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STUDIES ON THE SUBUNIT COMPOSITION OF THE M-PROTEIN OF SENDAI VIRUS

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1. Introduction

The M-proteins, also known as matrix or membrane proteins, are a class of proteins which occur in influenza, parainfluenza and rhabdoviruses. They are believed to just underlie the membrane of the virus [1,2]. Studies of these proteins have been hampered by the difficulty of isolating them without the use of denaturing solvents; the only representatives isolated without the use of such agents being those form the very similar parainfluenza-viruses Newcastle disease virus and Sendai virus [3,4], in which the isolation is based on the solubility of these proteins in a high ionic strength solvent. There is some evidence that the M-protein of these viruses when isolated is native [4]. This paper describes studies of the subunit composition of the M-protein of Sendai virus. These studies test, and tend to confirm, the suggestion form electron microscopy [4], that this protein has a structure based upon a dimer of its 35 000 polypeptide. The same conclusion is drawn for the 30 000, proteolytically cleaved derivative, previously described [5].

2. Materials and methods

Sendai virus and M-protein preparation and gel electrophoresis were as previously described [4,5].

2.1. *Gel permeation chromatography*

A column of 1 cm diameter × 60 cm length of Sephadex G-150 (Pharmacia) was run under a head of 20 cm running buffer. The eluate was fractionated by drop counting to give 50–60 fractions application of the sample to elution of ϵ-dinitrophenyl-lysine which was used as an internal standard for the column volume. The void volume of the column was determined with blue dextran. Fractions were assayed for protein according to [6].

2.2. *Velocity sedimentation*

A Spinco model E analytical ultracentrifuge, AnD rotor and 12 mm path-length, single sector cell were used with Schlieren optics. The M-protein has a limited solubility under the solvent conditions used (approx. 1 mg protein/ml in 1 M NaCl). At this level of concentration the detection and measurement of a protein peak using Schlieren optics is difficult. For this reason the cleaved form of the protein [5], which has a solubility of up to 3 mg/ml, was used exclusively for this experiment, at this concentration.

2.3. *Cross-linking*

Dimethyl suberimidate, prepared and used according to [7] was a gift of Dr N. M. Green. A sample of M-protein, as a low ionic strength precipitate, was washed several times to remove Triton X-100 and suspended in 0.2 M triethanolamine/HCl buffer, pH 8.5. One-third volume of 4 M NaCl was then added to dissolve the M-protein, and the cross-linking reagent added. Samples, 20 μl, were removed at intervals and added to 100 μl samples of upper electrophoresis buffer, supplemented with 1% SDS and 1% β-mercaptoethanol. Glycerol and bromophenol blue were then added and the samples subjected to SDS–polyacrylamide gel electrophoresis. Predialysis of the samples, to remove salt, was not found to improve the results significantly. Electrophoresis was through a column of 7.5% acrylamide and 0.3% *N,N*-methylenebisacrylamide.

3. Results and discussion

In a high ionic strength solvent, both the M-protein and its cleaved derivative are eluted from Sephadex G-150 essentially as a single peak (fig.1 and 2). Often some material appears in the excluded volume, this amount is variable though never exceeds the main peak. The molecular weights of the M-protein and its cleaved derivative were estimated, by comparison with standards (fig.3) to be 75 000 and 65 000, respectively. The error in the measurements is about 10% so the difference between the two values is not significant. These data accord essentially with expectation for a dimer of the M-protein poly-

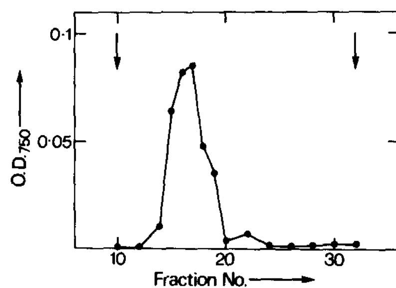

Fig.1. Elution profile of Sendai virus M-protein from Sephadex G-150. Solvent 1 M NaCl, 0.01 M potassium phosphate buffer, pH 7.2, no added detergent. Room temperature. Arrows indicate the positions of blue dextran (left) and ϵ-dinitrophenyl-lysine (right). Samples, 100 μl, were analyzed by Lowry protein assay and an absorbance of 0.1 is equivalent to about 50 μg/ml protein; the low solubility of the protein limits the amount which can be applied in a convenient volume, though the recovery is essentially quantitative.

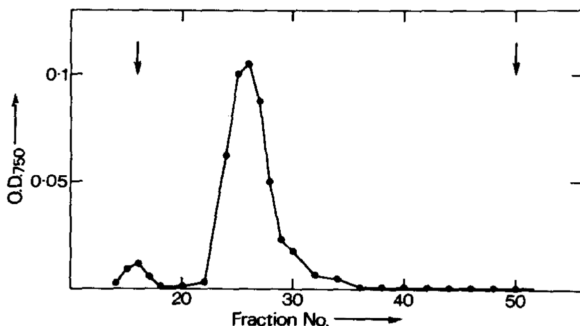

Fig.2. Elution profile of cleaved Sendai virus M-protein from Sephadex G-150. All conditions as fig.1.

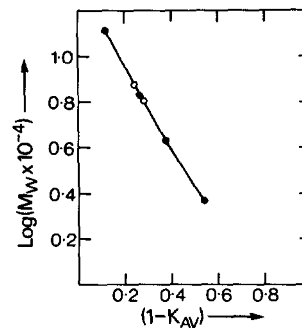

Fig.3. Estimation of the molecular weight of Sendai virus M-protein by gel permeation chromatography. Open circles M-protein (upper) and cleaved M-protein (lower). Filled circles are standards, being, in order of decreasing molecular weight, bovine serum albumin dimer, bovine serum albumin, ovalbumin, diisopropyl-phospho-trypsin.

peptide. The position of the main peak is not affected by the inclusion of Triton X-100 in the solvent up to a concentration of 0.5% v/v.

Sedimentation of the cleaved M-protein is shown in fig.4. The main peak is arrowed. The sharp drop in the Schlieren trace near the meniscus appears to be caused by flotation of some material, possibly detergent. There were a number of 'ripples' appeared during acceleration of the rotor, probably caused by

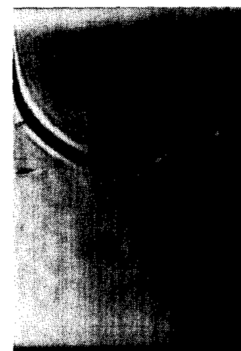

Fig.4. Schlieren pattern of velocity sedimentation of cleaved Sendai virus M-protein. Phase plate angle is 35°. Temperature 25°C. Solvent 1 M NaCl, 0.01 M potassium phosphate buffer, pH 7.2, 0.05% Triton X-100. This picture was taken 59 min after attainment of the full speed of 59 780 rev/min. Sedimentation is from right to left.

sedimentation of very high molecular weight material in the samples. The arrowed peak has an $s_{20,w}$ value of 4.2 ± 0.3 (this is after correction for the density and viscosity of the solvent). J. White (personal communication) has determined the sedimentation coefficient of both forms of the M-protein through high ionic strength sucrose gradients, and obtained preliminary measurements of Triton X-100 binding to them. Her results indicate an *s*-value of about 4.9 for the protein, while the level of Triton binding is such that it would not be expected to have a large influence on the sedimentation coefficient.

Let us take the usual 2/3 power relationship between sedimentation coefficient and molecular weight [8], and bovine serum albumin as standard (mol. wt 68 000, *s* 4.5). On this basis the monomer of the M-protein should have an *s* value of about 2.8, the dimer *s* 4.5 and the trimer *s* 5.9 etc. The slightly smaller cleaved form should have correspondingly lower values. On this basis the observed value favours the suggestion that the M-protein is sedimenting as a dimer. The two forms of the protein seem to be comparable, based on their forming the same structure in the electron microscope [5] and having similar behaviour on gel permeation and sucrose gradients (J. White, personal communication). Thus the M-protein appears to sediment as a dimer.

Scans of absorption versus mobility on SDS gels of the crosslinked protein are shown in fig.5. As crosslinking progresses some lower oligomers are formed with both the dimer and the tetramer predominating over the trimer. A striking feature of this experiment is the rate at which crosslinking proceeds to very high molecular weight forms; this suggests that an equilibrium involving higher oligomers may be occurring in the dissolved protein, though these forms are unlikely to predominate in view of the sedimentation and gel permeation data. That such an equilibrium may exist is perhaps unsurprising considering that the protein functions by forming a continuous shell beneath the virus membrane, and that in the absence of salt the protein precipitates. That the equilibrium may be based on dimers is suggested by the predominance of tetramer and dimer over trimer. The cleaved form of the protein gives similar results.

None of the experiments presented here indicate the presence of dissociation below the level of dimer in the presence of 1 M NaCl. Thus these experiments tend to the support the suggestion that the dimer is the basic subunit of the M-protein of Sendai virus.

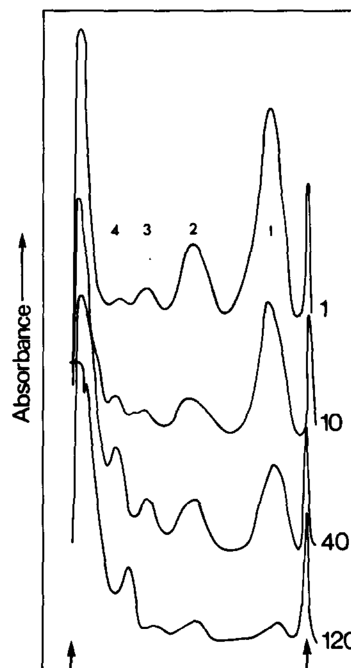

Fig.5. Crosslinking of Sendai virus M-protein with dimethyl suberimidate. Crosslinking was performed on a solution of 1 mg/ml protein by 0.7 mg/ml dimethyl suberimidate at 23°C in a solvent of 1 M NaCl, 0.15 M triethanolamine/HCl buffer, pH 8.5. Large figures refer to the time of crosslinking in minutes and small figures refer to the apparent polymerization state of the peaks. The samples were analyzed by SDS-gel electrophoresis, stained with Coomassie Brilliant Blue and scanned spectrophotometrically. Arrows refer to the top of the gel (left) and dye front (right).

Acknowledgements

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