmodified poly(I)·poly(C), would be consistent with binding of the modified compound near the receptor site. Several interesting results were uncovered.

1. Resistance to VSV was greater (as measured colorimetrically) in human cells exposed to poly(I)·poly(C) or poly(I)·mpC (1.2%) than in cells treated with complexes containing greater degrees of thiolation (Table 4). However, all modified polymers induced less *extracellular* interferon at 100 *μM* than unmodified poly(I)·poly(C) (compare groups 1, 4, and 7).

2. All human cells receiving two consecutive exposures to an inducer showed less antiviral activity than similar cells stimulated once; thus groups 2, 5, and 8 showed less viral protection than groups 1, 4, and 7. The reduction in protection was apparently not due to the cumulative concentration of inducer, since cells treated once with 2-fold higher dosages did not show this reduced protection (groups 3, 6, and 9). To determine why this loss of activity occurred, we then treated human cells with poly(I)·poly(C) for either (a) 1 hr, (b) 2 hr, (c) 1 hr, followed by washing and an additional 1-hr exposure to fresh poly(I)·poly(C) [poly(I)

·poly(C) → poly(I)·poly(C)], or (d) 1 hr, followed by washing and then incubation in buffer for 1 hr [poly(I)·poly(C) → buffer] prior to VSV challenge. A loss of activity occurred in both groups (c) and (d) (Table 5), suggesting that within 1 hr after the removal of unbound poly(I)·poly(C) a factor, presumably necessary for the chain of events ultimately leading to a stable antiviral state, was depleted from the cells by washing. (Control experiments measuring cell growth and quantitative uptake of supravital stains indicated that subtle cytotoxicity did not occur during the washing step.) Presumably the failure to restore antiviral activity completely with a second poly(I)·poly(C) exposure [group (c)] was due to the hyporesponsive state usually seen with multiple exposures to double-stranded RNAs (10).

3. The incubation sequence poly(I)·mpC → poly(I)·poly(C) (Table 4, groups 10 and 11) provided 1-2-fold greater final antiviral protection than was obtained with the sequence poly(I)·poly(C) → poly(I)·poly(C) (group 2), and gave protection similar to that obtained with the sequence poly(I)·mpC → poly(I)·mpC (group 5). Moreover, although the sequence poly(I)·poly(C) → poly(I)·poly(C) resulted in an apparent reduction in the anticipated extracellular interferon production, no diminution in extracellular interferon could be detected with the sequence poly(I)·mpC → poly(I)·poly(C) (groups 2, 10, and 11).

4. Double-stranded, thiolated complexes incubated simultaneously with poly(I)·poly(C) effected the protection anticipated by adding any of the complexes singly (compare groups 1, 4, and 7 with 12 and 13). Thus no apparent synergism could be detected, as was measured in the sequential addition experiments noted above. Finally, the single-stranded polymers alone provided no antiviral effect, emphasizing the absolute need for a double-helical complex (7). Preliminary incubation experiments verified that these single-stranded complexes not only were inactive as inducers but could not effectively compete for the attachment sites of poly(I)·poly(C) (groups 14–19).

In certain murine cells, particularly L-cells (a long-established line), a partially thiolated (1.7%) complex was somewhat less active as an inducer of extracellular interferon than unmodified poly(I)·poly(C) (Table 6) as measured using EMC as the challenge virus. Borrowing from the experimental approach developed in human cells (Table 4), a more detailed

TABLE 5

Effects of washing human fibroblasts after exposure to poly(I)·poly(C)

Group	Inducer			Concentration	Protection
				μM	%
(a)	Poly(I)·poly(C) (1 hr)			1	86
				10	94
				100	94
(b)	Poly(I)·poly(C) (2 hr)			1	87
				10	93
				100	100
(c)	Poly(I)·poly(C) (1 hr)	→	Poly(I)·poly(C) (1 hr)	1	60
				10	72
				100	73
(d)	Poly(I)·poly(C) (1 hr)	→	Buffer (1 hr)	1	39
				10	72
				100	72

TABLE 6

Interferon production by thiolated polynucleotides in mouse cells

Inducer (5-mercaptocytidylate content)	Concentration	Interferon	
		In L-cells	In mouse peritoneal exudates
	μM	*units/ml*	*units/ml*
		<4 (<4)[a]	<4 (<4)
Poly(I)·poly(C) (0%)	60	128 (128)	64 (64-128)
Poly(I)·mpC (1.7%)	60	32 (64)	32 (32-64)

[a] ( ) indicates results of a repeat experiment.

study in the L-cell showed, surprisingly, no loss of protection against VSV when cells were exposed to the inducer sequence poly(I)·poly(C)→poly(I)·poly(C) (Table 7, groups 1 and 2). This implied either that in the L-cell, unlike the human cell, poly(I)·poly(C) did not induce a factor which was depletable by a washing step or, alternatively, that exposure to a second inducer fully restored the antiviral state (i.e., the hyporesponsive state was not seen) (13). Partially thiolated poly(I)·poly(C) was fully as active as poly(I)·poly(C) in terms of intracellular protection (groups 1 and 3). Single-stranded products, as expected, were inactive inducers and furthermore did not abort subsequent induction by active inducers (groups 9, 11, 13, and 14). Finally, sequential addition experiments (groups 7, 8, 13, and 14) revealed no enhancement, or antagonism, in protection when unmodified and partially thiolated complexes were present at different times. Thus, in several important respects, murine L-cells behave differently from human cells, in which partially thiolated complexes displayed both (a) modestly enhanced antiviral activity [relative to poly(I)·poly(C)] and (b) an ability to abort the anticipated reduction in interferon yield seen when cells are multiply stimulated with poly(I)·poly(C).

DISCUSSION

We have shown that partially thiolated, double-stranded polymers are antiviral in both murine and human cells. Presumably part or all of the protection conferred is related to interferon production, since extracellular interferon can be regularly detected and only double-stranded complexes show activity. Indeed, complexes with lower degrees of thiolation (1.2–3.5%) are actually somewhat more active than unmodified poly(I)·poly(C) in human cells, although the enhancement in specific activity is modest (1–2-fold). With further thiolation at position 5 the enhancement is lost, but strong antiviral activity continues to exist in both murine and human cells. Since further thiolation actually strongly increases nuclease resistance,[3] the current study reaffirms the notion that enhanced nuclease resistance of double-stranded RNAs does not necessarily provide a superior interferon inducer (8), nor in fact is this property a critical one in inducer activity (14). Double-stranded RNAs are now known to trigger many reactions in biological systems in addition to interferon induction (for review, see ref. 7), and further studies are clearly necessary to determine whether the thiolated complexes may actually provide an improved therapeutic ratio in experimental viral chemotherapy systems.

In this study we also noted a difference in interferon induction by double-stranded RNAs between human and certain mouse cells, namely, the ability to deplete the full expression of the antiviral state in human cells by washing shortly after poly(I)·poly(C) exposure; the addition of fresh poly(I)·poly(C) complexes did not restore this full antiviral function to washed cells. Most interestingly, the use of poly(I)·mpC complexes abolished this depletion; i.e., the sequence poly(I)·mpC → poly(I)·poly(C) resulted in full antiviral activity. This obviously points to a difference in interferon triggering by poly(I)·mpC when compared with unmodified poly(I)·poly(C), which may prove practical in circumventing the hyporesponsiveness, or resistance to repeated stimulation with interferon inducers, usually encountered with repeated poly(I)·poly(C) treatments in experimental viral infections (10). Studies

[3] Unpublished observations.

TABLE 7

Effects of prior incubation with thiolated polymers on poly(I)·poly(C) antiviral activity in mouse L-cells

Group	Inducer I (5-mercapto-cytidylate content)		Inducer II (5-mercapto-cytidylate content)	Concen- of each	Protection
				μM	%
1	Poly(I)·poly(C) (0%)			1	29
				10	45
				100	87
2	Poly(I)·poly(C) (0%)	→	Poly(I)·poly(C) (0%)	1	23
				10	32
				100	82
3	Poly(I)·mpC (1.2%)			1	18
				10	51
				100	83
4	Poly(I)·mpC (1.2%)	→	Poly(I)·mpC (1.2%)	1	25
				10	39
				100	90
5	Poly(I)·mpC (9.3%)			1	10
				10	27
				100	76
6	Poly(I)·mpC (9.3%)	→	Poly(I)·mpC (9.3%)	1	14
				10	33
				100	72
7	Poly(I)·mpC (1.2%)	→	Poly(I)·poly(C) (0%)	1	32
				10	46
				100	97
8	Poly(I)·mpC (9.3%)	→	Poly(I)·poly(C) (0%)	1	24
				10	71
				100	67
9	Poly(C) (0%)			1	0
				10	0
				100	0
10	mpC (1.2%)			1	0
				10	0
				100	0
11	mpC (9.3%)			1	0
				10	0
				100	0
12	Poly(C) (0%)	→	Poly(I)·poly(C) (0%)	1	25
				10	57
				100	82
13	mpC (1.2%)	→	Poly(I)·poly(C) (0%)	1	31
				10	37
				100	100
14	mpC (9.3%)	→	Poly(I)·poly(C) (0%)	1	31
				10	54
				100	100

are now under way to determine whether inducer stability at its receptor site—as through a possible mixed disulfide bond with a protein receptor—may account for this unusual functional property of poly(I)·mpC.

REFERENCES

1. Bardos, T. J., Baranski, K., Chakrabarti, P., Kalman, T. I., Novak, L. & Mikulski, A. J. (1972) *Proc. Am. Assoc. Cancer Res.*, **13**, 359.
2. Mikulski, A. J., Bardos, T. J., Chakrabarti, P., Kalman, T. I. & Zsindely, A. (1973) *Biochim. Biophys. Acta*, **319**, 294-303.
3. Chandra, P. & Bardos, T. J. (1972) *Res. Commun. Chem. Pathol. Pharmacol.*, **4**, 615-622.
4. Srivastava, B. I. S. (1973) *Biochim. Biophys. Acta*, **335**, 77-84.
5. Strivastava, B. I. S. & Bardos, T. J. (1973) *Life Sci.*, **13**, 47-53.
6. Bardos, T. J., Chakrabarti, P., Mikulski, A. J. & Zsindely, A. (1973) *Proc. Am. Assoc. Cancer Res.*, **14**, 482.
7. Carter, W. A. & De Clercq, E., *Science*, in press.
8. Carter, W. A., Pitha, P. M., Marshall, L. W., Tazawa, I., Tazawa, S. & Ts'o, P. O. P. (1972) *J. Mol. Biol.*, **70**, 567-587.
9. De Clercq, E., Stewart, W. E. & De Somer, P. (1973) *Virology*, **54**, 278-282.
10. Bausek, G. H. & Merigan, T. C. (1970) *Proc. Soc. Exp. Biol. Med.*, **134**, 672-676.
11. Sulkowski, E., Odlyzko, A. M. & Laskowski, M., Sr. (1970) *Anat. Biochem.*, **38**, 393-400.
12. Eagle, H. (1959) *Science*, **130**, 432-437.
13. De Clercq, E. & De Somer, P. (1973) *J. Gen. Virol.*, **19**, 113-123.
14. Colby, C., Chamberlin, M. J., Duesberg, P. H. & Simon, M. I. (1971) in *Biological Effects of Polynucleotides*, (Beers, R. F., Jr., ed.), pp. 79-87, Springer, New York.