DEOXYCHOLATE-MEDIATED CLEAVAGE OF THE PROTEIN OF SENDAI VIRUS NUCLEOCAPSID DERIVED FROM INFECTED CELLS

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SUMMARY: Parental Sendai virus nucleocapsid-like particles (NLP) were discovered in particulate fraction of Ehrlish tumor cells prepared 2 hours after infection. NLP polypeptide patterns essentially depended on sodium deoxycholate (DOC) treatment of cell homogenate. When DOC treatment was omitted, two classes of parental polypeptides were revealed by polyacrilamide gel electrophoresis: the largest virion protein and the major nucleocapsid protein. DOC treatment influenced neither yield of the NLP in particulate fraction nor their buoyant density. However, the electrophoretic pattern of the protein was significantly changed: the largest protein was removed, and the most part of the major nucleocapsid protein was cleaved yielding two classes of proteins with lower molecular weights.

INTRODUCTION: Nucleocapsid protein of paramyxoviruses (Simian virus-5, Newcastle disease and Sendai viruses) with a molecular weight (MW) of about 60,000 daltons possesses an interesting property to be cleaved under special conditions yielding homogeneous products of MW about 40,000. This phenomenon has been described by Mountcastle et al.(1) in the case when paramyxovirus infected cells were removed from the glass by trypsin (but not by EDTA). Later we described the same phenomenon after incubation of Sendai virus nucleocapsids with Ehrlish tumor cell ribosomes (2). In this communication, we report that the cleavage of Sendai

virus nucleocapsid protein occurs when cytoplasmic extracts from infected cells are treated with DOC.

MATERIALS AND METHODS: Preparation and purification of Sendai virus labelled with /^{14}C/-amino acids were described elsewhere (2,3). Ehrlish tumor cells were infected with /^{14}C/-virus (100-200 ID_{50}/cell) and after two hours incubation at 37°C disrupted in Dounce homogenizer (10^8cells/ml) in hypotonic buffer (0.01 M NaCl; 0.002 M Mg-acetate; 0.01 M tris - HCl, pH 7.5). Cell homogenate was centrifuged at 2,000 rev/min for 10 min, supernatant ("S-2") was treated with 0.01 M EDTA, divided into two equal parts and one part was treated with 0.25% DOC. The cell extract was centrifuged at 15,000 g for 15 min, then the supernatants were layered on 30% sucrose (in hypotonic buffer lacking Mg^{2+}) and centrifuged in 50 Ti rotor of Spinco L 2-50 centrifuge at 42,000 rev/min for an hour. The pellets ("particulate fraction") were suspended in 0.01 M triethanolamine, pH 7.5 and used either for polypeptide analysis in sodium dodecylsulfate (SDS)-polyacrilamide gels in accordance with the method described by Laemmli (4) or for buoyant density analysis in cesium chloride gradients. In the latter case the samples were treated with 4% formaldehyde (5).

RESULTS AND DISCUSSION: As shown in Fig. 1a, b, Sendai virions and nucleocapsids were revealed as the particles with a buoyant density of 1.25 g/ml and 1.33 g/ml respectively. The parental virus material isolated from particulate fraction of infected cells has the same buoyant density as nucleocapsids (Fig. 1c, d). It is seen that DOC treatment of S-2 fraction influenced neither yield nor buoyant density of the parental NLP.

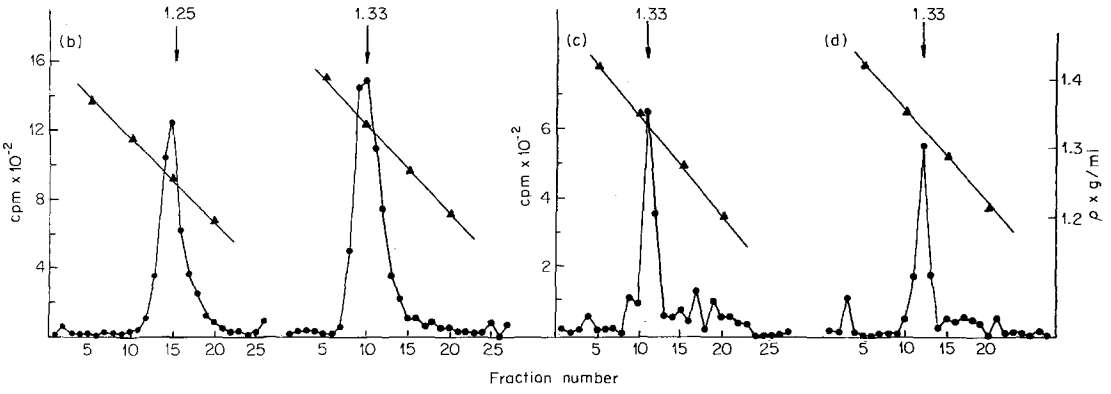

Figure 1. Buoyant density analysis of /^{14}C/-amino acid-labelled Sendai virions, nucleocapsids and intracellular NLP. Materials in 0.01 M tris-ethanolamine, pH 7.5 were treated with 4% formaldehyde and layered on the tops of CsCl gradients (1.5-1.1 g/ml). The gradients were centrifuged in SW 50.1 rotor of Beckman L2-65 centrifuge at 35,00 rev/min for 16 hours. 15 drop fractions were collected after centrifugation, buoyant density (8) and acid-insoluble radioactivity were determined (a) purified virions; (b) nucleocapsids (purified /^{14}C/-virions were treated with 0.5% DOC and the particles were sedimented through 30% sucrose); (c) and (d) S-2 fraction prepared 2 hr after infection of Ehrlish tumor ascitic cells with /^{14}C/-amino acid-labelled virus was divided into 1 ml samples. One sample was not treated (c) and the other was treated with 0.25% DOC (d). Particulate fractions were prepared and analyzed.

The polypeptide analysis in SDS-polyacrilamide gels (Fig. 2a, b) showed that Sendai virions contained 5 major proteins while nucleocapsids derived from virions by DOC treatment only 1 major class.

These results are in agreement with earlier determinations (6,7). NLP contained two classes of parental proteins: the largest virion protein (L) of MW 71,000 and the major nucleocapsid protein (NP) of MW 58,000 (Fig. 2c). Electrophoretic pattern was essentially altered when S-2 fraction was treated with 0.25% DOC. In this case (Fig. 2d) the L

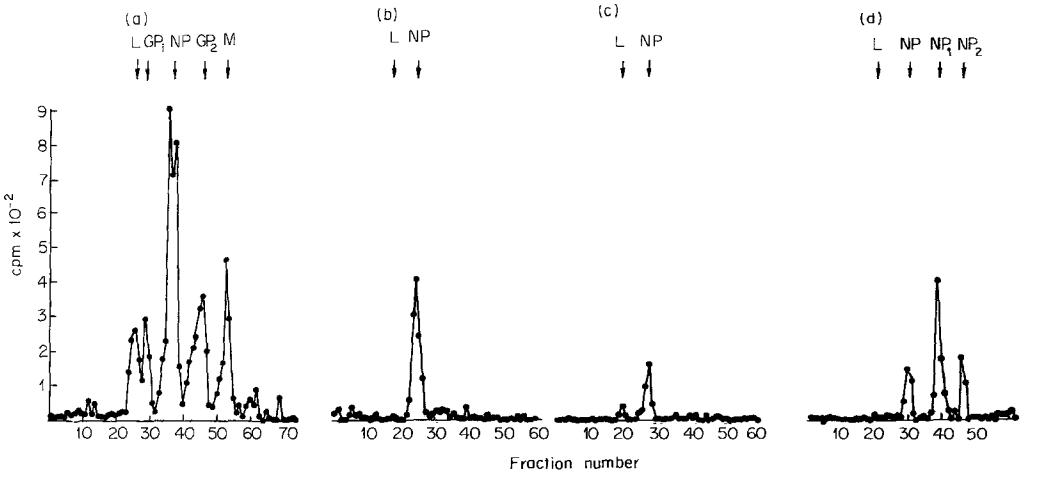

Figure 2. Polyacrilamide gel electrophoresis of /^{14}C/-amino acid-labelled Sendai virions, nucleocapsids and NLP. The method described by Laemmli (4) was used. 10 cm long 7.5% acrylamide gels with stacking gel of 2.5% acrylamide contained 0.2 % NN-methylene bisacrylamide in 0.375 M tris-HCl, pH 8.8 with 0.1% SDS. For polypeptide dissotiation the samples were suspended in 5 M urea, 2% SDS and 5% mercaptoethanol and heated in boiling water for 3 min. Electrophoresis was carried out for 3 hr at 5 mA/gel. After electrophoresis the gels were sliced into 0.75-1.25 mm slices, each slice was put into a tube with 0.1 ml 30% H_2O_2 and incubated overnight at 37°C. Solubilized materials were put on paper disks and counted after drying; (a) /^{14}C/-virions; (b) /^{14}C/-nucleocapsids; (c) NLP /^{14}C/-proteins without and (d) with prior DOC treatment of S-2 fraction (see Legend to Fig.1).

class was absent and two new classes were revealed: NP_1 (MW 49,000) and NP_2 (MW 42,000). Both new proteins are likely to be the products of the NP cleavage in vitro. The role of DOC in the cleavage is not clear at present. It may be suggested that the proteolysis is due to damaging of the cell lysosome membranes by detergent leading to liberation of proteases and their attack on NLP. However it cannot be ruled out that the L class protein of the NLP plays some protective role and its removal by DOC-treatment stimulates

the NP cleavage. Since the total molecular mass of the proteolysis products is higher than the molecular weight of NP polypeptide either a step-like proteolysis ($NP \rightarrow NP_1 \rightarrow NP_2$) or an ambiguity of cleavage ($2NP \rightleftharpoons \begin{matrix} NP_1 \\ NP_2 \end{matrix}$) may be suggested.

In any case, the homogeneous size of the cleavage products evidences that this process is highly specific. In this connection we cannot rule out that the same (or like) mechanism of the proteolysis is finally realized in infected cells and that this phenomenon is important for certain intracellular virus-specific processes.

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