Archives of Virology 52, 175—179 (1976)

Inactivation of Newcastle Disease Virus by β-Propiolactone

Brief Report

By

BARBARA GARLICK and R. J. AVERY

Department of Biological Sciences, University of Warwick, Coventry, England

With 3 Figures

Accepted June 1, 1976

Summary

Newcastle Disease Virus inactivated by β-propiolactone (β-PL) was found to lose RNA-dependent RNA polymerase activity. β-PL was shown to react with both virus proteins and RNA.

*

β-propiolactone is a highly reactive alkylating and acylating agent. It has been widely used to inactivate viruses, and has been especially useful for the production of vaccines, as it causes loss of infectivity while preserving antigenicity (5).

However, despite its widespread use, little is known about its mode of action. In order to elucidate the mechanism of the inactivation process, we have studied the effect of β-PL on the paramyxovirus Newcastle Disease Virus (NDV).

We have investigated the effects of β-PL on the infectivity of NDV and on the haemagglutinin, neuraminidase, haemolysin and RNA-dependent polymerase activities of the virus particle. In order to relate the changes observed in biological properties to those occuring at the molecular level we have also studied the interaction of [^{14}C] labelled β-PL with the components of the virus particle.

NDV-Texas was grown and purified as described by MOORE and BURKE (7) and assayed by plaque formation on primary chick embryo cell cultures prepared as described by MORSER *et al.* (8).

Virus was inactivated with β-PL by the method of SHEAFF *et al.* (10). Fig. 1 shows that infectivity decreased with increasing concentrations of β-PL and that the inactivation followed 1 hit kinetics. This result is in agreement with that obtained by SHEAFF *et al.* (10), who demonstrated a decline in infectivity of NDV with increasing concentrations of β-PL.

In order to determine whether β-PL was acting by simply disrupting virus structure a comparison was made of untreated and β-PL inactivated NDV by

electron microscopy. Virus samples were negatively stained by a standard method using 1 per cent phosphotungstic acid adjusted to pH 6 by addition of 1 N KOH (4). Treated and untreated particles were not detectably different.

To investigate which components of the virus particle were being inactivated and hence causing the loss of infectivity, the effects of β-PL on three properties of the virus surface were examined. These were the haemagglutinin, neuraminidase and haemolysin activities.

The haemagglutination of chick erythrocytes by NDV was measured in a standard microassay system. Neuraminidase activity was measured by the method of WEBSTER and LAVER (11), using fetuin prepared as described by GRAHAM (3). Virus was first dialysed against PBS at 4° C. Haemolysin activity was measured by the method of CLAVELL and BRATT (2).

Fig. 1 summarises the effect of β-PL on these properties as compared to its effect on infectivity. All the properties were inactivated by high concentrations of β-PL. However, at concentrations of β-PL which reduced infectivity by 4 $\log_{10}$ units, the haemagglutinin, neuraminidase and haemolysin activities were not detectably reduced. It therefore seems unlikely that the drop in infectivity was caused by the inability of the virus to adsorb to, or penetrate, the cell.

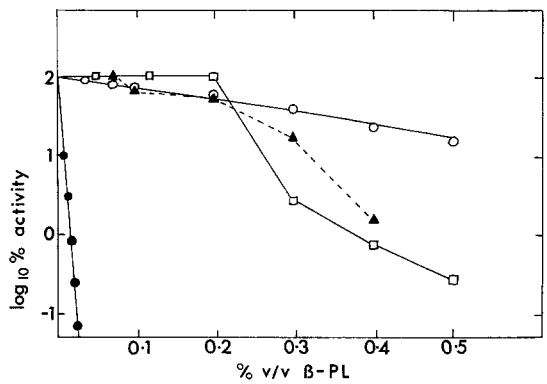

Fig. 1. Inactivation of NDV haemagglutinin □———□, neuraminidase ○———○, haemolysin ▲— — —▲, and infectivity ●———● by β-PL

We next examined the effect of β-PL on the RNA-dependent RNA polymerase activity of the virus, using the method of SHEAFF *et al.* (10), and employing 700 μg virus protein per assay. [^{3}H] GTP, specific activity 10 Ci/mmole, was supplied by the Radiochemical Centre, Amersham. RNA products were collected by precipitation with 2 ml 5 per cent TCA in 0.1 M sodium pyrophosphate using 50 μg yeast RNA as carrier. After 30 minutes at 4° C, precipitated RNA was collected on GF/C filter discs, washed 2 times with 4 ml 5 per cent TCA, 0.1 M sodium pyrophosphate, once with 4 ml ethanol, and once with 4 ml ether. The discs were dried and counted with toluene based scintillant in a Packard scintillation counter.

The effect of β-PL on the virion RNA—dependent RNA polymerase activity is shown in Fig. 2. The low concentrations of β-PL which had no effect on the surface properties of the virus did cause a decrease in polymerase activity. At the

lowest concentrations of β-PL, the rate of decline of polymerase activity was similar to that of infectivity, but less than that of infectivity at higher concentrations, presumably because virus particles which had been inactivated retain some ability to incorporate isotope.

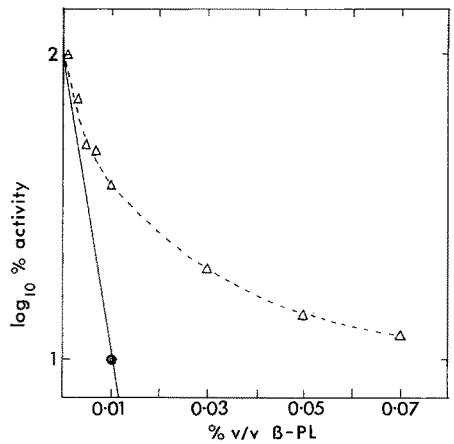

Fig. 2. Inactivation of NDV RNA polymerase △ — — —△ and infectivity •———• by β-PL

It seems clear that the step in virus replication most sensitive to the action of β-PL is the transcription of the virus genome. Thus transcription is the probable primary target of β-PL in causing a loss of virus infectivity.

To directly determine the site of action of β-PL, virus was treated with [^{14}C] β-PL, specific activity 2 mCi/mmole, obtained from the Radiochemical Centre, Amersham. After exhaustive dialysis against TN buffer (30 mM NaCl, 10 mM Tris HCL pH 7.3), viral RNA and protein were prepared. RNA was prepared by repeated extraction with phenol in the presence of 2 per cent SDS and 500 μg yeast carrier RNA, precipitated by the addition of ethanol at —20° C, and dissolved in standard saline citrate. Material from the phenol-aqueous interface was collected at each stage of the extraction, and protein precipitated from it by the addition of acetone to 90 per cent v/v at 4° C. The proteins were re-dissolved in 1 per cent SDS by heating in a boiling water bath.

Viral RNA and protein were assayed for reaction with [^{14}C] β-PL by precipitation for ½ hour in 5 per cent TCA at 4° C. The precipitates were collected, washed and counted as described earlier. Controls were set up to determine the background level of hydrolysed [^{14}C] β-PL retained on GF/C filters. The level of ^{14}C counts in samples of extracted RNA and protein before precipitation was determined. Controls contained equivalent amounts of counts from a [^{14}C] β-PL hydrolysate and of cold yeast RNA or cold bovine serum albumin. Susceptibility of viral RNA to T_1 and pancreatic ribonuclease digestion was assayed by the method of AVERY (1). RNA was also characterized by digestion with 0.3 M KOH for 16 hours at 37° C.

Table 1 shows the distribution of radioactivity between the RNA and protein preparations. These results suggested that both virus protein and RNA were reacting with β-PL.

Table 1. *TCA—precipitable* [^{14}C] *counts in NDV protein and RNA after treatment of virus with* [^{14}C] β-*PL. RNA was characterized by susceptibility to digestion by alkali and ribonuclease*

	TCA precipitable [^{14}C] c.p.m.
NDV RNA	26,200
T_1 and pancreatic RNase treated RNA	2,750
Alkali digested RNA	3,150
NDV protein	51,600

Viral proteins were analysed by electrophoresis on 9×0.5 cm 10 per cent polyacrylamide gels, prepared by the method of MOORE and BURKE (7). [^{14}C] β-PL treated virus was denatured by boiling for 2 minutes in the presence of 1 per cent SDS and 1 M urea. Denatured virus was loaded on top of the gel and electrophoresed for 4.5 hours at 3.5 ma per gel. Gels were stained with Coomassie blue, using the method of MAIZEL *et al.* (6), and scanned at 620 nm. Gels were then sliced, the slices digested with 200 μl 100 volume hydrogen peroxide, and counted using a Triton-toluene based scintillant.

Fig. 3 shows a typical gel profile. All the viral proteins are labelled, although not equally, and hence all were alkylated by β-PL.

As β-PL reacts with viral RNA and all the virus proteins (Fig. 3 and Table 1), it is not possible to say which of the components necessary for transcription is the most susceptible—the genome RNA, its surrounding nucleocapsid protein, or the RNA polymerase itself. However, the ^{14}C labelled β-PL is interacting with virus

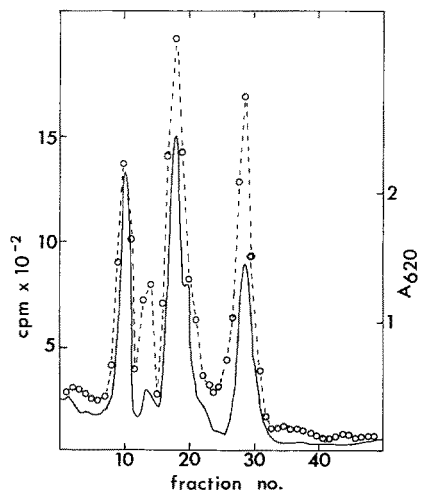

Fig. 3. Polyacrylamide gel electrophoresis of virus proteins from [^{14}C] β-PL treated virus. A 620—, c.p.m. —o—o—o

proteins (Fig. 3) at concentrations which do not affect at least some of their enzymatic activities (Fig. 1). In addition, PRINZIE *et al.* (9) have shown that RNA extracted from poliovirus inactivated with β-PL had lost its infectivity. Therefore it seems most likely that it is the alkylation of the RNA genome which reduces transcription by β-PL treated NDV, but this question can only be finally resolved when it becomes possible to dissociate and reconstitute the genome RNA and its associated nucleocapsid and polymerase proteins.

Acknowledgments

We thank the Agricultural Research Council for a grant which supported this work.

References

1. AVERY, R. J.: The subcellular localisation of virus-specific RNA in Influenza Virus-infected cells. J. gen. Virol. **24**, 77—88 (1974).
2. CLAVELL, L. A., BRATT, M. A.: Hemolytic interaction of Newcastle Disease Virus and chicken erythrocytes. II. Determining factors. Appl. Microbiol. **23**, 461—470 (1972).
3. GRAHAM, E. R. B.: Some aspects of the structure of the carbohydrate moiety of fetuin. Austral. J. Science **24**, 140—141 (1961).
4. HASCHEMEYER, R. M., MYERS, R. J.: Negative Staining. In: HAYAT, M. A. (ed.), Principles and Techniques of Electron Microscopy, Vol. II, 101—147. Van Nostrand Reinhold Company 1972.
5. LO GRIPPO, G. A.: Investigations of the use of beta-propiolactone in virus inactivation. Ann. New York Acad. Sci. **83**, 578—594 (1960).
6. MAIZEL, J. V., SUMMERS, D. F., SCHARFF, M. D.: SDS acrylamide gel electrophoresis and its application to the proteins of poliovirus and adenovirus-infected human cells. J. cell. Physiol. **76**, 273—288 (1970).
7. MOORE, N. F., BURKE, D. C.: Characterisation of the structural proteins of different strains of Newcastle Disease Virus. J. gen. Virol. **25**, 275—289 (1974).
8. MORSER, M. J., KENNEDY, S. I. T., BURKE, D. C.: Virus specific polypeptides in cells infected with Semliki Forest Virus. J. gen. Virol. **21**, 19—29 (1973).
9. PRINZIE, A. SCHONNE, E., DE SOMER, P.: Polio virus replication and cytopathogenicity in monolayer hamster cell cultures fused with β-propiolactone inactivated Sendai virus. Arch. ges. Virusforsch. **10**, 153—166 (1960).
10. SHEAFF, E. T., MEAGER, A., BURKE, D. C.: Factors involved in the production of interferon by inactivated Newcastle Disease Virus. J. gen. Virol. **17**, 163—175 (1972).
11. WEBSTER, R. G., LAVER, W. G.: Preparation and properties of antibody directed specifically against the neuraminidase of influenza virus. J. Immunol. **99**, 49—55 (1967).

Authors' address: Dr. BARBARA GARLICK, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, England.

Received March 3, 1976