Depressors of Interferon Synthesis: Inhibition of Interferon Synthesis by Chick Allantoic Mucopolysaccharide, Capsular Polysaccharide of Klebsiella pneumoniae and Heparin

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SUMMARY

Three kinds of polyanionic polysaccharides, chick allantoic mucopolysaccharide, capsular polysaccharide of Klebsiella pneumoniae and heparin depressed interferon synthesis induced by ultraviolet-irradiated Newcastle disease virus in chick embryo cells. The minimum concentrations causing complete inhibition of interferon synthesis were respectively 20, 0.5 and 50 µg./ml. The polysaccharides did not affect the action of exogenous interferon at concentrations sufficient to depress interferon synthesis. It seems unlikely that these polysaccharides were toxic for the cells, and that the depression of interferon synthesis was due to an inhibition of the early steps of virus-cell interaction.

INTRODUCTION

Kato et al. (1969) reported that the so-called enhancer of virus multiplication (Kato, Okada & Ohta, 1965a, b) detected in allantoic fluids of chick embryos infected with parainfluenza type 1 virus was a depressor of interferon synthesis (interferon depressor). The interferon depressor was heat-stable, undialysable and stable to ether. The stability to treatment with various enzymes made it unlikely that this factor is a protein, DNA or RNA.

In further studies it has been found that a mucopolysaccharide extracted from the allantoic fluid of hen’s eggs, the host antigen of Harboe and coworkers (Harboe, 1963a, b; Haukenes, Harboe & Mortensson-Egnund, 1965, 1966), has the ability to depress interferon synthesis in chick embryo cell cultures. Furthermore, two other mucopolysaccharides, the capsular polysaccharides of Klebsiella pneumoniae and heparin, have been found to possess a similar activity. These findings are described in the present report.

METHODS

Viruses. A small-plaque variant of the MIYADERA strain (MIYADERA/s) of Newcastle disease virus and Sindbis virus were used. Newcastle disease virus was propagated in chick embryos, Sindbis virus in chick embryo cell cultures (Kato et al. 1969).

Chick embryo cell cultures were used throughout these experiments. Cell culture techniques, the plaque assay of Sindbis virus and the interferon assay by the plaque
The inhibition method have already been described (Kato et al. 1969). The plaque assay method for Newcastle disease virus was the same as for Sindbis virus. It was read after incubation for 3 days. The interferon titres were expressed in PDD50 values (dose to give a 50% depression of the control plaque count).

Production of interferon induced by u.v. irradiated Newcastle disease virus (NDV<sub>w</sub> ). The procedures were described by Kato et al. (1969). However, the miyadera/s strain of Newcastle disease virus was used as interferon inducer. Since the experiments described in the preceding paper were completed, it was found that the capacity of cell cultures from 3-day-old embryos to produce interferon was greater than cells from 1- or 2-day-old embryos (Kato & Eggers, 1969). Such cells were therefore used and were pretreated for 24 hr with the materials to be tested for the production of interferon.

Polysaccharides. Highly purified mucopolysaccharide (host antigen) isolated from the allantoic fluid of chick embryos by the procedures of Haukenes et al. (1965, 1966) was supplied by Dr A. Harboe, National Institute of Public Health, Oslo, Norway. Capsular polysaccharide prepared from <i>Klebsiella pneumoniae</i> (Kasuya strain, type unknown) by the procedures of Batshon, Baer & Shaffer (1963) was supplied by Dr T. Kobayashi, Department of Bacteriology, Nagoya University School of Medicine, Nagoya, Japan. Klebsiella polysaccharides prepared from <i>K. pneumoniae</i> type A (3409/60), Type C (F.10.N.Y.), Type E (242) and Type 35 were made available by Dr J. Eriksen through Dr A. Harboe. Unless otherwise stated, Klebsiella polysaccharide purified from the Kasuya strain was used. Heparin was purchased from Deutsche Hoffmann-La Roche AG, Grenzach, Germany. The host antigen, capsular polysaccharide of <i>K. pneumoniae</i> and heparin were dissolved in phosphate-buffered saline (Dulbecco & Vogt, 1954) to concentrations of 1 mg./ml., 500 µg./ml. and 10 mg./ml., respectively. These stock solutions were diluted further with maintenance medium to obtain the desired concentrations.

Throughout this paper, chick allantoic mucopolysaccharide will be abbreviated CM, the capsular polysaccharide of <i>K. pneumoniae</i> CPK.

RESULTS
Toxicity of CM, CPK and heparin for chick embryo cells
Up to the concentrations of 500 µg./ml. of CM, 100 µg./ml. of CPK and 500 µg./ml. of heparin, these substances caused no visible damage in chick embryo cells within 24 hr.

Inability of CM, CPK and heparin to induce interferon in chick embryo cells
Because of the report (Kleinschmidt, Cline & Murphy, 1964) that statolon, a polysaccharide produced by the mould Penicillium stoloniferum, induced the production of interferon in chick embryo cells, the interferon-inducing capacity of CM, CPK and heparin was investigated. Monolayer cultures were incubated at 37° for 24 hr with maintenance medium containing 3·1, 6·3, 12·5, 25 and 50 µg./ml. of the substances. At the end of the incubation period medium was harvested and assayed for interferon. No interferon activity was detectable in any of these media. These polysaccharides did not, therefore, induce interferon synthesis in chick embryo cells.
**Interferon depressors**

Fig. 1. Depressing effect of chick allantoic mucopolysaccharide on interferon synthesis induced by NDV<sub>uv</sub> in chick embryo cells. The yield of interferon is expressed as per cent of the yield in control cultures.

Fig. 2. Depressing effect of the capsular polysaccharide of *K. pneumoniae* on interferon synthesis induced by NDV<sub>uv</sub> in chick embryo cells.
Effect of CM, CPK and heparin on interferon synthesis induced by NDV

Two-day-old cell cultures were incubated at 37° for 24 hr with 2 ml. of maintenance medium containing various concentrations of the compounds. For each variable three cultures were used. Control cultures were incubated with maintenance medium. At the end of the incubation period the fluids were removed, the cultures were washed with phosphate-buffered saline, and NDV was inoculated as the interferon inducer.

![Depressing effect of heparin on interferon synthesis induced by NDV in chick embryo cells.](image)

Table 1. Effect of capsular polysaccharides from various types of Klebsiella pneumoniae on interferon synthesis*

<table>
<thead>
<tr>
<th>Capsular polysaccharide from K. pneumoniae:</th>
<th>Yield of interferon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material for pretreatment</td>
<td>PDD 50</td>
</tr>
<tr>
<td>Type A</td>
<td>91</td>
</tr>
<tr>
<td>Type C</td>
<td>119</td>
</tr>
<tr>
<td>Type E</td>
<td>128</td>
</tr>
<tr>
<td>Type 35</td>
<td>128</td>
</tr>
<tr>
<td>Maintenance medium (control)</td>
<td>1260</td>
</tr>
</tbody>
</table>

* Chick embryo cells were preincubated at 37° for 24 hr with the polysaccharides at a concentration of 5 μg./ml. Control cultures were incubated with maintenance medium. Then, interferon synthesis induced by NDV was in each group of cultures was determined.

After incubation at 37° for 24 hr, the medium was harvested and assayed for interferon. The interferon-depressing effect of each substance followed a dose-response curve. The lowest concentration to cause a maximum depressing effect of CM, CPK and heparin were 20, 0.5 and 50 μg./ml. respectively (Fig. 1, 2, 3).
Similar experiments were also done using cells from 1-day-old embryos with some concentrations of the substances. The results were consistent with the above, although the net yields of interferon were generally lower (Kato & Eggers, 1969).

CPK is serologically type-specific (Wilson & Miles, 1966). The interferon-depressing activity is not however restricted to a particular type of *Klebsiella* (Table 1).

**Effect of CM, CPK and heparin on the action of exogenous interferon**

Fresh cultures of chick embryo cells were incubated at 37° for 24 hr with 2 ml. of maintenance medium containing each substance. The concentrations used for CM, CPK and heparin were 50, 0.5 to 50, and 50 μg./ml., respectively, which are sufficient to cause a maximum depression of interferon synthesis. At the end of the incubation period, the fluids were removed, the cultures were washed with phosphate-buffered saline and were divided into two groups. One group was incubated with 1 ml. of interferon at 37° for 3 hr. The other control group was incubated with maintenance medium. Two cultures were used per variable. Interferon had been prepared by treatment of chick embryo cells with NDV, and contained 710 PDD₅₀/ml. After removal of interferon, the cultures were inoculated with Sindbis virus at an input multiplicity of 10 p.f.u./cell. After an adsorption period of 1 hr at 37°, the cultures were washed twice with phosphate-buffered saline, 4 ml. of maintenance medium was added and the cultures were incubated at 37°. Eight hr after adding the virus, at which time a single cycle of
multiplication of Sindbis virus was completed (Kato et al. 1969), the media were harvested and assayed for virus yields. The reduction in the yield of Sindbis virus by interferon in cells pretreated with CM or heparin was similar to that in untreated cells (Fig. 4). In contrast, the reduction in the virus yield by interferon in cells pretreated with 50 µg./ml. of CPK was significantly less than that in cells treated with interferon only. However, pretreatment of cells with 0.5, 5 and 25 µg./ml. of CPK had no effect on the reduction of virus yield by interferon (Fig. 5).

Experiments were also done in which cells were simultaneously exposed to the polysaccharides and interferon. The results were like those described above.

We concluded that CM and heparin at a concentration of 50 µg./ml., and CPK at a concentration of 25 µg./ml. or less had no effect on the action of exogenous interferon. CPK at a concentration of 50 µg./ml. did affect slightly but significantly the action of interferon. However, this concentration was 100-fold higher than the minimum concentration which caused a maximum depression of interferon synthesis (Fig. 2).

The multiplication of Sindbis virus was neither enhanced nor inhibited by pretreatment with the polysaccharides. The lack of enhancement of Sindbis virus multiplication in a single cycle by the interferon depressors is consistent with previous results (Kato et al. 1969). The finding that virus multiplication was not inhibited by the
polysaccharides suggests that the interferon-depressing activity of these substances was not due to a nonspecific toxic effect on the cells.

Effect of CM, CPK and heparin on the multiplication of Newcastle disease virus.

The effect of pretreatment of chick embryo cells with these polysaccharides on the multiplication of Newcastle disease virus was investigated under the experimental conditions previously described (Kato et al. 1969) (Table 2). These treatments did not affect the multiplication of Newcastle disease virus; these results were consistent with those obtained with the interferon depressor detected in allantoic fluids of eggs infected with Sendai virus (Kato et al. 1969). Furthermore, since Newcastle disease virus multiplication was not inhibited by these three polysaccharides, it seems unlikely that the depression by CM, CPK and heparin of interferon synthesis by NDV$_{uv}$ was due to inhibition of adsorption, penetration or uncoating of NDV$_{uv}$.

Table 2. Effect of chick allantoic mucopolysaccharide, capsular polysaccharide of Klebsiella pneumoniae and heparin on multiplication of Newcastle disease virus*

<table>
<thead>
<tr>
<th>Material for pretreatment</th>
<th>Yield (p.f.u./ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chick allantoic mucopolysaccharide, 50 μg./ml.</td>
<td>2.0 × 10$^6$</td>
</tr>
<tr>
<td>Capsular polysaccharide of K. pneumoniae, 1 μg./ml.</td>
<td>1.8 × 10$^6$</td>
</tr>
<tr>
<td>Heparin, 100 μg./ml.</td>
<td>1.7 × 10$^6$</td>
</tr>
<tr>
<td>Maintenance medium (control)</td>
<td>1.8 × 10$^6$</td>
</tr>
</tbody>
</table>

* Chick embryo cells were preincubated with the test substances at 37° for 24 hr. Control cultures were incubated with maintenance medium. At the end of the incubation period Newcastle disease virus was inoculated at an input multiplicity of 20 p.f.u./cell. The cultures were incubated at 37° for 20 hr after virus inoculation, at which time a single cycle of multiplication of Newcastle disease virus had reached a peak.

DISCUSSION

The data presented demonstrate that chick allantoic mucopolysaccharide, capsular polysaccharide of K. pneumoniae and heparin have a depressing effect on the synthesis of interferon. These polysaccharides did not affect the action of interferon at concentrations sufficient to depress interferon synthesis. Although the biological origin of the substances is different, all three are polyanionic polysaccharides. Statolon, a polyanionic polysaccharide produced by the mould Penicillium stroliferum, has been reported to induce the production of interferon in vitro and in vivo (Kleinschmidt et al. 1964; Kleinschmidt & Murphy, 1965). However, recently it has been found that the interferon-inducing activity of statolon is associated with virus-like particles contained in the preparation of statolon (Ellis & Kleinschmidt, 1967). It is questionable, therefore, whether statolon is responsible for interferon induction.

Various synthetic anionic polymers are active in interferon induction (Merigan, 1967; Field et al. 1967; Kleinschmidt, 1968; Merigan & Finkelstein, 1968). Furthermore, as indicated by Merigan & Finkelstein (1968), most agents known to induce interferon have polyanionic components, including the polynucleotides of viruses, rickettsiae, mycoplasmas, bacteria, and the endotoxins. None of the polyanionic polysaccharides investigated in the present study exhibited interferon-inducing activity in chick embryo cells, but, on the contrary, all depressed interferon synthesis.
Although the mechanism of action of CM, CPK and heparin is not clear, it seems likely that the process susceptible to the depressing effect of these polysaccharides must happen after release of the virus nucleic acid from the nucleocapsid. It is tempting to consider the possibility that interferon-depressing polyanions may act as antagonists of interferon-inducing polyanions, although we have no direct evidence for this hypothesis.

We also point out that the interferon-inducing effect of polyanions depends on the cell species used. For example, the carboxylate copolymer complexed to organic cations is active in interferon induction in vitro only in peritoneal macrophages, but inactive in chick embryo fibroblasts, L cells, mouse spleen lymphocytes, or human fibroblasts (Merigan & Finkelstein, 1968).

On the basis of its properties (Kato et al. 1969) it seems probable that the interferon depressor detected in allantoic fluids of eggs infected with Sendai virus is the same substance as CM. Although CM is prepared from allantoic fluids of uninfected chick embryos (Harboe, 1963a, b; Haukenes et al. 1965, 1966), an interferon-depressing activity is not detectable in such fluids (Kato et al. 1969). This may be because the concentration of CM in normal allantoic fluids is low, i.e. approximately 1.5 μg./ml. (Harboe, 1963b; Haukenes et al. 1965), a concentration at which CM has only a very slight depressing effect on interferon synthesis (Fig. 1). Moreover, CM may be present in an inactive form in allantoic fluids of uninfected eggs.

Of the three polysaccharides described in the present paper, CPK on a weight basis appears to have the most potent depressing effect on interferon synthesis. This depressing effect is independent of the serological type of Klebsiella pneumoniae. It has been reported that CPK inhibits the multiplication of the pneumonia virus of mice (Horsfall & McCarty, 1947; Ginsberg & Horsfall, 1951) and mumps virus (Ginsberg, Goebel & Horsfall, 1948). This antiviral activity was observed in mice and chick embryos, but was not tested in cell cultures. On the other hand, we do not know whether the depressing effect of CPK on interferon synthesis is found also in mice and eggs. The solution to this apparent paradox may be found in different reactivities of various cell types, as discussed above.

It has been reported that CM and dextran sulphate potentiated the antiviral activity of interferon (Blough & Tudor, 1967). However, it is not obvious from this communication whether the potentiation is due to an effect on the action or the production of interferon. In the present study no potentiation of the action of interferon by the polysaccharides was observed.

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REFERENCES


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