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HUMAN INTERFERON AND CELL GROWTH INHIBITION. III. SEPARATION OF ACTIVITIES BY TREATMENT WITH SODIUM DODECYL SULPHATE

Helen Dahl

The Wilhelmsen Institute of Bacteriology, University of Oslo, Rikshospitalet, Oslo, Norway.

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SUMMARY: When human leukocyte interferon was treated with boiling sodium dodecyl sulphate antiviral activity without detectable effect on the growth of human amnion cells could be separated from the growth inhibitory activity by a single gel filtration. Similar results were obtained with mouse L-cell interferon. It is concluded that the two effects of interferon can be separated in distinct molecular entities.

INTRODUCTION: It is well documented that interferons in addition to antiviral activity exert numerous biological effects on the cellular level (1-8). In most investigations a strict correlation was found between non-antiviral and antiviral activities independent of degree of purification, and the agents responsible for the non-antiviral activities were physico-chemically indistinguishable from interferon (1-13). It was therefore concluded that all the effects of interferon reside in the same molecule. However, during the last few years reports have appeared indicating that the different effects of interferon may be separable phenomena mediated through different biological events. Thus, differences in sensitivity to actinomycin D (14), in cell sensitivity to the effects (15), in chromosome 21 regulation of activities (16), and in reversibility (17) have been reported. In agreement with these findings a physical separation of growth inhibitory activity from the antiviral effect of human leukocyte interferon by adsorption chromatography on albumin-agarose has been reported

Abbreviations: SDS, sodium dodecyl sulphate
MW, Molecular weight
BD, blue dextran
PR, phenol red

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from our laboratory (18). The results suggested that the growth inhibitory component was firmly attached to the antiviral component by electrostatic bonds. In contrast, Stewart and his colleagues (12) could not detect any separation of activities after drastic denaturation of interferon with SDS followed by electrophoresis. Also, in preparations of human fibroblast interferon purified to homogeneity (13) cell growth inhibition was found fully correlated with antiviral activity. The purification steps included denaturation with SDS. As SDS is known to destroy all non-covalent bonds, these results highly suggest that the two activities are caused by the same molecule. Therefore, in the light of our former results (18) indicating that the two activities can be separated, the effect of SDS-treatment on separation of interferon activities was investigated. The results presented here verify, that a near quantitative separation of activities indeed can be obtained by such treatment followed by gel filtration.

MATERIALS AND METHODS: Cells : The U-line of human amnion cells and mouse L-cells were grown in Eagle's medium (Grand Island Biological Co.) supplemented with 2% inactivated calf serum and 0.132% $NaHCO_3$. Human embryo lung cells in passage number 5-12 were grown in a mixture (50:50) of Eagle's medium and medium 199 (Grand Island Biological Co.) supplemented with 2% calf serum and 0.132% $NaHCO_3$. All cells used in this study were found negative for mycoplasma in repeated tests.

Interferon : A purified preparation of Sendai induced human leukocyte interferon was a gift from Dr.K.Cantell, Helsinki. It contained 0.7×10^6 units of interferon per mg protein. Mouse L-cell interferon was supplied by Dr.G.Bodo, Vienna. The preparation contained 1.3×10^6 units of interferon per mg protein. Antiviral activity was measured by a micromethod (19) using Vesicular stomatitis virus as challenge. Human embryo lung cells were used for human interferon and mouse L-cells for mouse interferon. All titers given are expressed as international units per 0.1 ml.

Test for effect on cell growth : In tests for cell growth inhibition U-amnion cells were used in the human system and mouse L-cells in the murine system. Routinely 5 parallel tubes were seeded with 10^5 cells in one ml of medium containing test material in dilution 1/25. Controls were given medium in stead of test material. The tubes were incubated for 3 days in 5% CO_2-atmosphere before treatment with trypsin-versene and counting. The results represent the average values of the parallel tubes and are expressed as per cent reduction in cell count as compared to the controls.

Sodium dodecyl sulphate treatment of interferon : Interferon, 10^5 units, was mixed with 0.9 ml of 3.3×10^{-3} M SDS. The mixture was kept in a boiling water bath for 1 minute and incubated at room temperature for 1 hour. After addition of MW markers (BD: MW > 5000, PR: MW < 1000) it was applied to a Sephadex G-25 column (1.5x28 cm) and eluted with distilled water in 3 ml fractions. Each fraction was tested for antiviral activity and for effect on cell growth.

Test for sodium dodecyl sulphate : Each fraction after gel filtration was tested for SDS content by a method (20) based on lysis of sheep red cells. Free as well as complex SDS in concentrations as low as 1 μg per ml will be detected by this method.

RESULTS AND DISCUSSION: The resulting elution profile when human leukocyte interferon was treated with SDS and gel filtered as described under methods is shown in Figure 1. Antiviral activity with no detectable effect on cell growth was eluted with the high MW marker (MW > 5000). In agreement with earlier findings (21) maximum growth inhibition was found in low MW fractions (MW≈1100) before the elution of low MW marker. In different experiments the antiviral peak fractions contained in total 10^5-1.8×10^5 antiviral units equivalent with a recovery of 100-180 per cent. The recovery of cell growth inhibitory activity was usually less, 50-70 per cent. As shown in Figure 2 mouse L-cell interferon could be separated in the same way as human interferon and with similar result.

As SDS is toxic for the cells in higher concentrations, it could be responsible for the effect on cell growth found in low MW fractions. However, neither of the fractions which inhibited the cell growth contained any detectable SDS. Some, probably complex, SDS was found in the antiviral fractions and surplus SDS was eluted together with the low MW marker. Neither of these fractions had any effect on cell growth. Therefore, it seems unlikely that SDS should be responsible for the measured cell growth inhibition.

A mock preparation of interferon prepared by Dr.K.Cantell in the same way as human leukocyte interferon was also treated with SDS and gel filtered as described. Neither of the fractions

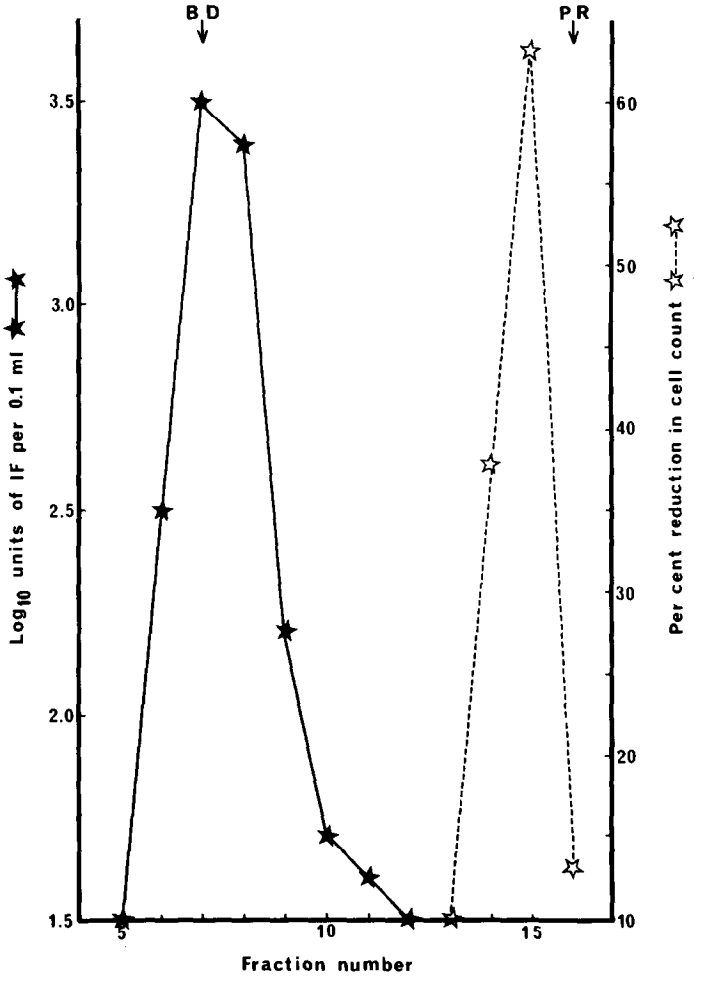

Figure 1: Separation of antiviral activity and growth inhibitory effect from human leukocyte interferon. Human leukocyte interferon was made 3.3×10^{-3}M with respect to SDS. The mixture was kept in a boiling water bath for 1 minute and incubated for 1 hour at room temperature. After gel filtration on Sephadex G-25 each fraction was tested for antiviral activity and for effect on cell growth in dilution 1/25.

showed any growth inhibitory effect or antiviral activity.

The isolated growth inhibitory component has been compared to the component isolated by chromatography on albumin-agarose (21). So far tested the two components isolated from human leukocyte interferon by so different methods seem to have identical physico-chemical characteristics. Both are cell growth inhibito-

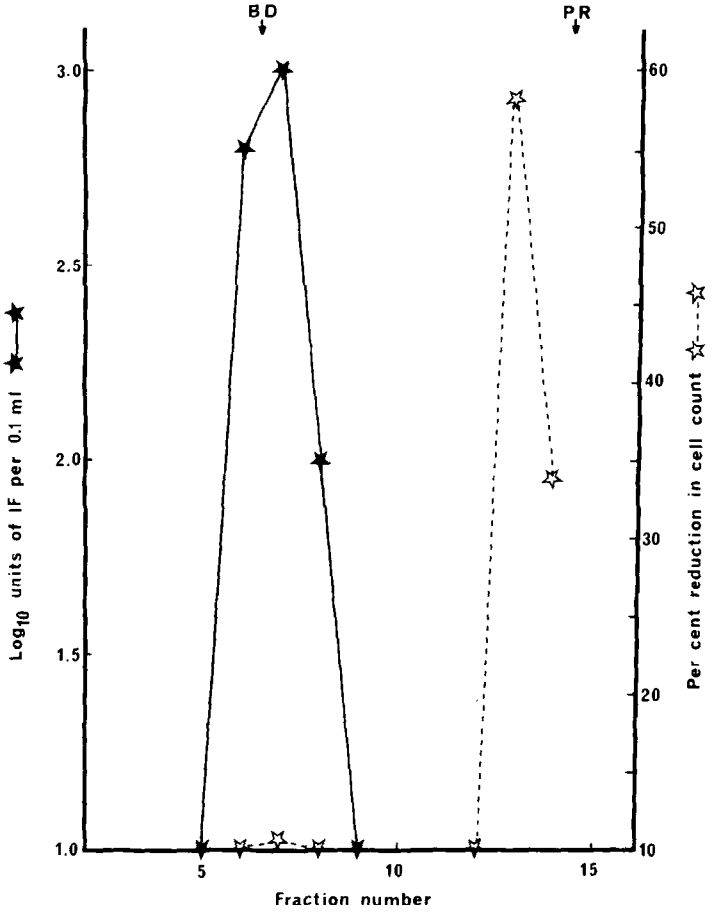

Figure 2: Separation of antiviral activity and growth inhibitory effect from mouse L-cell interferon. Mouse L-cell interferon was treated with SDS and gel filtered as described for human interferon. All fractions were tested for antiviral activity and for effect on cell growth in dilution 1/25.

ry without antiviral activity. They have the same MW and are unstable to pH 2 treatment with HCl, but stable to CCl_3COOH, trypsin and heating. Spectrophotometrically both have maximum absorption at 238 nm. These results and the isolation of an high titered antiviral component without effect on cell growth strongly support the suggestion (18) that the two effects of interferon can be dissociated and isolated as distinct molecular entities.

In the present study a continuous line of human amnion cells was used to test the effects on cell growth in the human system.

Identical results have been obtained with other human cell lines (to be published). The Daudi line of human lymphoblastoid cells - known to be highly sensitive to the growth inhibitory effect of both leukocyte and fibroblast interferon - is employed by several investigators in studies on cell growth inhibition. Preliminary results with Daudi cells indicate that this cell line is not sensitive to the isolated growth inhibitory component, but is strongly reduced in growth by the antiviral component. So far tested Daudi is the only cell line behaving in this way. At present this finding can not be explained, but it may suggest, that more than one mechanism may be involved in the cell growth inhibitory activity of interferon. However, the finding may explain why other investigators (12,13) using Daudi cells could not detect separation of activities even after drastic denaturation with SDS.

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