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ISOLATION OF SENDAI VIRUS F PROTEIN BY ANION-EXCHANGE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN THE PRESENCE OF TRITON X-100

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SUMMARY

Purified Sendai virions were treated with Triton X-100. The detergent extract containing the fusion protein (F) and the haemagglutinin-neuraminidase protein (HN) was subjected to anion-exchange high-performance liquid chromatography on a Mono Q (Pharmacia) column with 0.1 % Triton X-100 in phosphate-buffered saline. HN was not retained by the column while elution with a salt gradient resulted in several peaks containing mainly or only F protein.

INTRODUCTION

Sendai virus is a paramyxovirus ranging in size from 150 to 250 nm. Its nucleocapsid is surrounded by a lipid envelope which is associated with a protein responsible for hemagglutination and neuraminidase activities HN, the fusion protein F and the internalized matrix protein M. The F protein plays a crucial role in haemolysis, cell fusion and infectivity[1–3]. It consists of two disulphide-bonded polypeptide chains F_1 and F_2 with molecular weights of 50,000 and 13,500 daltons, respectively[4,5].

Treatment of purified Sendai virions with a mild detergent, *i.e.* Triton X-100, in a buffer of low salt concentration results in the selective extraction of the HN and F proteins. Several methods have been used to separate the HN and F proteins[6–10]. One of these methods, ion-exchange chromatography with eluents containing detergent, was never entirely satisfactory as a single purification step.

In this study the purification of fusion protein F in one chromatographic step by anion-exchange high-performance liquid chromatography (HPLC) is described.

EXPERIMENTAL

Virus

Sendai virus was grown in the allantoic sac of 10-day-old embryonated chicken eggs. Allantoic fluid was harvested after 48 h of incubation at 36°C. Debris was pelleted at 2000 *g* for 30 min. Virions were purified by sucrose-gradient centrifugation.

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Detergent extraction

Purified virions (46.5 mg/ml) in 5 m*M* Tris–HCl (pH 7.23) were disrupted by treatment with Triton X-100 (BDH, Poole, Great Britain) at a final concentration of 2% (v/v) for 15 min at room temperature. The detergent:viral protein ratio was 0.9 (w/w). After centrifugation at 100,000 *g* for 90 min the resulting supernatant was dialysed against 0.02 *M* sodium phosphate buffer, pH 7.2.

Anion-exchange HPLC

The retentate containing mainly F and NH protein was subjected to anion-exchange HPLC. A Mono Q HR 5/5 (50 × 5 mm I.D.) column (Pharmacia, Uppsala, Sweden) was used for chromatography with a system consisting of a Waters M 6000A pump, an LKB 11300 Ultrograd gradient mixer, a Rheodyne 7125 injector and a Pye Unicam LC-UV detector set at 260 nm. The gradient from 0.15 *M* to 1.5 *M* sodium chloride in 0.02 *M* sodium phosphate (pH 7.2) containing 0.1% Triton X-100 was made by low-pressure mixing using the LKB gradient mixer in 24 min at a flow-rate of 1 ml/min. Peak fractions were collected in low protein absorption (minisorp) tubes (Nunc, Roskilde, Denmark). To remove most of the Triton the tubes were covered with dialysis-membrane tubing and dialysed overnight at 4°C against water with Bio-Beads SM2 (Bio-Rad Labs., Richmond, CA, U.S.A.)[11].

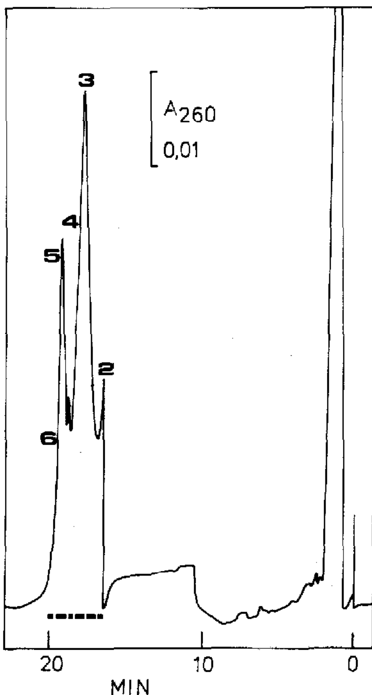

Fig. 1. Anion-exchange HPLC of a Triton X-100 extract of purified Sendai virions. A gradient from 0.15 *M* to 1.5 *M* sodium chloride in 0.02 *M* sodium phosphate (pH 7.2) containing 0.1 % Triton X-100 was used at a flow-rate of 1 ml/min. Absorbance was monitored at 260 nm. Fractions were collected manually as indicated by numbers and bars. Identical volumes were analysed by SDS–polyacrylamide gel electrophoresis (see Fig. 2).

Analysis

Dialysed fractions were analysed by sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis in 10% slab gels[12]. Polypeptide bands were visualized with a silver-staining method[13].

Protein was measured by the Lowry method[14].

Amino acid analysis was performed with a Kontron Liquimat III amino acid analyser. Proteins were hydrolyzed in 6 *M* hydrochloric acid for 20 h at 110°C in sealed and evacuated glass tubes.

RESULTS AND DISCUSSION

Triton X-100 (0.1%, v/v) was used in the eluent. Since the absorbance of the aromatic ring in Triton X-100 does not allow detection at 280 nm, which would have

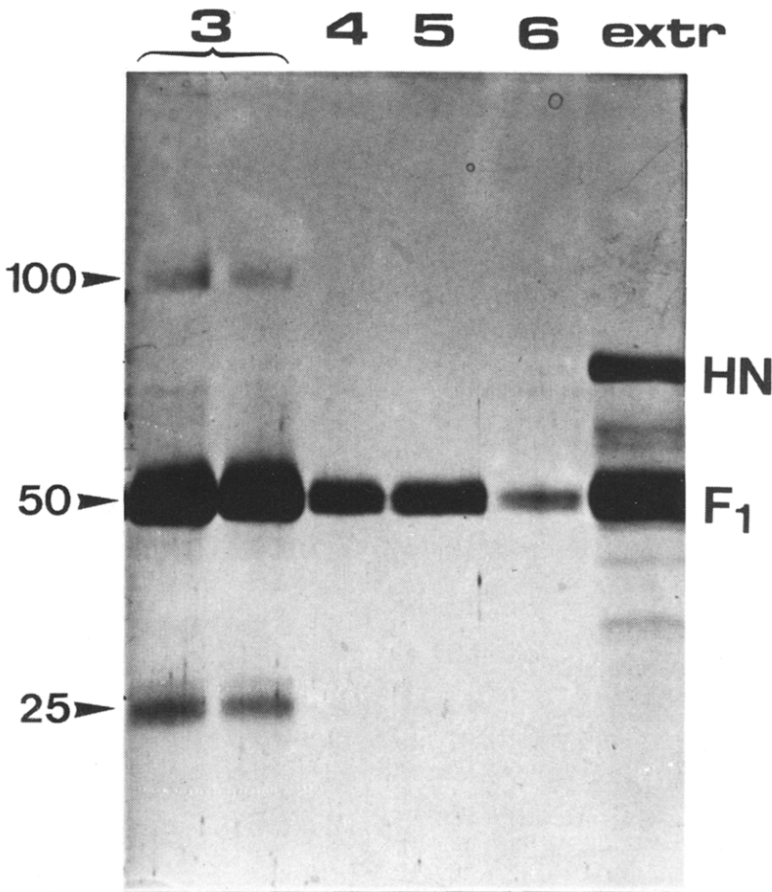

Fig. 2. SDS–polyacrylamide gel electrophoresis of fractions 3 (divided in two parts), 4, 5 and 6 from the anion-exchange HPLC of the Triton X-100 extract of Sendai virus shown in Fig. 1. Extr = the Triton extract, HN = haemagglutinin-neuraminidase; F_1 = the 50,000-daltons molecular weight part of the fusion protein F; the molecular weights of a few polypeptide bands are given in kilodaltons and are indicated by arrows.

been a more suitable wavelength for the detection of protein, the absorbance was monitored at 260 nm.

Triton X-100 treatment of Sendai virions at low salt concentration results in extraction of mainly HN and F protein. These extracts were subjected to anion-exchange HPLC which resulted in the elution pattern shown in Fig. 1. Identical volumes (50 μl) of each fraction (2–6) were analysed on 10% SDS–polyacrylamide gels after reduction of the samples. The reduction with mercaptoethanol results in cleavage of the disulphide bonds between F_1 and F_2. Thus the purity of the F protein eluting from the anion-exchange column was assessed by the presence of a single polypeptide band of the F_1 protein (M_r, 50,000). Peaks eluted by the salt gradient contained mainly F protein ; especially peak 3 contained a relatively large amount of F protein (ca 15 μg). Some minor bands with molecular weights of approximately 100,000 and 25,000 daltons were often present in this particular peak fraction. The reason for this is not clear, but this could be the result of dimerization and degradation, respectively. Peaks which eluted after this relatively broad peak also contained F protein and were pure as judged by SDS–polyacrylamide gel electrophoresis (see Fig. 2). The F protein heterogeneity might well be caused by differences in charge resulting from the presence of acidic oligosaccharide chains attached to the protein[7].

Since Brij 35 does not absorb at 280 nm, the same chromatography was performed with 0.1% (v/v) Brij 35 in the eluent. This resulted in a similar separation, although some tailing of the peaks was observed.

The identity of the F protein was further confirmed by a procedure described by van der Zee *et al.*[15]. F protein was reduced with dithiothreitol and after analysis by reversed-phase HPLC it turned out that F_1 and F_2 protein were present as the predominant components. Finally the amino acid composition of 60 μg F protein was determined. The analysis showed many similarities with the composition determined by Gething *et al*[8] although some distinct differences were observed, for instance about half the amount of glutamic acid was found.

The anion-exchange HPLC method presented in this study was shown to be a rapid method for the purification of viral proteins in the presence of non-ionic detergent and might be very suitable for the isolation of similar membrane proteins.

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