Mechanisms of DEAE-Dextran Enhancement of Polynucleotide Induction of Interferon[1] (35440)

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In previous reports (1, 2) it has been shown that diethylaminoethyl dextran (DE AE-dx) strongly enhances the production of interferon (IF) and antiviral resistance which is induced by polynucleotides such as polyinosinic·polycytidylic acid (In·Cn) (3). Studies on the mechanisms underlying the enhancing effect of this compound (2) showed that DEAE-dx exerts (a) a substantial protection of the inducer against extracellular RNase and (b) a cell-mediated effect which may be related to increased permeability for the large inducer molecules. It has not been established which of the two has a greater influence.

To help determine the relative importance of these factors we examined the enhancing effect of DEAE-dx on a RNase-resistant form of In·Cn.

Materials and Methods. Polyinosinic and polycytidylic acids (P-L Biochemicals) were processed using pyrogen-free materials to obtain the double-stranded complex, In·Cn (3). They were sterilized by filtration through Millipore filters. DEAE-dx (Pharmacia, M. W. 2×10^6) was dissolved in 0.15 *M* phosphate-buffered saline (PBS) and the pH was adjusted to 7.2 with NaOH.

Mouse L cells were used as previously described (1) both for production and for titration of IF. The fluids were harvested for IF assay 12 hr after the exposure to inducer. Antiviral resistance was determined by inhibition of yield of GD-VII virus hemagglutinin (C. Buckler, H. Oie, D. Hill, and S. Baron, unpublished observations) after a single growth cycle (4).

The formation of a complex between In·Cn and DEAE-dx was performed by mixing equal volumes of different concentrations of the two substances as indicated in results. The sensitivity to RNase was tested as previously described (2) using pancreatic RNase (Worthington RAF, 1 μg/ml) in 0.15 *M* NaCl.

Results. Formation of an RNase-resistant form of In·Cn by complexing In·Cn with DEAE-dx complex. Preliminary experiments showed that when In·Cn and DEAE-dx were mixed at a weight ratio of less than 1:10 a very heavy precipitate occurred. However, if the concentration of DEAE-dx was increased to a ratio of 1:10 or greater, no formation of precipitate was observed and the mixture acquired only a slight opalescence. In further experiments a solution of In·Cn containing 200 μg/ml was mixed at 4° with the same volume of a solution of DEAE-dx (2 mg/ml). The centrifugation of the mixture at 100,000*g* for 4 hr in a Spinco model L refrigerated centrifuge resulted in a well defined pellet which was carefully rinsed with PBS, resuspended in the same volume of PBS using a Dounce homogenizer, and centrifuged again. The final pellet was resuspended in PBS and was used in the experiments. Practically all of the In·Cn present in the mixture was recovered in the pellet. The optical density (OD) at 243 and 263 mμ of the starting material, supernates, and sedimented fraction demonstrated that no RNA was released from the pellet into the wash

[1] This work was supported in part by Grant 11510/56 of Consiglio Nazionale delle Ricerche. It was presented in preliminary form at the International Association of Microbiology Societies 31st Symposium, Standardization of Interferon and Interferon Inducers, London, October, 1969.

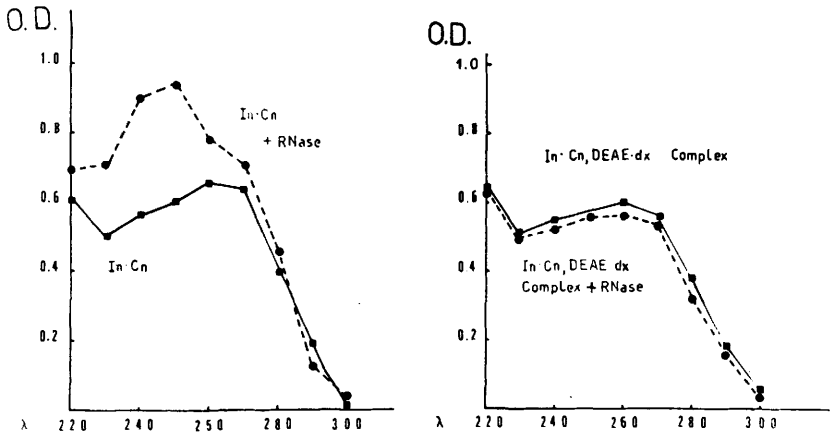

FIG. 1. The effect of complexing In·Cn with DEAE-dextran on resistance to ribonuclease. Details in text.

fluids. When the same concentration of In·Cn alone or DEAE-dx alone was centrifuged, no pellet was observed. These data were interpreted to indicate that under these conditions a relatively clear, suspended complex between In·Cn and DEAE-dx is formed. If this complex was resistant to RNase then it could be used to help determine whether an RNase-resistant form of In·Cn is enhanced as an interferon inducer by DEAE-dx treatment of cells.

RNase resistance of the In·Cn–DEAE-dx complex. In order to test the sensitivity to RNase of the In·Cn included in the complex in comparison with the uncomplexed In·Cn, the two materials, each containing 50 μg of In·Cn, were treated with pancreatic RNase (Worthington RAF, 1 μg/ml) at 37° in the presence or absence of DEAE-dx (200 μg/ml). The pH of the mixture was 7.2. The resulting degraded material was measured 30 min later on the basis of increase of OD.

As shown in Fig. 1, the OD increased in the uncomplexed In·Cn treated with RNase. Although not shown in Fig. 1, this increase was prevented by the addition of DEAE-dx. However the OD of the complex did not increase even in the absence of added DEAE-dx indicating that the complex was not degraded by RNase.

The residual resistance-inducing activity of the two kinds of material, after treatment with RNase, was determined in L cells. The results are shown in Table I. It can be seen that the resistance-inducing activity of In·Cn was destroyed by RNase treatment but the presence of DEAE-dx prevented this effect.

TABLE I. Effect of RNase on Antiviral Activity of In·Cn and In·Cn–DEAE-dx Complex, With and Without Extra DEAE-dx.

Inducer	Treatment before induction	Log_{10} inhibition of GD-VII virus (HA yield) at conc of inducers (μg/ml)[a]		
		5.0	0.5	0.05
In·Cn	30 min at 37°	2.1	2.1	2.1
	RNase (1 μg/ml)	0.6	0.3	0.0
	RNase + DEAE-dx[b]	2.1	2.1	2.1
In·Cn–DEAE-dx	30 min at 37°	2.1	2.1	2.1
	RNase	2.1	2.1	2.1
	RNase + DEAE-dx	2.1	2.1	2.1

[a] Assayed in the presence of DEAE-dx.

[b] 50 μg/ml final concentration of DEAE-dx added to In·Cn prior to RNase.

TABLE II. Induction of IF Antiviral Resistance by In • Cn and by the In • Cn–DEAE-dx Complex in the Presence or Absence of Extra-Added DEAE-dx.

Inducer	Conc of In • Cn (μg/ml)	Addition of DEAE-dx (200 μg/ml)	IF yield (units/ml)	GD-VII virus HA yield $\log_{10}$	$\log_{10}$ inhibition of GD-VII virus yield (HA)
None		—	<10	2.7	None
		+	<10	2.7	None
In • Cn–DEAE-dx complex	300	—	7000	<0.6	>2.1
	30	—	400	<0.6	>2.1
	3	—	<10	2.4	0.3
	30	+	4000	<0.6	>2.1
	3	+	2000	<0.6	>2.1
In • Cn	30[a]	—	<10	2.7	None
	30	+	3000	<0.6	>2.1
	3	+	2000	<0.6	>2.1

[a] In other experiments, 300 μg/ml of In • Cn gave results identical to those observed for 30 μg/ml.

In contrast the resistance-inducing activity of the complexed form of In•Cn was completely resistant to RNase even in the absence of added DEAE-dx and the addition of more DEAE-dx did not modify its behavior.

Determination of IF-inducing activity of the RNase-resistant complex. The IF-inducing activity of the complex in comparison with uncomplexed In•Cn was studied in L cells in the presence or the absence of additional DEAE-dx. The results are shown in Table II.

In general the complex was more active as an IF inducer than was In•Cn alone and the addition of DEAE-dx to the medium increased the IF-inducing capacity of the complex. Specifically 300 and 30 μg/ml of In•Cn induce no IF or antiviral resitance. In comparison the same concentrations of In•Cn in the complex induced high levels of IF and antiviral resistance. When DEAE-dx was added to the culture medium containing the RNase-resistant complex at In•Cn concentrations of 30 or 3 μg/ml, there was a marked enhancement of IF production and antiviral resistance.

The inhibitor produced was shown to have the properties of IF. It was (i) resistant to pH 2 treatment; (ii) trypsin sensitive; (iii) active on viruses other than GD-VII; (iv) inactive on heterologous chick embryo cells; (v) without antiviral effect when mixed directly with GD-VII virus; (vi) active after washing the cells with which it had reacted; and (vii) inactive in L cells pretreated with actinomycin D, 1 μg/ml.

Discussion and Summary. The present results demonstrate that, in the presence of an excess of DEAE-dx, In•Cn does not precipitate but it complexes with the polycation in a way which makes it resistant to pancreatic RNase. The formation of this type of complex may occur with other polycationic substances. Preliminary experiments show that high concentrations of neomycin produce a soluble complex, and poly-*d*-lysine at a change ratio of >30:1 also produces a soluble In•Cn-polycation (J. M. Rice, personal communication). It was also found that the induction of IF by the RNase-resistant complex was potentiated by the addition of DEAE-dx to the cell cultures. This indicates that protection of In•Cn against enzymatic degradation is not the sole mechanism by which IF production can be enhanced by DEAE-dx. It implies that DEAE-dx causes a large part of its enhancement by an effect on cells. Conceivably this could involve increased cellular uptake of the In•Cn as DEAE-dx does for other RNAs (5–7) but the need for such uptake has not been established. Additional evidence for a cell-mediated action of DEAE-dx comes from the previous observation that pretreatment of

cells with DEAE-dx gave a maximal enhancing effect which could be abolished by brief contact of the treated cells with the polyanionic dextran sulfate (2).

An RNase-resistant form of In·Cn may be useful in stimulating IF under conditions where RNase destruction of In·Cn is an important factor, such as in certain *in vivo* applications. This view is supported by the finding that sera from different species contain different amounts of depolymerizing enzymes and that some of them, including human serum, exert a substantial destructive effect on In·Cn (6).

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Received Oct. 13, 1970. P.S.E.B.M., 1971, Vol. 136.