Interferon Inducing Activity of Polyinosinic Acid

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SUMMARY

Although poly(I) is generally considered to be inactive as an interferon inducer, we have found several authentic poly(I) preparations to be effective inducers. Their interferon inducing ability varied considerably from one cell system to another. In human diploid fibroblasts, primed with interferon and superinduced by cycloheximide and actinomycin D, all active poly(I) samples proved nearly as effective in inducing interferon as poly(I).poly(C). In primary rabbit kidney cell cultures, the active poly(I) samples were either as active, or 3 to 30 times less active than poly(I).poly(C). In intact rabbits they were 100 times less active than poly(I).poly(C). Except for one particular sample, all active poly(I) preparations were inferior to poly(I).poly(C) when assayed for interferon induction in interferon-treated mouse L cells; in DEAE-dextran-treated L cells, they induced little, if any, interferon. The poly(I) inducers of interferon were considerably more susceptible to degradation by T1 ribonuclease, pancreatic ribonuclease and human serum nuclease(s) than was poly(I).poly(C) when assayed under the same conditions. Due to their limited half-life time in biological fluids, poly(I) analogues such as those described here may offer a greater safety margin in clinical use than poly(I).poly(C).

INTRODUCTION

Although double-strandedness is considered to be a major prerequisite for the ability of polynucleotides to induce interferon (De Clercq, 1974), some 'single-stranded' homopolynucleotides such as poly(I) and poly(C)* have been reported to induce interferon in both cell cultures and rabbits (Baron et al. 1969). The activities of the single homopolymers varied considerably from one batch to another (Baron et al. 1969), but a marked increase in interferon induction was observed if the homopolymers poly(I) and poly(C) were mixed with polybasic substances such as DEAE-dextran, methylated albumin, neomycin, streptomycin, protamine or poly(D-lysine) (Billiau et al. 1969; Pitha & Pitha, 1974).

Whether the homopolymers themselves rather than double-stranded RNA contaminants present in the homopolymer preparations were responsible for the induction of interferon by

* Abbreviations: Poly(I), poly(inosinic acid); poly(C), poly(cytidylic acid); poly(A), poly(adenylic acid); poly(U), poly(uridylic acid); poly(rT), poly(ribothymidylic acid); poly(s*C), poly(2-thiocytidylic acid); poly(dIz), poly(2'-azido-2'-deoxyinosinic acid); poly(I)x, poly(O)3x+ and poly(C)3x+ preparations with sedimentation values (s20,w) of 2.5S, 13.2S and 3.1S respectively; PRK, primary rabbit kidney; HSF, human skin fibroblast.
poly(I) and poly(C) became a matter of conjecture (Field et al. 1968). Baron et al. (1969) proved experimentally that at least some of their active poly(I) and poly(C) preparations were not contaminated by double-stranded RNA [poly(I).poly(C)].

Although poly(I) proved consistently more active than poly(C) and, in some conditions, even as active as poly(I).poly(C) (Pitha & Pitha, 1974), the interferon inducing potential of poly(I) has not received due attention. Recently, we found two newly synthesized poly(I) preparations particularly effective in inducing interferon in PRK cell cultures (Thang et al. 1977). Other poly(I) preparations, made under identical conditions, did not induce interferon. The purity of all these poly(I) preparations was greater than 99.5%, and all preparations showed the same u.v. and circular dichroism spectra and behaved identically in forming complexes with either poly(C), poly(A) or poly(A).poly(U) (Thang et al. 1977). However, the active poly(I) preparations reacted with antibodies to double-stranded RNA, whereas the inactive samples failed to do so (Stollar et al. 1978). The interferon inducing properties of the active poly(I) samples are further described in the present report.

**METHODS**

*Polyonucleotides.* The poly(I) samples were synthesized with purified *Escherichia coli* polynucleotide phosphorylase (PNPase) covalently linked to CNBr-activated agarose beads. After fixation of the enzyme, the agarose beads were inactivated with ethanolamine, then washed extensively with 0.1 M-carbonate, pH 9.0, 1 M-NaCl and finally equilibrated with 0.1 M-tris-HCl, pH 8.0. The activity of the agarose-bound PNPase was approx. 1 unit per mg agarose wet weight. The polymerization of IDP was performed under the following conditions: 0.1 M-tris-HCl, pH 8.0, 10 mM-IDP, 5 mM-MgCl₂, 0.1 mM-EDTA and 1 unit PNPase/ml. The incubation mixture was stirred gently at 37 °C for 12 h. The agarose-bound PNPase was recovered by centrifugation. The polymer [poly(I)] was separated from the monomer (IDP) by ethanol precipitation and Sephadex G-100 filtration, and finally dialysed successively against 0.01 M-phosphate buffer, pH 7.4 (containing 0.15 M-NaCl), 1 mM-EDTA, pH 7.4, and distilled water. The poly(I) was then lyophilized and stored at −20 °C.

The physico-chemical properties (including the apparent sedimentation coefficients) and the immunochemical properties of the poly(I) preparations have been described previously (Thang et al. 1977; Stollar et al. 1978). Some poly(I) samples were provided by Choay Laboratories (Paris, France) (batches 315, 332 and 338), Miles Laboratories (Elkhart, Indiana) or P-L Biochemicals (Milwaukee, Wisconsin). Prior to use, the poly(I) samples were dissolved in Dulbecco’s phosphate buffered saline (PBS) at 1 mg/ml and stored at 4 °C. The ‘standard’ poly(I).poly(C) complex was constituted from its homopolymers which were both obtained from P-L Biochemicals. The sedimentation coefficients (s₂₀,₀) of poly(I) P-L and poly(C) P-L were 9.4S and 10.0S, respectively. Poly(I).poly(C) P-L, poly(I) P-L and poly(C) P-L were stored at −20 °C at 1 mg/ml in PBS.

*Enzymes.* Pancreatic ribonuclease (bovine pancreatic ribonuclease A crystallized five times, 80 Kunitz units/mg) was purchased from Sigma Chemical Co. (St Louis, Missouri) and T1 ribonuclease (470000 units/mg) from Worthington Biochemical Corp. (Freehold, New Jersey).

*Interferon induction.* The methodology for measuring interferon production has been described previously: for interferon induction in primary rabbit kidney (PRK) cells (super-induced with cycloheximide and actinomycin D), see De Clercq et al. (1974); for interferon induction in human skin fibroblast (HSF) cells, VGS strain (primed with interferon and
Interferon induction by poly (I)

superinduced with cycloheximide and actinomycin D), see De Clercq & Torrence (1977); for interferon induction in mouse L-929 cells (primed with interferon or pre-treated with DEAE-dextran), see De Clercq & De Somer (1975); for interferon induction in rabbits, see De Clercq et al. (1976). All interferon data represent average values for three or more separate determinations on different occasions, and all interferon titres are expressed in terms of the appropriate research reference standards.

Pyrogenicity. Pyrogenicity measurements were performed in rabbits, as described in the Pharmacopée Française [IXth edn., vol. II, pp. 235–238 (1972)]. The polynucleotides were injected intravenously (three rabbits per group). Each rabbit received 0.5 mg polynucleotide [0.1 mg for poly(I), poly(C)] in 0.5 ml non-pyrogenic isotonic NaCl solution (warmed up to 38.5 °C before injection). The initial temperature was determined as the average of two measurements made with a 30 min interval during the 40 min period before the injection. After the injection, the temperature was measured every 30 min over 3 h. The pyrogenic response of the rabbit was defined as the difference between the highest temperature recorded during this 3 h period and the initial temperature.

Treatment with enzymes. The sensitivity of the polynucleotides toward nucleases was monitored by measuring the residual ability of the polymers to induce interferon in PRK cells.

Degradation by T1 ribonuclease. Polynucleotide solutions at 10 µg/ml [in Eagle’s minimal essential medium (MEM)+1 mM-EDTA] were incubated for 1 h at 37 °C in the presence of varying T1 ribonuclease concentrations. For poly(I) CV 2 and poly(I).poly(C), the polynucleotide–enzyme mixtures were then applied to PRK cells and assayed for interferon induction, as described above. For poly(I) P-L, the polynucleotide–enzyme digest was first incubated for another 1 h at 37 °C with 10 µg/ml of poly(C) and then applied to PRK cells. For poly(C), the polynucleotide–enzyme digest was directly incubated on the cells for 1 h; this incubation period was followed by another 1 h incubation of the cells with 10 µg/ml of poly(I) P-L, after which the cells were processed for interferon induction.

Degradation by pancreatic ribonuclease A. A similar procedure was employed. Polynucleotide solutions of 10 µg/ml in MEM + 1 mM-EDTA were incubated for 1 h at 37 °C in the presence of varying concentrations of pancreatic ribonuclease A. For poly(I) o9, poly(I) CV 2 and poly(I).poly(C), the polynucleotide–enzyme mixtures were directly assayed for interferon induction in PRK cells. For poly(I) o9, the incubation of the cells with the polymer–enzyme digest was followed by another 1 h incubation of the cells with 10 µg/ml of poly(C), after which the cells were processed for interferon induction. For poly(C), the polymer–enzyme digest was first incubated for 1 h at 37 °C with 10 µg/ml of poly(I) P-L before being assayed for interferon induction. For poly(I) P-L, the polynucleotide–enzyme mixture was directly incubated on the cells for 1 h; after the cells had been treated for another 1 h period with 10 µg/ml of poly(C), they were processed for interferon induction.

The design of the experiments described above rests on the premise that poly(I) and poly(C), when added to the cells with a 1 h interval, reunite at the cellular level to form the interferon inducing complex poly(I).poly(C) (De Clercq & De Somer, 1972).

Degradation by nucleases present in human serum. The polynucleotides were first incubated for 1 h at 37 °C in the presence of varying concentrations of human serum and then assayed for residual interferon inducing activity in HSF cells.
RESULTS

Primary rabbit kidney cells

Out of nine different poly(I) preparations tested previously, only two samples [designated poly(I) o9 and CV 2] induced a significant amount of interferon when assayed at 10 μg/ml in PRK cells superinduced with cycloheximide and actinomycin D (Thang et al. 1977). Table 1 (a) presents the dose-response relationship of the interferon titres obtained with these two poly(I) preparations. At all doses tested, they were 3 to 10 times less active than the ‘standard’ poly(I).poly(C) preparation. Two additional poly(I) preparations (designated 428 and 429) have now been obtained which induced high amounts of interferon in PRK cells (Table 1 a). One of these preparations, poly(I) 429, was even more active than poly(I).poly(C) when assayed at the lower dosage range (0.1 to 1 μg/ml).

Human diploid fibroblast cells

In human skin fibroblasts which were primed with interferon and superinduced by cycloheximide and actinomycin D, several poly(I) preparations (CV 2, 428, 429) were almost as active, or even more active, in inducing interferon as poly(I).poly(C) (Table 1 b). At higher dose levels (10 to 100 μg/ml), these poly(I) preparations and poly(I).poly(C) had comparable interferon inducing activities. At a lower dose (0.1 μg/ml), only poly(I) 429 and poly(I).poly(C) proved effective in inducing interferon. Other poly(I) preparations (10, 11, 332, 427 and Miles) were entirely ineffective in inducing interferon in human cells.

Mouse L-929 cells

Four poly(I) samples (o9, CV 2, 428 and 429) induced interferon in interferon-primed L-929 cells at concentrations of 2 to 20 μg/ml or greater (Table 1 c). Other poly(I) preparations, e.g. poly(I) P-L, were ineffective as inducers, even at concentrations of 250 μg/ml. At the higher dose levels (10 to 250 μg/ml), the interferon inducing activity of poly(I) 429 slightly exceeded that of poly(I).poly(C). Both interferon responses tended to level off from a polymer concentration of 10 μg/ml onward. At the lower dosage range (0.08 to 0.4 μg/ml), poly(I).poly(C) induced interferon, whereas poly(I) 429 did not (Table 1 c).

The interferon inducing potency of poly(I).poly(C) in interferon-treated L cells is accompanied by a marked toxic alteration of the cells (De Clercq & De Somer, 1975). For poly(I).poly(C), toxicity became (microscopically) visible at a concentration of 0.08 μg/ml and reached its maximum (100% destruction of cell monolayer) at a concentration of 10 μg/ml. For poly(I) P-L, no toxicity was observed even at 250 μg/ml. For poly(I) o9 and CV 2, cytotoxicity was observed at doses of 50 and 250 μg/ml. For poly(I) 428 and 429, cytotoxicity was noted for concentrations equal to or higher than 10 and 2 μg/ml, respectively. Hence, no clear-cut dissociation between activity and toxicity was achieved with any of the active poly(I) preparations.

In view of the high interferon inducing potency of poly(I) CV 2 and poly(I) 428 in human cells, their relatively poor interferon inducing behaviour in mouse L cells is rather unexpected. It should be noted, however, that various other polynucleotides, while nearly as active as poly(I).poly(C) in human diploid fibroblasts, show little, if any, interferon inducing activity in mouse L cells. Typical examples are: (i) poly(A).poly(U) and poly(A).poly(rT) [see De Clercq et al. (1975) and De Clercq & Torrence (1977)]; (ii) poly(I)2.5-poly(C)13e and poly(I)2.5-poly(C)13e [see Stewart & De Clercq (1974) and De Clercq & Torrence (1977)]; (iii) poly(dIz).poly(C) (E. De Clercq et al. unpublished data); (iv) poly(I).poly(s2C) (Reuss
Table 1. Interferon induction in different cell cultures

<table>
<thead>
<tr>
<th>Polynucleotide*</th>
<th>0.01</th>
<th>0.1</th>
<th>1</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(a) In PRK cells superinduced with cycloheximide and actinomycin D</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(I) 09</td>
<td>&lt; 1.0</td>
<td>1.0</td>
<td>2.7</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Poly(I) CV 2</td>
<td>&lt; 1.0</td>
<td>1.0</td>
<td>2.3</td>
<td>3.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Poly(I) 428</td>
<td>&lt; 1.0</td>
<td>1.0</td>
<td>1.6</td>
<td>2.3</td>
<td>3.0</td>
</tr>
<tr>
<td>Poly(I) 429</td>
<td>1.0</td>
<td>2.7</td>
<td>4.0</td>
<td>4.0</td>
<td>4.3</td>
</tr>
<tr>
<td>Poly(I).poly(C) P-L</td>
<td>&lt; 1.0</td>
<td>1.7</td>
<td>3.0</td>
<td>4.0</td>
<td>4.3</td>
</tr>
</tbody>
</table>

| **(b) In HSF cells primed with interferon and superinduced with cycloheximide and actinomycin D** |
|---|---|---|---|---|---|
| Poly(I) CV 2            | < 1.0| 1.0 | 3.0 | 3.7 | 4.0 |
| Poly(I) 428             | < 1.0| 2.9 | 4.0 | 4.0 |    |
| Poly(I) 429             | < 1.0| 2.9 | 4.0 | 4.0 |    |
| Poly(I).poly(C) P-L     | 1.0  | 2.5 | 3.7 | 4.0 | 4.3 |

<table>
<thead>
<tr>
<th>Interferon titre (log10 units/ml) obtained at polynucleotide concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(c) In L-929 cells primed with interferon</strong></td>
</tr>
<tr>
<td>Poly(I) 09</td>
</tr>
<tr>
<td>Poly(I) CV 2</td>
</tr>
<tr>
<td>Poly(I) 428</td>
</tr>
<tr>
<td>Poly(I) 429</td>
</tr>
<tr>
<td>Poly(I).poly(C) P-L</td>
</tr>
</tbody>
</table>

| **(d) In L-929 cells pretreated with DEAE-dextran** |
| Poly(I) CV 2          | < 0.5 | 0.5 | 0.85 |    |
| Poly(I) 428           | < 0.5 | 0.5 | 1.95 | 2.18 |    |
| Poly(I) 429           | < 0.5 | 0.5 | 3.11 | 3.75 | 3.87 |
| Poly(I).poly(C) P-L   | < 0.5 | 1.89 | 3.11 | 3.75 | 3.65 |

* Other poly(I) preparations (10, 11, 332, 427, Miles, P-L) did not induce interferon when assayed at the concentrations shown above.

et al. 1976) [see De Clercq & Torrence (1977)]. When assayed in parallel with poly(I), poly(C) in interferon-primed L-929 cells, poly(I).poly(s2C) induced only 15 units/ml of interferon at a concentration of 50 µg/ml and none at 10 µg/ml.

In accordance with previous findings (Dianzani et al. 1968, 1971; De Clercq & De Somer, 1975), DEAE-dextran markedly enhanced the poly(I).poly(C)-induced interferon response in L-929 cell cultures (Table I d). As polybasic substances such as DEAE-dextran have also been reported to potentiate the interferon stimulating capacity of single homopolymers (Billiau et al. 1969; Pitha & Pitha, 1974), one could have anticipated high interferon inducing activity of the active poly(I) samples when tested in L-929 cells pre-treated with DEAE-dextran. Yet, poly(I) CV 2, 428 and 429 showed little, if any, interferon inducing activity under these conditions (Table 1 d). Likewise, poly(I).poly(s2C) was unable to induce interferon when assayed at 50 µg/ml in L cells which had been treated with DEAE-dextran (data not shown). The reason(s) why poly(I) CV 2 and poly(I).poly(s2C), unlike poly(I).poly(C), failed to stimulate interferon formation in DEAE-dextran treated L-cells, even though they equalled poly(I).poly(C) in inducing interferon in other cell systems (e.g. human fibroblasts), remain at present unresolved.
Table 2. Interferon induction in rabbits

<table>
<thead>
<tr>
<th>Polynucleotide*</th>
<th>Dose (µg/rabbit)</th>
<th>1.5 h</th>
<th>3 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(I) 09</td>
<td>1000</td>
<td>4.0</td>
<td>3.3</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.8</td>
<td>1.6</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>&lt; 1.0</td>
<td>&lt; 1.0</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>Poly(I) CV 2</td>
<td>1000</td>
<td>4.5</td>
<td>4.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3.5</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>&lt; 1.0</td>
<td>&lt; 1.0</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>Poly(I).poly(C) P-L</td>
<td>1</td>
<td>3.8</td>
<td>2.8</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>&lt; 1.0</td>
<td>&lt; 1.0</td>
<td>&lt; 1.0</td>
</tr>
</tbody>
</table>

* Other poly(I) preparations (10, 11, P-L) did not induce interferon when injected at 1000 µg/rabbit.

Rabbits

Both poly(I) 09 and poly(I) CV 2 proved effective in stimulating the appearance of interferon in the bloodstream of rabbits, injected intravenously with 100 µg polynucleotide (Table 2). However, as little as 1 µg poly(I).poly(C) P-L was needed to give an interferon response that was of similar magnitude to that observed upon administration of 100 µg of poly(I) 09 or CV 2. Hence, in the rabbit model, poly(I).poly(C) was a 100-fold better interferon inducer than the active poly(I) samples 09 and CV 2.

The poly(I) preparations (09, CV 2, Miles and P-L) were pyrogenic in rabbits when administered intravenously at 0.5 mg per animal. The increase in body temperature (sum of pyrogenic responses of three rabbits) was 3.5, 4.4, 3.9 and 3.5 °C, respectively. Poly(I) 10, while inactive as interferon inducer, proved non-pyrogenic in rabbits. Poly(I).poly(C), however, was highly pyrogenic, even when injected at 0.1 mg per rabbit (sum of pyrogenic responses for three rabbits was 4.8 °C).

Sensitivity to degradation by T1 ribonuclease

Poly(I) P-L, poly(C) P-L, poly(I).poly(C) and the active interferon inducer poly(I) CV 2 were compared for their sensitivities to hydrolysis by T1 ribonuclease. As the respective susceptibilities to enzymic hydrolysis were monitored by residual interferon inducing activity, the T1 ribonuclease susceptibility of poly(I) P-L and poly(C) P-L (which are inactive as interferon inducers in PRK cells) could only be assessed after the complementary homopolymer [poly(C) P-L and poly(I) P-L, respectively] had been added to the incubation mixture or to the cell cultures (see Methods). Poly(I) P-L and poly(I) CV 2 exhibited the same degradation profile as a function of T1 ribonuclease concentration, whereas poly(I).poly(C) was approx. 30 times less susceptible to degradation than was poly(I) (Fig. 1). In accordance with the well-known substrate specificity of T1 ribonuclease, poly(C) was totally resistant to breakdown by this enzyme (Fig. 1).

Sensitivity to degradation by pancreatic ribonuclease

In another series of tests, poly(I) P-L, poly(C) P-L, poly(I) 09, poly(I) CV 2 and poly(I).poly(C) were analysed for their susceptibilities to degradation by pancreatic ribonuclease A (see Methods). As shown in Fig. 2, and in agreement with the known substrate specificity of pancreatic ribonuclease, the ability of poly(C) to form an active interferon inducing complex
Interferon induction by poly (I)

Fig. 1. Sensitivity of poly(I) CV 2 (○—○), poly(I) P-L (△—△), poly(C) P-L (▲—▲) and poly(I) P-L (●—●) to degradation by T1 ribonuclease, as monitored by residual interferon inducing activity in PRK cells. Poly(I) P-L and poly(C) P-L were assayed for interferon induction after addition of poly(C) or poly(I), respectively (see Methods).

Fig. 2. Sensitivity of poly(I) CV 2 (○—○), poly(I) 09 (assayed directly (■—■) or after addition of poly C (■—■)], poly (I) P-L (△—△), poly(C) P-L (▲—▲) and poly(I) poly(C) P-L (●—●) to degradation by pancreatic ribonuclease A, as monitored by residual interferon inducing activity in PRK cells. Poly(I) P-L and poly(C) P-L were assayed for interferon induction after addition of poly(C) or poly(I), respectively (see Methods). Poly(I) CV 2 and poly(I) poly(C) P-L were assayed directly.

with poly(I) was destroyed at very low concentrations of pancreatic RNase. However, due to a relative lack of specificity for nucleotide substrates, pancreatic ribonuclease A may also degrade poly(A) and poly(I) (Beers, 1960) and consequently, the interferon inducing activity of poly(I) 09 and poly (I) CV 2 was gradually destroyed by increasing concentrations (Fig. 2). Nevertheless, poly(I) 09, as well as poly(I) P-L, after being treated with pancreatic ribonuclease, could still form an entirely active interferon inducing complex with poly(C) (Fig. 2). These observations indicate that poly (I) 09 was not chopped by the action of
pancreatic RNase into fragments too small to be able to form an active interferon inducing complex with poly(C) (Tytell et al. 1970; Carter et al. 1972; Stewart & De Clercq, 1974). Our observations also indicate that the pancreatic RNase-treated poly(I) 09 and poly(I) CV 2, when used alone as inducer, either lost the active part of their structure or became too short to be able to trigger the cellular receptor site for interferon induction. As postulated before (Thang et al. 1977), part of poly(I) 09 and CV 2 molecules may be tied up, by an as yet undefined post-synthetic event, into a sort of looped structure. This looped structure may be recognized by both antibodies directed against double-stranded RNA (Stollar et al. 1977) and the putative receptor site for interferon induction.

Sensitivity to degradation by serum nucleases

Human serum and other sera contain the necessary nuclease(s) to degrade both single- and double-stranded RNAs. Poly(I).poly(C) is hydrolysed to acid-soluble material and loses its pyrogenicity when incubated in the presence of human plasma (Nordlund et al. 1970; Stern, 1970). As evaluated by residual interferon inducing activity in HSF cells (primed with interferon and superinduced by metabolic inhibitors), poly(I) 429 was approx. four times more susceptible and poly(I) CV 2 was 10 to 20 times more susceptible to the inactivating effect of human serum than was poly(I).poly(C) (Fig. 3). One may assume, therefore, that poly(I) CV 2 and poly(I) 429 would have a shorter half-life time in the human body than poly(I).poly(C).

DISCUSSION

Several authentic poly(I) preparations (with no CMP or any other nucleotide other than IMP present in alkaline hydrolysates of the polymers) were capable of inducing interferon, although to an extent which varied considerably from one cell system to another. In human diploid cells, primed with interferon and superinduced with metabolic inhibitors, the interferon inducing potency of the active poly(I) preparations approached that of poly(I).poly(C).
Since polycationic substances such as DEAE-dextran failed to potentiate, and even diminished, the interferon inducing ability of the active poly(I) preparations described here, the structure of these preparations may fundamentally differ from those poly(I) samples which have been accredited with interferon inducing properties in previous studies (Billiau et al. 1969; Pitha & Pitha, 1974).

Although the precise physico-chemical basis for the interferon inducing activity of our poly(I) preparations has not been elucidated, the hypothesis has been proposed that part of the poly(I) molecules is fixed by a covalent linkage between two hypoxanthine residues into a sort of looped structure (Thang et al. 1977). Apparently, this particular structural configuration is recognized by several biological probes, namely (i) the cellular receptor site for interferon induction, and (ii) antibodies directed towards double-stranded RNA (Stollar et al. 1978).

Could active poly(I) preparations, such as poly(I) CV 2 and poly(I) 429, be considered as potentially useful interferon inducers in man? According to Ts'o et al. (1976) and Carter et al. (1976), the time required for a polynucleotide inducer of interferon to trigger the interferon response may be considerably shorter than the time required to induce other, often undesirable, biological side effects. On this premise, one might develop an interferon inducer of superior efficacy to poly(I).poly(C). Such an inducer should persist in biological fluids for a time sufficiently long to trigger the interferon response but not so long that it induces additional undesirable physiological responses. Our active poly(I) samples may meet these requirements. In analogy with the mismatched analogues of poly(I).poly(C), poly(I).poly(C13, U) and poly(I).poly(C20,G) (Carter et al. 1972; Ts'o et al. 1976), the active poly(I) preparations CV 2 and 429 are (nearly) as active as poly(I).poly(C), at least in inducing interferon in human diploid cell cultures (Table 1 b) and yet poly(I) CV 2 and 429 are significantly more susceptible to degradation by nucleases (including nucleases present in human serum) than is poly(I).poly(C) (Fig. 1 to 3). It remains to be seen, however, whether compounds like poly(I) CV 2 and 429 can reach their target cells in the human body before being hydrolysed.

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