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PURIFICATION OF NEWCASTLE DISEASE VIRUS BY CHROMATOGRAPHY ON CONTROLLED-PORE GLASS BEAD COLUMN

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Summary. — Newcastle disease virus (NDV) purified by sucrose density gradient centrifugation was chromatographed on a controlled-pore glass (CPG) bead column. By this procedure, the contaminating ribonuclease activity was reduced by 94—96% and the specific viral haemagglutinating activity increased from 3- to 5-fold. Purified NDV moved in two fractions in free electrophoresis.

Key words: virus purification; controlled-pore glass; virus-associated enzymes

Introduction

Enveloped RNA viruses have associated to their coat certain enzymes occurring in the plasma membranes of the host cells (Pristašová, 1980). Virus purification procedures based on sedimentation methods are insufficient to remove these enzymatic activities. Several viruses have been successfully purified by column chromatography on controlled-pore glass (CPG), e.g. influenza (Bresler *et al.*, 1975), avian myeloblastosis (Darling *et al.*, 1977), tick-borne encephalitis (Krasilnikov *et al.*, 1977), rabies (Hiatt *et al.*, 1971), bacterial (Gschwender *et al.*, 1969) and plant (Marcinka, 1972; Barton, 1977) viruses.

We used CPG chromatography for NDV purification and checked the homogeneity of purified virus by free electrophoresis.

Materials and Methods

Virus. Newcastle disease virus (NDV; genus *Paramyxovirus*) strain Kansas was propagated in chick embryos as described (Rosenbergová and Pristašová, 1972) and purified from allantoic fluid by differential and discontinuous and continuous sucrose density gradient centrifugation (Rosenbergová *et al.*, 1981).

CPG chromatography. CPG with a mean pore size of 150 nm (Bio-Glass 1500, BioRad) was mixed with de-aired 1% aqueous solution of polyethylene glycol 20000 (PEG) and kept for 12 hr at room temperature to reduce the sorption properties of CPG (formation of hydrogen bonds between PEG and electronegative active centres on CPG surface). Excess CPG was removed by several decantations with distilled water. A 58 × 1.4 cm column was filled with thus treated CPG and equilibrated with de-aired elution buffer, either NaCl-Tris (0.06 M NaCl, 0.01 M Tris-HCl, pH 7.6) or NaCl-phosphate (0.06 M NaCl, 0.01 M phosphate buffer, pH 7.6). From 0.5 to 1 ml of virus was placed on the column. The virus was eluted at a flow rate of 25 ml per hr. The eluate

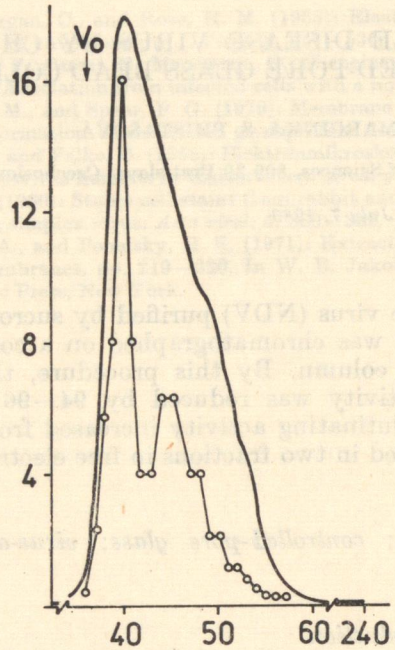

Fig. 1.

NDV elution profile after CPG column chromatography
—— A_{280nm} (UV-monitor record)
○ Virus titre
Abcissa: fraction number; ordinate: HA units per ml (in thousands)

was centrifuged for 90 min at 40000 × g and the pellet resuspended in a volume corresponding to that of the sample placed on the column. Purity of the eluted virus was evaluated based on its protein contents determined according to Lowry *et al.* (1951). Virus infectivity was assayed by the plaque method and the titres were expressed in plaque forming units (PFU) per ml. The haemagglutinating and ribonuclease activities were determined according to Rosenbergová and Pristašová (1972). The enzymatic activity was expressed in $A_{260\ nm}$ of nucleotides released from 40 μg cellular RNA in 1 hr in a standard assay. The virus preparations were also checked by free electrophoresis in sucrose density gradients (Rosenbergová *et al.*, 1981).

Results

The elution profile on CPG chromatography of NDV purified by sucrose density gradient centrifugation is illustrated in Fig. 1. The curve of absorbancy at 280 nm shows that proteins were eluted from the column in a comparatively broad peak immediately after the void volume. The haemagglutinating (HA) activity of the virus occurred in the same region, but the respective elution curve showed two maxima. Fractions 37—46 from the column (Fig. 1) were pooled, centrifuged for 90 min at 40000 × g and the sediment was resuspended in NaCl-Tris in a volume corresponding to that of the sample placed on the column. As compared to the original titres, the HA titre of the eluted virus remained unchanged and the infectivity titre related to mg protein decreased by 0.3 log units. The protein content in the eluate decreased by 60%. Since no protein was detected in the eluate on further elution from the column, we assume that the remaining protein was firmly bound to the column.

Table 1. Efficienty of NDV purification by CPG column chromatography

Purification procedure	mg protein per column	HA units per ml $\times 10^{-3}$	mg protein per ml	RNase activity $A_{260\ nm}$	HA units per mg protein	Purification factor	% residual enzyme
Sucrose gradient	—	256	12.4	0.644	20.6	1.0	100.0
1× CPG, NaCl-Tris	11.0	256	4.4	0.114	58.0	2.8	17.7
1× CPG, NaCl-Tris	11.0	256	3.8	0.132	67.5	3.28	20.2
2× CPG, NaCl-Tris	4,4	256	2.5	0.052	102.0	4.95	8.1
Sucrose gradient	—	256	8.5	0.495	30.0	1.0	100.0
1× CPG, NaCl-Tris	4.8	256	2.5	0.030	102.0	3.3	6.4
1× CPG, NaCl-phosphate	4.8	128	3.0	0.021	42.7	2.42	4.3

In studying the effects on virus chromatography of the amount of protein placed on the column and of the elution buffer, we found (Table 1) that in the range from 5 to 11 mg protein the purification factor was approximately the same (about 3). This factor increased to 5 after rechromatography. From the point of view of virus recovery, the anionic phosphate buffer proved to be less suitable than the cationic Tris buffer. On elution with NaCl-Tris, the HA activity of the virus was preserved, while in NaCl-phosphate buffer the HA titre decreased by 50—80%. The ribonuclease activity assay showed that the relative decrease in enzymatic activity depended on the amount of virus placed on the column. On chromatography of 11 mg protein, the ribonuclease activity in the eluate decreased by 80%, with 4—5 mg protein it decreased by 96—98%.

The individual purification steps were checked by free electrophoresis. Virus purified only by differential centrifugation moved in a single peak in the buffer used; proteins moved in several fractions (Fig. 2-I). Sucrose density gradient centrifugation removed most of the fast-moving proteins the virus population was separated into two fractions moving at different speeds (Fig. 2-II) and coinciding with the protein peaks. The slow-moving proteins shown in Fig. 2-II were removed by CPG chromatography.

Discussion

Purification of NDV by CPG column chromatography made it possible to remove from the virus proteins which could not be separated from it by sedimentation methods. Ribonuclease activity used as a marker of protein contamination was lowered to 2—4% in NDV purified by CPG chromatography. The amount of proteins irreversibly bound to CPG (60—70%) was surprisingly high and cannot be explained by adsorption of only cellular

proteins because polypeptides found in NDV purified by sedimentation in a gradient are mainly viral (Montcastle *et al.*, 1971). We assume that under the conditions used also a part of viral material was adsorbed on to the CPG column. The HA titre of the purified virus in NaCl-Tris elution buffer did not decrease, but this phenomenon could also be explained by dissociation of viral aggregates.

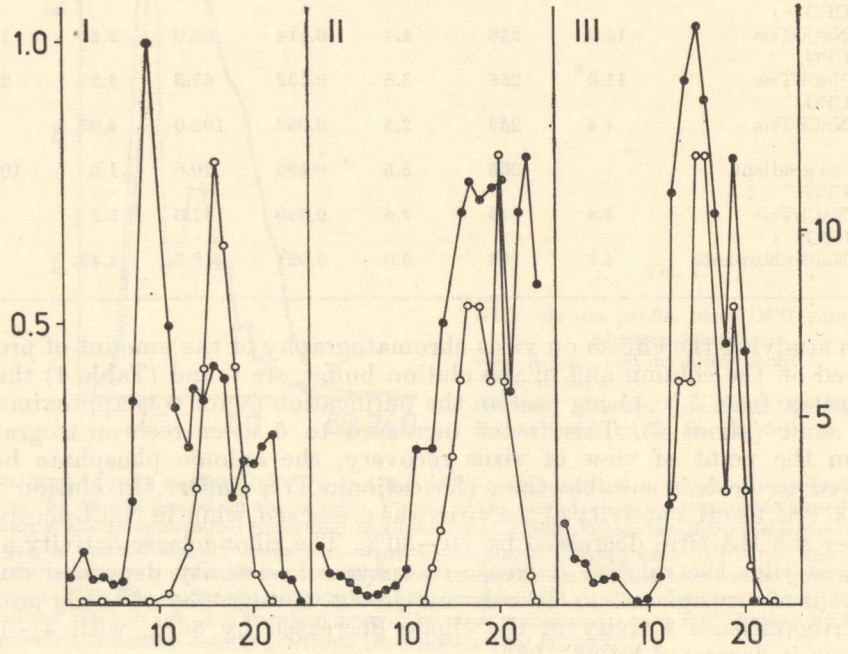

Fig. 2.

Electrophoretic pattern of NDV purified by differential centrifugation (I), sedimentation in sucrose density gradient (II) or CPG chromatography (III)

Abscissa: fraction number; left ordinate: absorbancy ($A_{280\ nm}$ - $A_{310\ nm}$; ●); right ordinate: virus titre (HA units per ml, in thousands; ○)

Treatment of CPG by PEG 20000 does not completely eliminate the electrostatic forces of glass beads (Krasilnikov *et al.*, 1977). This was confirmed by the present results according to which that part of proteins which was not eluted in the viral peak remained irreversibly bound to the carrier. We cannot definitely conclude, therefore, based on which separation principle the two HA fractions were obtained from the column.

Pleomorphism of paramyxovirus particles was demonstrated by electron microscopy (Horne and Waterson, 1960; Rott and Schäfer, 1961). Two density fractions were found on Sendai virus sedimentation in an isopyknic

gradient (Kiselev *et al.*, 1969). We found by electrophoresis of NDV purified by density gradient sedimentation and CPG chromatography that NDV population is not homogeneous as to its electric charge and is separated in the electric field into two HA fractions. The problem of electrophoretic forms of NDV is being studied further.

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