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EFFECT OF DETERGENTS ON THE STRUCTURE OF INTEGRAL MEMBRANE PROTEINS OF SENDAI VIRUS STUDIED WITH SIZE-EXCLUSION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND MONOCLONAL ANTIBODIES

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SUMMARY

The integral membrane proteins of Sendai virus, the fusion protein F (M_r = 65 000) and the haemagglutinin-neuraminidase protein HN (M_r = 68 000), were used as a model protein mixture. They were subjected to size-exclusion high-performance liquid chromatography on Superose 6HR columns with eluents containing various additives in order to solubilize the proteins. The effect of the additives on the structure of the membrane proteins was investigated with conformation-dependent monoclonal antibodies, either directed against F or HN protein, and by determination of the haemagglutinating capacity of the HN protein. The results show that the structure of the HN protein is more easily disturbed by eluents than that of the F protein. When the elution conditions are mild, *e.g.*, 0.1% octylglucoside, the structure of both proteins is conserved but no separation is obtained. Elution with a buffer containing 0.05% sarkosyl (dodecyl methylglycine sodium salt) did not affect the structure and resulted in pure F protein. Pretreatment of the Amberlite XAD-2-treated Sendai virus envelope extract with 4% sodium dodecyl sulphate (SDS) and elution with 0.1% SDS in 50 m*M* sodium phosphate (pH 6.5) altered the structure of the HN protein but resulted in purification of the tetramer and the dimer of the HN protein, and the monomer of the F protein.

INTRODUCTION

Integral membrane proteins show a great tendency to aggregate, and their solubilization is of primary importance in purification procedures involving chromatography. These proteins are embedded in a bilayer of phospholipid molecules. Detergents resemble the phospholipids in many aspects, and they can be used to

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extract membrane proteins from the lipid bilayer[1]. Alternatively, organic solvents can be used[2].

Size-exclusion high-performance liquid chromatography (SE-HPLC) is a non-interaction type of chromatography, *i.e.*, under the right conditions there is no interaction of protein molecules with the column material, and has been used for separations of proteins without denaturation[3–6]. However, for SE-HPLC of integral membrane proteins, additives must be present in the eluent in order to prevent aggregation. Several additives have been used: sodium dodecyl sulphate (SDS)[7–9], sarkosyl[10], triethylamine Triton X-100[11], Brij 35[12], 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate (CHAPS)[13], Tween-20[13], octylglucoside[14], guanidine hydrochloride[8,15] and deoxycholate[16]. These additives may affect the conformation of proteins to different extents[17]. We have conducted a comparative study with several of the eluents used by these authors using Sendai virus envelope proteins as a model. Sendai virus is a paramyxovirus of mice. Its envelope is composed of a lipid bilayer in which two integral membrane proteins are embedded, the fusion protein F (M_r = 65 000) and the haemagglutinin-neuraminidase protein HN (M_r = 68 000). The F protein consists of two components, F_1 (M_r = 50 000) and F_2 (M_r = 13 000–15 000), which are connected by disulphide bridges. This protein can be found in tetrameric, dimeric or monomeric form, depending on the medium in which it is solubilized. When relatively high concentrations of SDS (>4%) are present, the F protein is present in a monomeric form[18–20]. The HN protein is also present as a monomer, dimer and tetramer (HN, HN_2 and HN_4, respectively). HN_2 and HN_4 can be converted into the monomeric form by treatment with a reducing agent. Occasionally, truncated forms, *i.e.*, without the membrane-spanning region of the HN dimer are observed. The multimeric forms of the HN and F proteins appear as spikes on the outside of the virus particle.

To study the effect of various additives in the eluent on the conformation of each Sendai virus protein individually, immunological activity profiles were determined, using monoclonal antibodies directed exclusively against the native conformation of Sendai virus F and HN protein. In addition, the biological activity of the HN protein was determined in a haemagglutination assay.

EXPERIMENTAL

Detergent extraction of Sendai virus

Sendai virus was cultured in 10-day-old embryonated chicken eggs. The allantoic fluid was harvested 72 h after inoculation. Cell debris was removed by low-speed centrifugation (30 min at 2000 *g*). The virus particles were pelleted by ultracentrifugation (1 h at 70 000 *g* at 5°C). The pellet was resuspended in 10 m*M* Tris–HCl (pH 7.2), and the protein concentration was determined[21].

Extraction of Sendai virus glycoproteins without complete disruption of the virus particle is possible only with a limited number of detergents, *e.g.*, Triton X-100. Detergent extraction was performed by the addition of 1 ml 10 m*M* Tris–HCl (pH 7.2), containing 4% Triton X-100, to a virus preparation of 40 mg protein in 1 ml 10 m*M* Tris–HCl (pH 7.2). After a 20-min incubation at room temperature, the extraction procedure was terminated by centrifugation for 1 h at 70 000 *g*. The extracted virus proteins HN and F are present in the supernatant (the Sendai virus envelope extract), which was stored in aliquots of 200 μl at −80°C.

Sample preparation for SE-HPLC

To avoid interference of the detergent used for extraction with those present in the eluents, Triton X-100 was removed by the addition of 300 mg of Amberlite XAD-2 to 1 ml of Sendai virus envelope extract. After a 30-min incubation at room temperature, the Amberlite XAD-2 was removed by low-speed centrifugation. This results in more than 90% recovery of the Sendai virus glycoproteins. Prior to injection, one volume of the supernatant was incubated for 20 min with an equal volume of concentrated (2×) buffers used for elution of the Superose 6 HR 10/30 columns (see below for composition of buffers).

Size-exclusion HPLC

Chromatography was performed on two tandem-linked Superose 6HR 10/30 (300 mm × 10 mm I.D.) columns (Pharmacia, Uppsala, Sweden). The chromatography system consisted of a M 6000A pump (Waters, Etten-Leur, The Netherlands) or a 2150 pump (LKB, Zoetermeer, The Netherlands), a Rheodyne 7125 injector (Inacom, Veenendaal, The Netherlands) and a Waters 441 detector or a Pye Unicam LC-UV detector (Philips, Eindhoven, The Netherlands).

The following eluents were used: 6 *M* guanidine hydrochloride in 50 m*M* sodium phosphate (pH 6.5), 0.25% deoxycholate in 10 m*M* sodium phosphate (pH 8.1), 0.1% Brij 35 in 50 m*M* sodium phosphate (pH 6.5), 0.1% triethylamine (pH 3.0) with 0.1% decyl polyethylene glycol-300, 20% acetonitrile in 50 m*M* sodium phosphate (pH 6.5), 0.1% lauryldimethylamine oxide in 50 m*M* sodium phosphate (pH 6.5), 0.25% taurocholate in 10 m*M* sodium phosphate (pH 7.4), 0.03% Tween 20 in 50 m*M* sodium phosphate (pH 6.5), 0.1% decyl polyethylene glycol-300 in 50 m*M* sodium phosphate (pH 6.5), 0.1% SDS in 50 m*M* sodium phosphate (pH 6.5) (Figs. 1 and 2), 0.25% CHAPS in 100 m*M* sodium phosphate (pH 6.5), 0.1% octylglucoside in 50 m*M* sodium phosphate (pH 6.5) (Fig. 4) and 0.05% sarkosyl in 10 m*M* Tris–HCl (pH 7.5) supplemented with 0.6 *M* sodium chloride (Fig. 3).

Fractions were collected in 70 mm × 11 mm Minisorp tubes (Nunc, Roskilde, Denmark). Aliquots (50 μl) of each fraction were taken for analysis by SDS-polyacrylamide gel electrophoresis (PAGE). The remaining part of the fractions was then dialyzed against water by covering the Minisorp tubes with a square piece of dialysis membrane tubing, and the tubes were closed by fitting a slice of silicone tubing over the dialysis membrane. After dialysis, the fractions were freeze-dried in the tubes. The freeze-dried fractions were analyzed in an enzyme-linked immunosorbent assay (ELISA) for reaction with monoclonal antibodies, directed against conformational epitopes present on Sendai virus HN and F protein.

SDS-PAGE

The eluate fractions were analyzed on 8% SDS-polyacrylamide gels[22]. Polypeptide bands were visualized by silver staining[23]. Reference proteins were myosin (M_r = 210 000), phosphorylase b (M_r = 92 500), bovine serum albumin (BSA) (M_r = 68 000), immunoglobulin G (IgG) heavy chain (M_r = 50 000), ovalbumin (M_r = 43 000) and trypsin inhibitor (M_r = 20 000).

Monoclonal antibodies against Sendai virus

The production of monoclonal antibodies directed against Sendai virus pro-

teins HN and F has been described elsewhere[24]. To select monoclonal antibodies reactive with ordered and less-ordered HN and F proteins, the reactivity of 24 monoclonal antibody preparations with denatured (boiled in 4% SDS) and non-denatured Sendai virus envelope extract was investigated in an ELISA. Monoclonal antibodies HN 851 were selected as reactive with non-denatured HN protein. These were used to screen the fractions obtained after elution on the Superose 6HR with different buffer systems for the presence of intact HN protein. They are involved in the inhibition of different biological functions of the virus protein[24], like inhibition of haemagglutination, inhibition of haemolysis and virus neutralization.

In addition, monoclonal antibodies F 1.216 were selected which are directed against Sendai virus F protein and react predominantly with non-denatured F protein. They cannot block biological functions of the virus. Both monoclonal antibodies are of the IgG1 subclass.

Enzyme-linked immunosorbent assay

The ELISA was performed for three different purposes.

(1) Selection of monoclonal antibodies directed against denatured and non-denatured HN and F proteins. The reactivity of the monoclonal antibodies was determined with denatured (boiled in 4% SDS for 2 min) and non-denatured virus envelope extract. Both extracts were serially diluted in 50 m*M* sodium carbonate (pH 9.6) (coating buffer), added to wells of ELISA plates (Dynatech, Denkendorf, F.R.G.) and coated overnight. The plates were washed three times with phosphate-buffered saline (pH 7.2), containing 0.2 *M* sodium chloride, 0.3% Tween 20 and 1 mg/l SDS (washing buffer). The proteins were allowed to react with a 1:1000 dilution of monoclonal antibodies HN 851 and F 1.216 in washing buffer, supplemented with 0.5% BSA (dilution buffer). After incubation for 1 h at room temperature and washing, peroxidase-labelled anti-mouse IgG (ShaM/IgG/PO, Pasteur), diluted 1:1000 in dilution buffer, was added. The plates were incubated for 1 h at 37°C. After washing, the peroxidase activity was visualized by adding 100 μl per well of 50 m*M* sodium phosphate buffer (pH 5.6), containing 0.2 mg per ml *o*-phenylenediamine dihydrochloride (Eastman Kodak, Rochester, NY, U.S.A.) and 0.006% hydrogen peroxide. The reaction was terminated by adding 50 μl per well of 2 *M* sulphuric acid, and the optical density at 492 nm ($O.D._{492}$) was measured in a microplate photometer. Optical density values below 0.2 were considered as negative.

(2) Effect of incubation. The influence of the eluents used for SE-HPLC on the structural conformation of HN and F proteins was determined as the residual reactivity with monoclonal antibodies HN 851 and F 1.216 after a 2-h incubation of 30 μl of the concentrated (2 ×) eluents with 30 μl of Amberlite XAD-2-treated virus envelope extract. The incubation mixture was serially diluted, starting with a 100-fold dilution in coating buffer, and the ELISA was performed as described above. The dilution of the envelope extract that resulted in an $O.D._{492}$ of 0.8 was taken as a measure of the reactivity with the monoclonal antibodies.

(3) Specific immunological activity. The HPLC fractions were analyzed for the presence of intact HN and F proteins by determining the reaction with monoclonal antibodies HN 851 and F 1.216. The lyophilized HPLC fractions were dissolved in demineralized water and diluted in coating buffer (10 μg of protein per ml). The plates were coated with 1 μg of protein per well of each fraction, and the reaction

with monoclonal antibodies HN 851 and F 1.216 was determined as described above. The O.D.$_{492}$ per μg of protein was taken as a measure of the specific immunological activity.

Haemagglutination assay

One of the biological activities of native HN protein is the ability to agglutinate erythrocytes of different animal species. To investigate whether this biological activity was affected, the incubation mixtures of Amberlite XAD-2-treated Sendai virus envelope extract and the different eluents were tested for haemagglutination. After 2 h of incubation, the mixtures were serially diluted in phosphate-buffered saline in microtitre plates, and 50 μl per well of a 0.6% suspension of guinea pig erythrocytes were added. After incubation overnight at 4°C, the 50% haemagglutination titres were determined.

RESULTS AND DISCUSSION

Selection of monoclonal antibodies directed against conformational epitopes on HN and F proteins

The reaction of eighteen HN-specific monoclonal antibodies was determined with denatured (boiled for 2 min in the presence of 4% SDS) and non-denatured (not boiled and no SDS added) Sendai virus envelope extract. Eight of these monoclonal antibodies reacted with both extracts. Of the remaining ten monoclonal antibodies, two did not show detectable reactivity with the extracts, and eight reacted only with the non-denatured Sendai virus envelope extract. From these eight monoclonal antibodies, HN 851 was selected, since it had the highest reactivity with non-denatured Sendai virus envelope extract in the ELISA. Besides this, HN 851 can block several biological functions of this glycoprotein[24].

Six monoclonal antibodies against F protein were tested with the extracts. Of these, two monoclonal antibodies reacted to a much lower extent with the denatured than with the non-denatured Sendai virus envelope extract. Monoclonal F 1.216 was selected for our study, since small amounts of intact F protein (250 ng) were still detectable. Monoclonal antibodies F 1.216 do not interfere with the known biological functions, *e.g.*, fusion activity, of the F protein[24].

Eight out of eighteen monoclonal antibodies directed against HN protein and four out of six directed against F protein showed similar reactivities with either denatured or non-denatured Sendai virus proteins. This suggests that they may be directed against continuous stretches of the polypeptide chain.

Incubation of Amberlite XAD-treated Sendai virus extract in different eluents

The effect of incubation in eluents with different additives is shown in Table I as the dilution of the envelope extract that resulted in an O.D.$_{492}$ of 0.8. Eluents with 0.1% octylglucoside and 0.05% sarkosyl are mild with respect to denaturation of HN, and under these conditions the structure of this glycoprotein is largely unaffected. After incubation with 6 *M* guanidine-hydrochloride in 50 m*M* phosphate (pH 6.5), no reaction with the HN monoclonal antibodies was observed. This means that incubation in guanidine hydrochloride disrupts the structure required for the reaction with HN 851. All the other eluents listed in Table I had at least some effect

TABLE I

INCUBATION OF AMBERLITE XAD-2-TREATED SENDAI VIRUS ENVELOPE EXTRACT WITH DIFFERENT ELUTION BUFFERS FOR SE-HPLC AND THE EFFECT ON THE REACTIVITY WITH MONOCLONAL ANTIBODIES DIRECTED AGAINST NATIVE SENDAI VIRUS HN AND F PROTEIN

Incubation buffer	*Reactivity with**		*HA titre***
	HN 851	*F 1.216*	
6 *M* Guanidine hydrochloride in 50 m*M* sodium phosphate (pH 6.5)	<100	4480	<100
0.25% Deoxycholate in 10 m*M* sodium phosphate (pH 8.1)	380	8000	102.400
0.1% Brij 35 in 50 m*M* sodium phosphate (pH 6.5)	760	8000	102.400
0.1% Triethylamine (pH 3.0) + 0.1% decyl polyethylene glycol-300	920	6400	102.400
20% Acetonitrile in 50 m*M* sodium phosphate (pH 6.5)	1000	7680	102.400
0.1% Lauryldimethylamine oxide in 50 m*M* sodium phosphate (pH 6.5)	1040	4320	102.400
0.25% Taurocholate in 10 m*M* sodium phosphate (pH 7.4)	1360	7840	102.400
0.03% Tween 20 in 50 m*M* sodium phosphate (pH 6.5)	1400	9600	102.400
0.1% Decyl polyethylene glycol-300 in 50 m*M* sodium phosphate (pH 6.5)	1440	8320	102.400
0.1% SDS in 50 m*M* sodium phosphate (pH 6.5)***	1760	8320	102.400
0.25% CHAPS in 100 m*M* sodium phosphate (pH 6.5)	1760	7680	102.400
0.1% Octylglucoside in 50 m*M* sodium phosphate (pH 6.5)§	2080	8960	102.400
0.05% Sarkosyl, in 10 m*M* Tris-HCl (pH 7.5) + 0.6 *M* sodium chloride§§	2880	6080	102.400

* Dilution of Amberlite XAD-2-treated Sendai virus envelope extract resulting in an $O.D._{492}$ of 0.8.

** Dilution of Amberlite XAD-2-treated Sendai virus envelope extract giving 50% haemagglutination.

*** For SE-HPLC, see Figs. 1 and 2.

§ For SE-HPLC, see Fig. 4.

§§ For SE-HPLC, see Fig. 3.

on the reaction with monoclonal antibodies HN 851, which is probably due to conformational changes induced by additives in the different eluents.

Haemagglutination of erythrocytes is one of the biological properties of the HN protein. The haemagglutination titres after incubation in the various buffers were determined (Table I) and the results show that only incubation in 6 *M* guanidine hydrochloride inhibits the haemagglutination activity. Either the structure involved in the haemagglutination reaction is more stable than that of the epitope and insensitive to the other eluents tested, or partial changes in conformation still allow haemagglutination.

The reactivity with monoclonal antibodies F 1.216 indicates that although the structure of the F protein is affected by the various eluents, this occurs to a much lesser extent than with the HN protein. Incubation with sarkosyl, a mild detergent with respect to its effect on the conformation of HN protein, results in some structural alteration of the F protein. In this case, sarkosyl is not the mildest detergent, and the results show that the envelope extract can be diluted further when it is incubated in Tween 20 or octylglucoside.

The immunological activity after incubation of the Amberlite XAD-2-treated envelope extract in SDS was investigated further by comparing samples either incubated at room temperature or heated for 2 min at 100°C, in either low (0.1%) or high (4%) concentrations of SDS. The results are shown in Table II. With 0.1% SDS and without boiling the conformation remains largely intact, since an high immunological activity and also haemagglutination activity is observed. Boiling of the sample and incubation in 0.1% SDS does affect the structure of the HN protein, since no immunological activity and haemagglutination activity was found. However, the F protein appears to be more rigid than the HN protein, since after boiling in 0.1% SDS it is still reactive with monoclonal antibodies F 1.216 directed against the intact structure.

SE-HPLC with different eluents

All eluents mentioned in Table I were used for SE-HPLC of a Sendai virus

TABLE II

PRETREATMENT OF AMBERLITE XAD-2-TREATED SENDAI VIRUS ENVELOPE EXTRACT

Incubation buffer: 0.1% SDS in 50 m*M* phosphate buffer (pH 6.5).

Pretreatment		*Reactivity with**		*HA titre***
4% SDS	*Boiling*	*HN 851*	*F 1.216*	
–	–	1760	8320	102 400
–	+	< 100	7040	< 100
+***	–	< 100	< 100	< 100
+	+	< 100	< 100	< 100

* Dilution of Amberlite XAD-2-treated Sendai virus envelope extract resulting in an $O.D._{492}$ of 0.8.

** Dilution of Amberlite XAD-2-treated Sendai virus envelope extract giving 50% haemagglutination.

*** Pretreatment for SE-HPLC in Fig. 2.

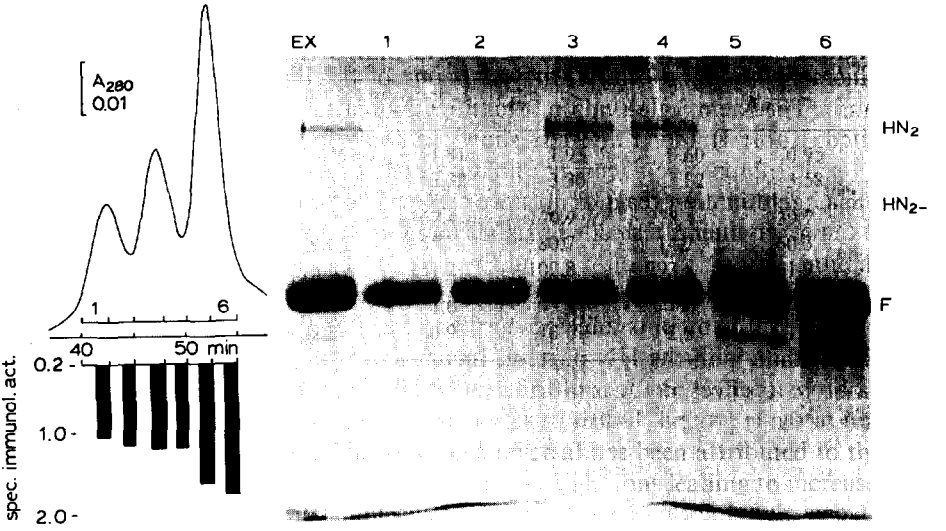

Fig. 1. Size-exclusion HPLC of Sendai virus membrane proteins on two tandem-linked Superose 6 (300 mm × 10 mm I.D.) columns. The column was eluted with 0.1% SDS in 50 m*M* sodium phosphate (pH 6.5) at a flow-rate of 0.5 ml/min. The absorbance was monitored at 280 nm. Fractions (1–6) were analyzed on 8% SDS gels. EX = Sendai virus envelope extract. The positions of the HN_2, HN_2- (the truncated form of HN_2) and F proteins are indicated (from ref. 17 with permission). The specific immunological activity is indicated as $O.D._{492}$ per μg of protein below each of the fractions (1–6). The right column represents the reactivity with the monoclonal antibodies F 1.216 directed against intact F protein (black area).

envelope extract[17] pretreated as described in the Experimental. From these, four conditions were selected and the eluate fractions were studied with monoclonal antibodies HN 851 and F 1.216: (1) 0.1% SDS in 50 m*M* sodium phosphate (pH 6.5), without pretreatment of the Amberlite-treated envelope extract (Fig. 1); (2) 0.1% SDS in 50 m*M* sodium phosphate (pH 6.5), with pretreatment (4% SDS added to the Amberlite-treated envelope extract) (Fig. 2); (3) 0.05% sarkosyl in 10 m*M* Tris–HCl (pH 7.5) plus 0.6 *M* sodium chloride (Fig. 3) and (4) 0.1% octylglucoside in 50 m*M* sodium phosphate (pH 6.5) (Fig. 4).

The elution patterns are shown in Figs. 1–4, together with the analysis of the eluate fractions by SDS-PAGE and the immunological profiles. Fig. 1 shows that all three peaks contain F protein, probably the tetramer, dimer and monomer, which are found on the SDS gel as a monomer because the samples were boiled in 4% SDS prior to electrophoresis. In addition, the second peak contains the dimer of the HN protein. The immunological analysis shows that in all fractions there is a considerable reactivity with monoclonal antibodies F 1.216, directed against native F protein. Despite the presence of HN (Fig. 1, fractions 3 and 4 from the SDS gel), no reactivity is observed with monoclonal antibodies HN 851, directed against intact HN. In Fig. 2 the results are shown of adding 4% SDS to the Amberlite-treated envelope extract prior to chromatography. Peak 1 (fractions 1 and 2) contains the tetramer of HN, 2 (fractions 3 and 4) the dimer of HN and 3 (fractions 5 and 6) the monomeric form of the F protein. A high immunological reactivity was found for the reaction between

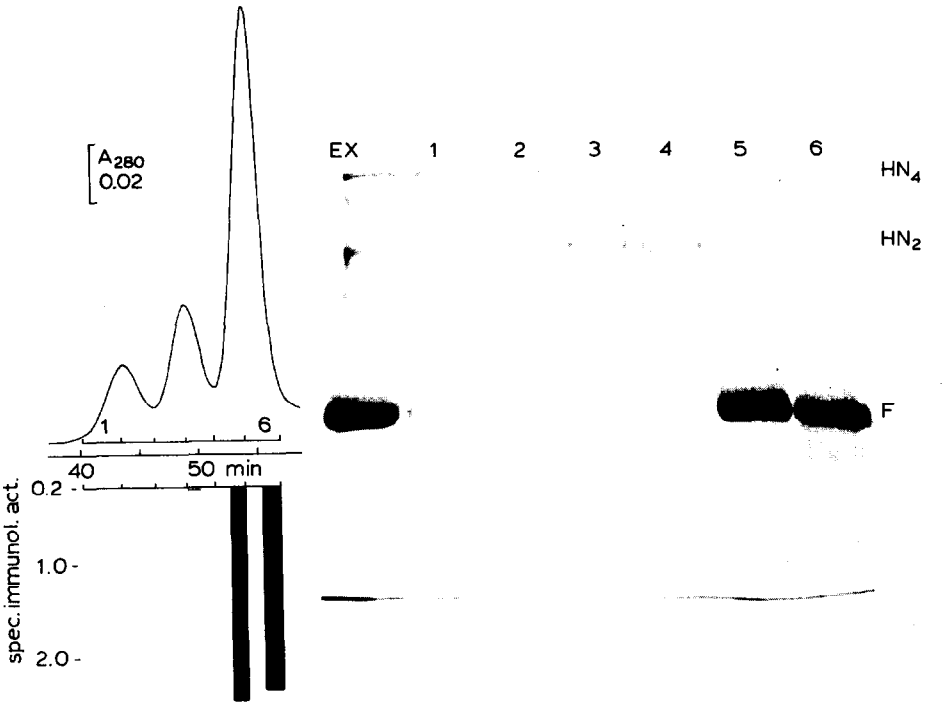

Fig. 2. Size-exclusion HPLC of Sendai virus membrane proteins on two tandem-linked Superose 6 (300 mm × 10 mm I.D.) columns. The extract was dissolved in 4% SDS, and the column was eluted with 0.1% SDS in 50 m*M* sodium phosphate (pH 6.5) at a flow-rate of 0.5 ml/min. The absorbance was monitored at 280 nm. Fractions (1–6) were analyzed on 8% SDS gels. The positions of the HN_4, HN_2 and F proteins are indicated. The specific immunological activity is indicated below each fraction (1–6). The left column represents the reactivity with the monoclonal antibodies HN 851 directed against intact HN protein (hatched area) and the right column that with the monoclonal antibodies F 1.216 directed against intact F protein (black area).

the F protein, containing fractions 5 and 6, and the monoclonal antibodies F 1.216, directed against intact F protein, but hardly any or no reaction of the HN-containing fractions with monoclonal antibodies directed against intact HN. The higher reactivity of the F protein compared to that in the incubation experiment (Table II, line 3) may be caused by the shorter incubation time in 4% SDS prior to SE-HPLC (20 *versus* 120 min). Fig. 3 shows the elution pattern, the analysis by SDS-PAGE and the immunological profile of an envelope extract subjected to SE-HPLC in 0.05% sarkosyl. According to SDS-PAGE, a relatively large amount of F protein is present in the first three peaks, and this F protein is still immunologically active. The tetramer and the dimer of the HN protein are present in fractions 2 and 3, respectively, and are still reactive with monoclonal antibodies HN 851, directed against intact HN protein. It has been reported[25] that sarkosyl does not denature membrane proteins of *Spiroplasma citri*. Considering the high immunological activity of the HN tetramer and dimer compared to chromatography in 0.1% SDS, our results are in agreement with this finding. Fig. 4 shows the results of using 0.1% octylglucoside in 50 m*M* sodium phosphate (pH 6.5) as the eluent. No separation was obtained (fractions 1–

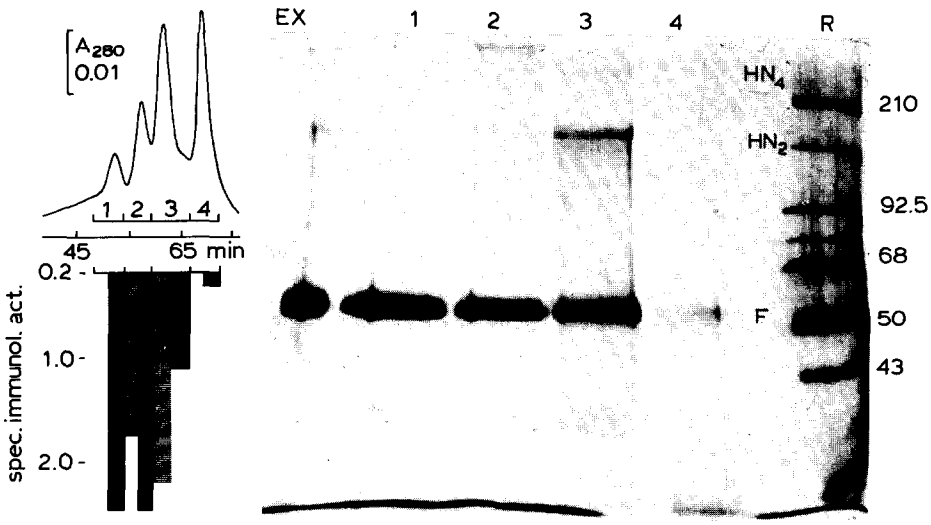

Fig. 3. Size-exclusion HPLC of Sendai virus membrane proteins on two tandem-linked Superose 6 (300 mm × 10 mm I.D.) columns. The column was eluted with 0.05% sarkosyl in 10 m*M* Tris–HCl (pH 7.5), containing 0.6 *M* sodium chloride, at a flow-rate of 0.5 ml/min. The absorbance was monitored at 280 nm. Fractions (1–4) were analyzed on 8% SDS gels. The positions of the HN_4, HN_2 and F proteins are indicated; R = reference proteins (molecular weight is in kilodaltons) (from ref. 17 with permission). The specific immunological activity is indicated below each fraction (1–4). The left column represents the reactivity with the monoclonal antibodies HN 851 directed against intact HN protein (hatched area) and the right column that with the monoclonal antibodies F 1.216 directed against intact F protein (black area).

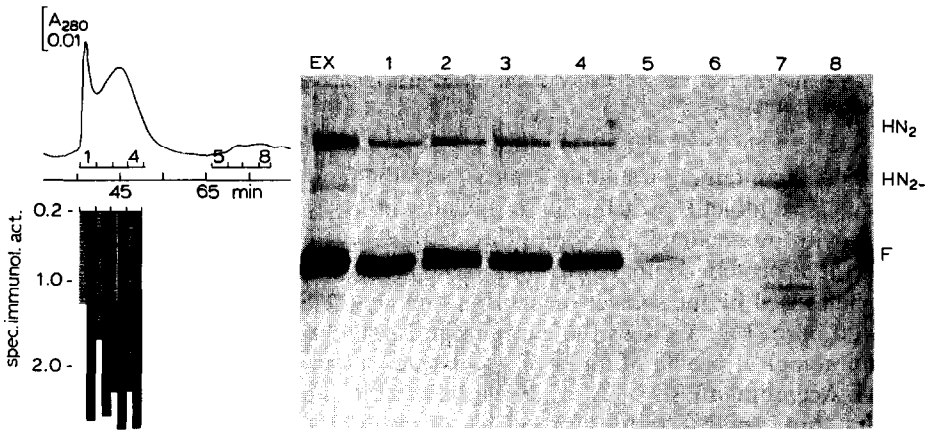

Fig. 4. Size-exclusion HPLC of Sendai virus membrane proteins on two tandem-linked Superose (300 mm × 10 mm I.D.) columns. The column was eluted with 0.1% octylglucoside in 50 m*M* sodium phosphate (pH 6.5) at a flow-rate of 0.5 ml/min. The absorbance was monitored at 280 nm. Fractions (1–8) were analyzed on 8% SDS gels. The positions of the HN_2, HN_{2-} and F proteins are indicated (from ref. 17 with permission). The specific immunological activity is indicated below each fraction (1–4). The left column represents the reactivity with the monoclonal antibodies HN 851 directed against intact HN protein (hatched area) and the right column that with the monoclonal antibodies F 1.216 directed against intact F protein (black area).

4), but a high reactivity of both monoclonal antibodies HN 851 and F 1.216 with fractions 1–4 was found.

An alternative explanation for the presence of F protein alone or both HN and F protein in many eluate fractions may be provided by the results obtained by Mascher and Lundahl[26]. They assumed that monomer– and dimer–detergent complexes of the glucose transporter from human red cells were formed depending on the amount of lipid present.

The structural alterations resulting from SE-HPLC with different eluents were measured as specific immunological activity. A high reactivity of the monoclonal antibodies with either HN or F protein does not imply that there may not be small local changes, distant from the site at which the antibody and protein interact. However, large structural alterations in any part of the molecule are expected to have long-range effects and will therefore lower the reactivity of monoclonal antibodies with the protein.

The specific immunological activity was determined using equal amounts of protein from the eluate fractions. The highest activity was found with octylglucoside, however no separation between HN and F proteins was obtained (Fig. 4). Almost equally high immunological activity was found with sarkosyl in the eluent and a better separation was obtained. Elution with a buffer containing SDS affects predominantly the structure of the HN protein and to a much lesser extent that of the F protein (Figs. 1 and 2).

CONCLUSIONS

The results show that, depending on the additives selected, there is a sensitive balance between separation or aggregation on the one hand and conservation of native conformation or partial denaturation on the other hand. A relatively mild detergent like octylglucoside at a concentration of 0.1% [below the critical micelle concentration (CMC)] does not affect the structural integrity of the proteins, but is not capable of preventing aggregation. Higher concentrations, above the CMC (0.73%), may result in separation, but may affect the biological activity[26]. Detergents that are denaturing, *e.g.*, SDS, may affect the conformation of a particular protein, *e.g.* the HN protein, while the structure of another protein, *e.g.*, the F protein remains unaffected. In between, there may be detergents like sarkosyl that are sufficiently mild so as not to affect the structure of the HN and F proteins and still sufficiently strong to prevent aggregation.

Another important factor that may determine the choice of a particular detergent as additive in SE-HPLC of integral membrane proteins is, of course, the resulting chromatogram. Therefore, the elution conditions should depend on which protein is to be purified and whether it must be immunologically or biologically active.

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