AN ENDONUCLEASE ACTIVITY ASSOCIATED WITH PREPARATIONS OF CHICK INTERFERON

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Received February 18, 1971

SUMMARY: The viscosity of solutions of calf thymus DNA is decreased after incubation with preparations of chick interferon of low protein content induced either by UV-inactivated NDV virus or by Polyinosonic:polycytidylic acid (poly I:C). The time course of the reaction suggests an endonuclease activity and preliminary analysis of products confirms this. The activity is resistant to pH 2 for 5 days, is not found in mock interferon preparations and is associated with interferon preparations purified by chromatography on CM-Sephadex.

Attempts made to detect nuclease activity in interferon preparations have been reported to be unsuccesful (1). Endonuclease activity is not always detectable by following liberation of small molecules, especially when optimum conditions are not known, whereas decrease in viscosity of DNA solutions provides a sensitive method for detection of endonuclease activity. Using this method, we have obtained evidence for the presence of an endonuclease activity in preparations of chick interferon.

MATERIALS AND METHODS

Cells. Primary cultures of chick embryo fibroblasts (CEF) were prepared from 10 day old embryos and grown in reinforced Eagles medium containing 5% calf serum. Bicarbonate concentration was 2.2 g/l and petri dishes were grown in an atmosphere of 5% CO_2.

Viruses. Vaccinia virus was grown and titrated in CEF. Newcastle Disease virus (NDV) was grown in the allantoic cavity

of 10 days embryonated eggs and assayed as plaques on CEF. For production of interferon, NDV preparations were irradiated with UV as described by Paucker (2).

Preparations of Interferon. (a) Viral-induced. Five day old cultures of CEF were washed twice with phosphate buffered saline (PBS) and UV-irradiated NDV was added containing less than 10 plaque forming units (p.f.u.)/ml but with an infecting ratio of 200 p.f.u. per cell calculated from the initial titre. After 2 hr at 37°, the virus was removed, the cells washed twice with PBS and medium without serum added. The medium was harvested from the cultures after incubation for 24 hr at 37°, adjusted to pH 2 and kept 5 days at 4° before readjusting the pH to neutrality. Mock interferon preparations were prepared using UV-irradiated allantoic fluid in place of NDV. (b) Poly I:C induced interferon. Using a solution of poly I:C (10 µg/ml) and DEAE-dextran (10 µg/ml) in place of UV-NDV, interferon was prepared as described above. Interferon was assayed in a plaque assay using vaccinia virus (3) and one unit is defined as the amount required to reduce the number of plaques by 50%.

Viscosity measurements. The relative viscosity (η) of solutions of calf thymus DNA, prepared by the method of Kay et al (4), was measured using an Oswald viscosimeter. After mixing the DNA and interferon preparations, η was measured immediately (η_0) and at various intervals (η_T). When the reaction rate is constant then $\log\frac{\eta_0}{\eta_T} = KT$, where K is a constant (5).

Paper Chromatography. of reaction mixtures was done as described by Felix et al (6), while paper electrophoresis was performed on Whatmen No. 3 MM paper at pH 3.5 using 0.05 M sodium citrate in the apparatus and under the conditions proposed by Markham and Smith (7).

RESULTS AND DISCUSSION

Effect of interferon preparations on viscosity of solutions of calf thymus DNA. Preparations of chick interferon were concentrated twenty fold by adsorption to Doucil at pH 5.0 and elution in 0.6 M phosphate pH 7.4(8). This step effected only a 10 fold purification, but the protein content of the preparations was low(approx. 100 µg/ml) and the specific activity of the interferon was 9,000 units/mg protein in the case of viral induced material and 1,800 units/mg. protein in the case of poly I:C induced iterferon. Mock interferon preparations were also concentrated on Doucil and contained the same amount of protein. Samples of these interferon preparations were used in the work reported below. Solutions of calf thymus DNA containing Mg^{++} were incubated with interferon and mock interferon prepa-

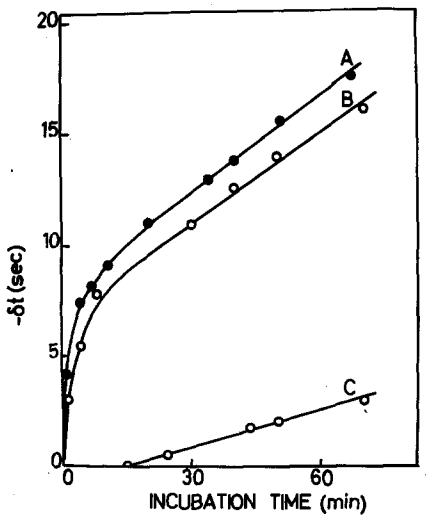

Fig. 1. 3.5 ml of a 1 mg/ml solution of calf thymus DNA in 0.1M phosphate buffer pH 5.0 containing 5×10^{-3}M Mg^{++} was mixed with 0.5 ml of Doucil concentrated chick interferon induced by UV-inactiviated NDV (curve A) Poly I:C (curve B) or with Doucil concentrated mock interferon (curve C). At intervals, the time taken for the mixture to flow between the two marks on the viscometer was measured (t). The ordinate -dt represents the decrease in this time at each point. Curve A, 660 units interferon curve B 150 units.

rations and the viscosity measured at various times. Fig. 1 shows that the viscosity of the DNA solution decreased after incubation with viral-or poly-I:C induced but was practically unaffected by preparations of mock interferon.

Conditions affecting the rate of reaction. The optimum pH was found to be 6.3 and divalent cations were shown to be essential to the reaction, since it was completely inhibited by ethylenediamine tetraacetate (EDTA). Using 0.1 M sodium acetate buffer (optimal concentration) at pH 6.3, Co^{++} and Mn^{++} were shown to be effective, Mg^{++} less so and Ca^{++} not at all.

Evidence for endonuclease activity. The rapid initial decrease in viscosity upon addition of interferon preparations to a DNA solution (fig. 2a) is in itself evidence for endonucleolytic activity. This indication is strengthened by the observation that, as the reaction progresses, traces only of trichloro-

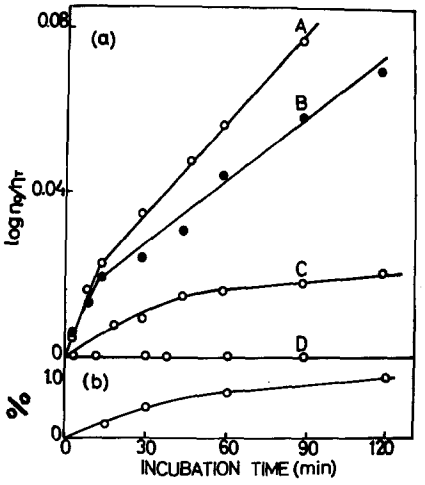

Fig. 2. (a) change in relative viscosity (η) of 1 mg/ml solutions of calf thymus DNA in 0.1M acetate buffer pH 6.3 after incubation with interferon (curves A,B,D) or mock interferon (curve C). Mn^{++} (curves B and C), Co^{++} (curve A) or EDTA (curve D) were added to a final concentration of 5×10^{-3} M.

(b) Acid soluble material produced during incubation of DNA with interferon using conditions as for curve B in figure 2 (a). Ordinate gives percentage of OD_{260} of original DNA solution rendered TCA soluble.

acetic acid(TCA) soluble products are released, not exceeding 1% of the total substrate even after 2 hr of incubation (fig. 2b). The acid-soluble material has not been characterized, but the absorption spectrum which at first shows a peak at 270 nm, shifting to 260 nm as the reaction progresses could indicate an initial predominance of pyrimidines in the small nucleotides released. Paper chromatography showed that mononucleotides were not formed, while paper electrophoresis confirmed that the DNA was split since there was movement of the UV-absorbing material from the origin under conditions where the DNA incubated with mock interferon moved only slightly. Further confirmation of the endonucleolytic degradation of the substrate was obtained by gel filtration of the reaction mixture through Sephadex G-200 that excludes polymers of molecular weight $2X10^5$ or higher. Twenty five percent of the DNA incubated with viral induced but not with mock interferon preparations was retarded on the column, indicating that hydrolysis did occur. Of the hydrolysis products more than 70% were only slightly retarded indicating a molecular weight not much less than $2X10^5$.

Relation of the endonuclease activity to Interferon. The material concentrated on Doucil was chromatographed on CM-sephadex (1) using a pH gradient for elution. Interferon-containing fractions to which bovine albumen had been added, were combined and reconcentrated by absorption to and elution from Doucil. Figure 3 shows that the endonuclease activity was still present in the interferon preparation. Again, even though the initial rate of reaction was fast, there was a marked decrease after 30 minutes incubation.

From the above evidence it seems clear that an endonucelase is released into the medium by cells producing interferon.

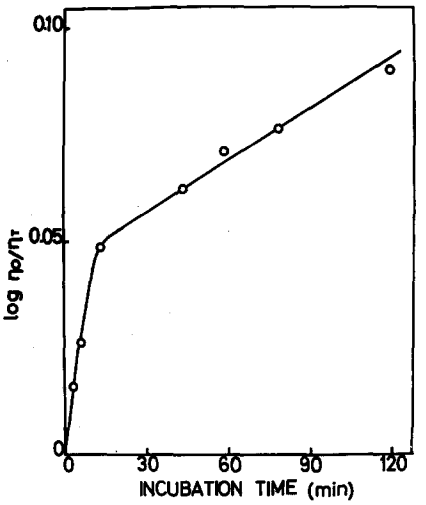

Fig. 3. Change in relative viscosity of solutions of calf thymus DNA after incubation with the concentrated Interferon-containing eluent from CM-sephadex. Conditions as for curve B figure 2 (a). Units of interferon=100.

Whether the enzyme is related specifically to interferon and its action as yet remains to be seen. It is possible that cells treated with UV-NDV virus or poly I:C may secrete or leak proteins to a greater degree than untreated cells. It should be emphasized that virus was not multiplying in these cells which showed no cytopathological effect. Even so the circumstantial evidence that the enzyme activity is not inactivated at pH 2, is not present in mock interferon preparations and is not separated from interferon by chromatography on CM-sephadex merely allows the possibility that the enzyme activity may be related to the interferon molecule. Certainly other proteins are present in the chromatographed preparations, some of which can be separated from interferon by acrylamide gel electrophoresis and some of which probably cannot (9). It will be difficult to determine conclusively whether the two activities belong to the same molecule.

Even if the nuclease activity is not a property of the in-

terferon protein, it could still play a role in interferon-associated viral resistance. The interferon preparations used represent medium, harvested from cells 24 hr after the stimulus to interferon production. At this time cells were resistant to infection so that any other proteins essential to the antiviral state would be present in the cells and possibly in the medium.

One hypothesis which can be considered is that the endonuclease activity demonstrated here is associated with the interferon protein which acts on host nucleic acid giving a product which is an inducer of viral resistance and interferon production. Prelimary results indicate that ribonucleic acids are also split by preparations of chick interferon.

ACKNOWLEGEMENT

The authors are grateful to Professor A. Symeonidis for helpful discussions and continued support, to Dr. N. Papakyriazis for the preparation of poly I:C induced interferon and to Mrs. G. Karamanlidou for expert technical assistance. The work was supported by grant DRG 1040 from the Damon Runyon Memorial Fund for Cancer Research.

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