Binding of Polyriboinosinic-Polyribocytidylic Acid with Cultured Cells

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Summary

Homogenates prepared from polyriboinosinic-polyribocytidylic acid copolymer [poly(rI)·poly(rC)]-treated cells exhibited antiviral activity in chick embryo, L and rabbit kidney cells. The antiviral activity in the homogenate co-sedimented with cellular membrane material and was shown to be poly(rI)·poly(rC) by a hybridization competition test with immobilized polyribocytidylic acid. The results indicate that poly(rI)·poly(rC) binds firmly to cellular membrane. These studies, however, could not differentiate between specific binding leading to the interferon induction and non-specific binding possibly unrelated to the induction of interferon.

Introduction

Animals and cultured cells are induced for interferon synthesis upon exposure to some species of polyribonucleotides. However, the underlying mechanisms through which the cells recognize these polynucleotides as interferon inducers are not understood. Poly(rI)·poly(rC) was reported to combine with cells in the presence of polycations such as diethylaminoethyl (DEAE)-dextran (1, 2, 4). This conclusion was based on the results of experiments following the fixation of radioactivity of added tritiated copolymer. Indirect evidence suggested that the bulk of the bound poly(rI)·poly(rC), however, was not related to the essential process of triggering the interferon response.

Recent findings that insolubilized forms of poly(rI)·poly(rC) were capable of stimulating interferon in cultured cells (13, 11, 3), have suggested that the site of cell-polyribonucleotide interaction is probably at the outer side of the cell membrane.

Studies reported here were aimed to characterize the antiviral activity found in a homogenate of poly(rI)·poly(rC)-treated cells. The active principle was identified to be the input copolymer bound to cellular membrane component(s),
providing confirmatory evidence for the binding and persistence of the copolymer on cells.

Materials and Methods

Cells

Mouse L cells were grown in Eagle’s minimal essential medium containing 3 times the normal concentration of amino acids (EMEM), with 5 per cent fetal bovine serum and antibiotics. The preparation and cultivation of primary chick embryo (PCE) cells has been described previously (7). In some initial experiments, an established line of rabbit kidney (RK-1) cells (unpublished) was employed. They were grown in EMEM containing 5 per cent calf serum and antibiotics.

Viruses

The GDVII strain of mouse encephalomyelitis virus was propagated in actinomycin D (0.5 μg/ml)-treated BHK-21 cells. Infectivities of GDVII seed pools were determined by hemagglutinin (HA) production end point in test tube cultures of BHK-21 cells. Sindbis virus was grown and assayed by plaque formation in PCE cells. The level of the antiviral state in RK-1 cells was determined by the vaccinia virus plaque reduction. This virus (DIE or IHD strain) was grown in the chorioallantoic membrane of embryonated hen’s eggs.

Synthetic Polyribonucleotides

Poly (rI) · poly (rC), poly (rC), poly (rA), poly (rU) and poly (rI) were purchased from Miles Laboratories, Elkhart, Indiana. Tritium labeled poly (rI) was synthesized by polymerization of inosine -8-H3-5 diphosphate (4.0 Ci/m Mole, Radiochemical Centre, Amersham England) with polynucleotide phosphorylase (EC 2.7.7.8, Boehringer, Mannheim, Germany) as described by Goldberg (5). The radioactive nucleotide substrate was usually diluted with cold inosine diphosphate (1:100). The molecular weight of the product was not determined.

Homogenization of Cells and Preparation of Membrane Fraction

L or RK-1 cells in 750 ml Roux flasks or 200 ml bottles were exposed to various concentrations of poly (rI) · poly (rC) dissolved in EMEM in the presence of 10 μg/ml of DEAE dextran for 1—2 hours at 37°C, and washed 6 times with Hanks’ buffered salt solution (BSS). They were then collected by scraping with a rubber policeman and suspended in a hypotonic tris-HCl buffer (0.01 M trisaminomethane-HCl, 0.01 M KCl, 0.0015 M MgCl2, pH 7.4; RSB) and sedimented by low speed centrifugation. A measured amount of packed cells was transferred into a Dounce homogenizer (tight fitting) and disrupted by 12—15 strokes in an equal volume of RSB, except where otherwise stated. Larger fragments of cells and nuclei were removed by centrifugation at 450 × g for 15 minutes. The resulting supernatant is referred to as ‘cytoplasmic homogenate’.

Although in most experiments the homogenate was either used immediately or further processed, it was shown that storage at —80°C did not alter the biological characteristics of the homogenate. In some earlier experiments, poly (rI) · poly (rC)-treated, washed cells were disrupted by 3 cycles of freezing and thawing, which yielded essentially the same results. In order to obtain the membrane fraction, the cytoplasmic homogenate in RSB (0.1—0.5 ml) was layered on top of 5 ml of a continuous (or, in some later experiments, discontinuous) ribonuclease-free sucrose gradient (0.5—2.0 m) and spun at 150,000 × g for 5 hours in the Hitachi RPS-40 rotor. Samples were collected either from the top (employing a Hitachi sampling device) or from the bottom (punching a hole in the bottom of the tube). In preparative runs, 1.0 ml of the homogenate was placed on 25 ml of a 0.5—2.0 m discontinuous sucrose gradient and spun at 50,000 × g overnight.

Measurement of Antiviral Activity of Poly (rI) · Poly (rC) or of the Cytoplasmic Homogenate

Antiviral activities were determined by mixing serially diluted material with 10 μg/ml of DEAE dextran, and measuring the levels of antiviral resistance induced by the mixtures in treated cells. Two different systems were employed. One which was pre-