IDENTIFICATION OF SENDAI VIRUS NEURAMINIDASE AND HEMAGGLUTININ SUBUNITS

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Received 10 December 1974

1. Introduction

The polypeptides of Sendai virus have been examined by SDS* polyacrylamide gel electrophoresis and about 6 polypeptides have been separated (1–4) (compare also fig.3). The most abundant in virions is the nucleocapsid polypeptide, which is for the moment the only polypeptide identified with certainty [2,3,5].

Two glycoproteins belonging to the virion surface have been found, with a mol. wt of 65 000 and 53 000, respectively [2]. According to Kingsbury [3], the 65 000 glycoprotein could be the hemagglutinin, whereas the smaller glycoprotein probably represents the viral neuraminidase. On the other hand, Maeno et al. [6] have identified two kinds of Sendai virus surface glycoproteins, one of which is associated with HA activity, having a molecular weight of 124 000, and another one, associated with neuraminidase activity, having a mol. wt of 114 000.

Since up to now no definite identification of either hemagglutinin or neuraminidase has been made [3], the present paper describes the identification of these two Sendai virus subunits and the determination of their mol. wts.

* *Abbreviations:* SDS = sodium dodecyl sulfate; EDTA = ethyle diamine tetraacetic acid; HA activity = hemagglutination activity; PBS = phosphate buffered saline.

2. Materials and methods

Sendai virus was grown and purified according to the procedure of Mountcastle, Compans and Choppin [2]. The virus nucleocapsid was isolated and purified from Triton X-100 and sodium deoxycholate-disrupted virus [7]. The glycoproteins situated at the surface of the virus were isolated by treatment of the virus with Tween and ether [8].

Neuraminidase activity was determined according to Aminoff's method [9]. Fetuin (Colbiochem) was used as substrate. Protein was estimated by the method of Lowry et al. [10] using as a standard bovine serum albumin.

Hemagglutinin estimations were done by the standard method described by the WHO Expert Committee on Influenza [11].

Dissociation of viral proteins and electrophoresis on polyacrylamide gels for molecular weight estimations was performed as follows: samples containing approximately 100 μg of virus, virus components or standard proteins in 0.04 M Tris–HCl, 0.02 M acetate, 0.002 M EDTA pH 7.8 buffer were mixed with urea, SDS and β-mercaptoethanol up to a final concentration of 5 M, 1% and 2%, respectively. The mixtures were then heated at 65°C for 10 min and after cooling they were applied directly to polyacrylamide gels. The gel concentration was of 10% acrylamide and 0.27% bis-acrylamide and contained 0.1% SDS. The gels were prepared in glass tubes with an internal diameter of 6.5 mm. The reservoirs contained 0.04 M Tris–HCl, 0.02 M sodium acetate, 0.002 M EDTA, pH 7.8 buffer, supplemented with 0.5% SDS.

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As mol. wt markers of the gels we used: bovine serum albumin (67 000), soya bean trypsin inhibitor (21 000) and cytochrom *c* (12 400).

Coomassie Brilliant Blue 0.25% dissolved in 50% (v/v) methanol, 5% (v/v) acetic acid was used as protein stain.

Carbohydrate groups associated with polypeptides, generally called glycoproteins [1–3], were detected using the improved periodic acid–Schiff method of Kapitony and Zebrowski [12].

3. Results and discussion

3.1. *Sendai virus glycoproteins*

Fig.1 shows the SDS gel electrophoresis of glycoproteins solubilized by Tween and ether from the surface of Sendai virus. Three prominent glycoproteins were found, with mol. wts of 75 000, 65 000 and 53 000. With the exception of the 75 000 compound, the other two glycoproteins had been described by Mountcastle, Compans and Choppin [2]. A fourth compound, although in a quite small amount, could always be seen on the gels in which the total virus

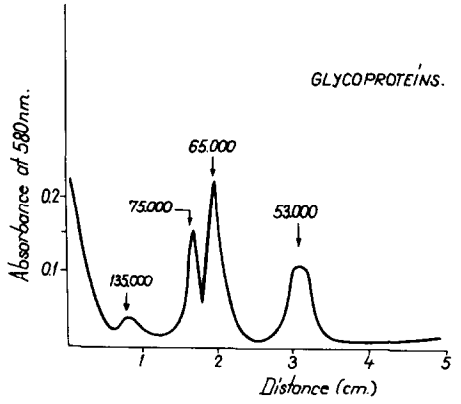

Fig.1. Densitometric tracing at 580 nm of Sendai virus glycoproteins separated by SDS gel electrophoresis and stained with periodic acid–Schiff reagent [12]. Virus surface glycoproteins were obtained by Tween and ether treatment of the virus [8], dialysed 3 days against PBS [4] and finally concentrated with Acrylex-10000 (Chinoin). Approximately 100 μg of glycoproteins were dissociated with urea, SDS and β-mercaptoethanol and applied to SDS polyacrylamide gel electrophoresis as described in Materials and methods.

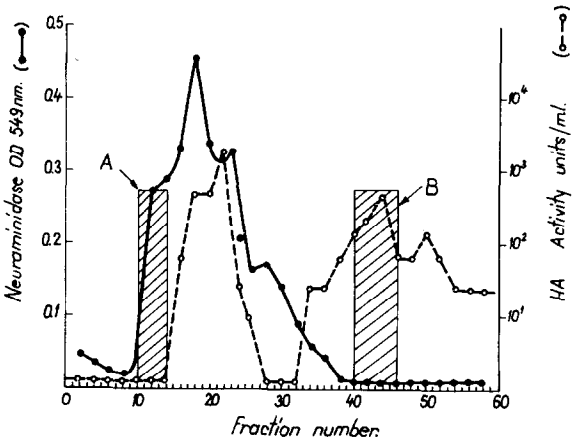

Fig.2. Sendai virus glycoproteins fractionation on Sephadex G-200 column. Glycoproteins were separated, dialysed and concentrated as indicated in the legend of fig.1. Ten ml of glycoproteins, containing 1.3 μmol NANA/mg protein neuraminidase activity and 10^5 HA units/ml, were applied to a Sephadex G-200 column. Bed dimensions: 2.5 × 40 cm. Flow rate: 40 ml/hr. The void volume was of 54 ml. Elution was performed with PBS and 2-ml fractions were collected (starting with the 17^{th} fraction). Each fraction was tested for protein content, HA and neuraminidase activity. Fractions containing only neuraminidase activity (area indicated on the figure with A) and those containing only HA activity (area B) were concentrated and analysed by SDS polyacrylamide gel electrophoresis (compare fig.3).

polypeptides or glycoproteins were examined. It has a molecular weight of 135 000 and probably represents the dimer form of the 65 000 glycoprotein which failed to be dissociated during the urea, SDS and β-mercaptoethanol treatments.

3.2. *Identification of neuraminidase and hemagglutinin subunits*

When glycoproteins are submitted to gel filtration on Sephadex G-200 column, neuraminidase and HA activities are distributed almost in the same fractions eluted from the column (fig.2). This indicates that the greatest part of neuraminidase and hemagglutinin are associated. However, there are some fractions which contain only neuraminidase or HA activities. The fractions containing only neuraminidase activity and no detectable HA activity were pooled (compare area A in fig.2), concentrated, and analysed by SDS gel electrophoresis for characterization of polypeptide content. Fractions containing the released HA

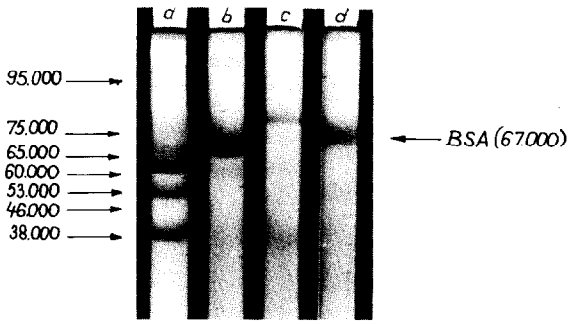

Fig.3. Polyacrylamide gel electrophoresis of a) whole Sendai virus; b) hemagglutinating material eluted from Sephadex G-200 column (compare area B in fig.2); c) neuraminidase activity-containing material (compare area A in fig.2); d) bovine serum albumin. Dissociation of virus, virus components or standard proteins to polypeptides is described under Materials and methods. Gels a) and d) were stained with Coomassie Brilliant Blue, whereas gels b) and c) with periodic acid–Schiff reagent [12].

activity were also pooled (area B in fig.2), concentrated and characterized by SDS gel electrophoresis Fig.3 shows that the hemagglutinating material contains a glycoprotein with a mol. wt of 65 000. The neuraminidase activity-containing material is also a glycoprotein, whose mol. wt is of 75 000.

It has been suggested previously that the larger glycoprotein with a mol. wt of 65 000 represents the viral hemagglutinin, since 'hemagglutinin is thought likely to be the most abundant surface protein and since the larger glycoprotein is the principal one that adsorbs to erythrocytes' [3]. Thus, our results are in perfect agreement with this observation, establishing directly the mol. wt of the hemagglutinin subunit.

On the other hand, we found that the neuraminidase subunit had a molecular weight of 75 000 and not of 53 000 [3], or of 114 000 [6] as suggested by other authors. The presence of a glycoprotein with a molecular weight of 53 000 was always found between the glycoproteins separated from the virus surface by Tween and ether treatment (compare fig.1). We do not know yet whether this glycoprotein can or cannot be related to the virus neuraminidase. It is of interest to mention that the neuraminidase-containing material which contains the 75 000 glycoprotein has a specific activity of 13 μmol NANA/mg protein, which is about 12 times higher than that found in the glycoprotein solution.

The biological activities of Sendai virus associated with the virus surface are destroyed by detergents [4]. Therefore, a successful characterization of the molecules possessing these activities depends on the artificial re-assembly of detergent-solubilized particles by the removal of the detergent. At least 3 days of dialysis against PBS were necessary to remove the Tween 80 from the glycoprotein solution. During this time, a kind of artificial assembly of glycoprotein particles occurred. This process has been studied recently by Hosaka and Shimizu [4]. Most of the particles include both neuraminidase and hemagglutinin subunits in different proportions, as illustrated by the gel filtration experiment (compare fig.2), and a limited number of particles include only neuraminidases or hemagglutinins. A somewhat similar situation was previously reported in a gel filtration experiment of Sendai virus glycoproteins on Sephadex G-200 columns [6] or Sepharose 6B columns [13]. The artificial assembly process during dialysis cannot be controlled, consequently the chromatographic profile of glycoproteins submitted to gel filtration is not totally reproducible. However, in 4 out of 7 different experiments, we were able to isolate fractions containing only neuraminidase and HA activities. In all these cases the fractions possessing HA activity contained a glycoprotein with a mol. wt of 65 000 and the neuraminidase activity-posessing fractions contained a glycoprotein with a mol. wt of 75 000, a fact which supports our conclusion that these glycoproteins represent the hemagglutinin and neuraminidase subunits, respectively. Finally, it should be mentioned that the separation of these two components is due only to the different aggregation properties of neuraminidase and hemagglutinin, since otherwise it would have been impossible to separate molecules with molecular weights of 65 000 and 75 000 on the Sephadex G-200 column.

References

[1] Content, J. and Duesberg, P. H. (1970) J. Virol 6, 707.
[2] Mountcastle, W. E., Compans, R. W. and Choppin, P.W. (1971) J. Virol. 7, 47.
[3] Kingsbury, D. W. (1972) Curr. Top. in Microbiol. 59, 1.

[4] Hosaka, Y. and Shimizu, Y. K. (1972) Virology 49, 627.
[5] Popa, L.M., Repanovici, R., Samuel, I., Burducea, O., Weiner, M. and Portocală, R. (1974) Rev. roum. Virol. 25, in press.
[6] Maeno, K., Yoshida, T., Inuma M., Nagai, J., Matsumoto, T. and Asai, I. (1970) J. Virol. 4, 492.
[7] Mountcastle, W. E., Compans, R. W., Caliguiri, L. A. and Choppin, P. W. (1970) J. Virol. 6, 677.
[8] Becht, H. and Rott, R. (1972) Med. Microbiol. Immunol. 158, 67.
[9] Aminoff, D. (1961) Biochem. J. 81, 384.
[10] Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265.
[11] WHO Expert Committee on Influenza (1953) Techn. Rep. 64.
[12] Kapitany, K. A. and Zebrowski, E. J. (1973) Annal. Biochem. 56, 361.
[13] Brostrom, M. A., Bruening, G. and Bankowski, R. A. (1971) 46, 856.