Parainfluenza Virus Surface Projections: Glycoproteins with Haemagglutinin and Neuraminidase Activities

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The parainfluenza virus SV5 has been found to contain six proteins with mol. wts ranging from about 41,000 to 76,000 (Caliguiri, Klenk & Choppin, 1969; Mountcastle, Compans & Choppin, 1970). The nucleocapsid of the virus particle is composed of a single polypeptide chain with a mol. wt. of about 61,000 (Mountcastle et al. 1970); the other five proteins are presumably associated with the virus envelope. Two of the proteins are covalently linked to carbohydrate (Klenk, Caliguiri & Choppin, 1970). We report here that these glycoproteins comprise the spikes or projections on the surface of the SV5 virus particle.

The W3 strain of SV5 (Choppin, 1964) was grown in MDBK cells (Choppin, 1969) in reinforced Eagle's medium (Bablanian, Eggers & Tamm, 1965) with 2 % calf serum. Cells were inoculated at a multiplicity of about 10 p.f.u./cell. For labelling of virus proteins, 3 parts of reinforced Eagle's medium without amino acids and 1 part of standard reinforced Eagle's medium were used, and 1 μc/ml. of [14C]amino acids, 5 μc/ml. of [3H]amino acids or 5 μc/ml. of [3H]glucosamine were added as indicated. Glucosamine has been shown to be specifically incorporated into the carbohydrate of SV5 (Klenk et al. 1970) and Sindbis virus (Strauss, Burge & Darnell, 1970). After about 48 hr, the supernatant medium was harvested, centrifuged for 20 min. at 3000 g to remove cell debris, and mixed with an equal volume of saturated ammonium sulphate. After stirring at 4 ° for 30 min. the precipitate was pelleted at 3000 g for 30 min., resuspended in Eagle's medium, layered over a 15 to 40 % (w/w) potassium tartrate gradient, and centrifuged at 23,000 rev./min. for 2 hr in a Spinco SW 25-1 rotor. The virus formed a visible band which was collected and dialysed against 0.1 M-tris + HCl buffer, pH 7.0, before enzyme treatment.

A solution of a protease from Streptomyces griseus (Protease Type VI, Sigma Chemical Co, St Louis, Missouri) in 0.1 M-tris buffer, pH 7.0 was added to the purified virus suspension to give a final concentration of 500 haemagglutinating units (HAU) of virus and 2 mg. of enzyme/ml. The mixture was incubated at 37 ° for about 2 hr, and the digestion of spikes was monitored in the electron microscope. Control virus preparations were incubated at 37 ° without the enzyme. When the removal of spikes was judged to be essentially complete, the enzyme-treated virus was separated from enzyme and digestion products in a potassium tartrate gradient as described above, and control preparations were also recentrifuged in a second gradient. For measurement of infectivity and haemagglutinin, virus preparations were dialysed overnight against reinforced Eagle's medium; for determinations of protein and neuraminidase assay, preparations were dialysed against water. Neuraminidase activity was determined by the method of Aminoff (1961) as described previously (Compans et al. 1970).

Treatment with Protease Type VI resulted in an essentially complete removal of the spikes from the surface of the virus particle. Fig. 1 to 3 show untreated virus particles which have well-defined surface spikes about 100 Å in length, and, by contrast, the smooth-surfaced particles obtained after protease treatment. Papain, subtilopeptidase A, bromelain, and ficin were found to be less satisfactory, in that the spikes were relatively resistant to these enzymes. The spikeless particles are sufficiently stable to withstand banding in a potassium tartrate
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gradient without apparent morphological damage. The biological activities of spikeless virus particles were compared with those of control preparations (Table I). The infectivity of protease-treated virus was reduced about a million-fold, and haemagglutinin and neuraminidase activities were abolished. Thus, these activities appear to involve the virus spikes. The loss of infectivity is presumably secondary to failure of adsorption after loss of haemagglutinin. It was shown previously that treatment of Newcastle disease virus with

Fig. 1 to 3. SV 5 particles negatively stained with sodium phoshotungstate.

Fig. 1. Untreated SV 5 virus particles showing the layer of surface projections or spikes characteristic of paramyxoviruses. The particle on the right, which is penetrated by the stain, shows an electron-lucent membrane beneath the spike layer. The helical nucleocapsid is partially extruded from the particle.

Fig. 2, 3. SV 5 particles after treatment with protease showing smooth-surfaced spikeless particles. The particles which have been penetrated by the stain show an electron-lucent membrane surrounding the nucleocapsid. This membrane often shows outpocketing, giving the particles bizarre shapes.
pronase removed surface projections with loss of haemagglutinin activity (Calberg-Bacq, Reginster & Rigo, 1967).

The polypeptides present in control and protease-treated SV5 virus were compared by polyacrylamide gel electrophoresis. The procedures employed in the preparation of polyacrylamide gels, dissociation of the virus with sodium dodecyl sulphate and mercaptoethanol, electrophoresis of proteins, and processing of gels for determination of radioactivity have been described previously in detail (Caliguiri et al. 1969; Mountcastle et al. 1970). Fig. 4 compares the proteins of enzyme-treated particles with those of a control sample from the same SV5 preparation which was labelled with [14C]amino acids and [3H]glucosamine. The electrophoretic pattern of the control virus preparation (Fig. 4a) corresponds to the pattern described previously (Caliguiri et al. 1969; Klenk et al. 1970); the glucosamine label is associated with proteins 2 and 4, indicating that these are glycoproteins. These two glycoproteins are completely absent in the enzyme-treated particles, indicating that they are components of the virus spikes (Fig. 4b). The small peak following protein 6 probably represents the product of incomplete degradation of spike proteins in this experiment. Such a peak is not always present after enzyme treatment (Fig. 5).

Table 1. Comparison of biological activities of SV5 virus before and after removal of spikes

<table>
<thead>
<tr>
<th>Activity/mg. protein</th>
<th>Virus</th>
<th>p.f.u.</th>
<th>HAU</th>
<th>Neuraminidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4·4 × 10^{10}</td>
<td>5,632</td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td>Protease-treated</td>
<td>9.8 × 10^{a}</td>
<td>&lt;48</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

The neuraminidase and haemagglutinin of influenza virus appear to be two kinds of spikes present on the surface of the particle (Laver & Valentine, 1969; Webster & Darlington, 1969), and the present results indicate that SV5 haemagglutinin and neuraminidase are activities of the virus spike proteins. It has also been reported recently that these activities are associated with the spikes on haemagglutinating virus of Japan (HVJ) (Maeno et al. 1970). It is not yet certain which of the two SV5 glycoproteins is associated with which biological activity.

Fig. 4b shows that, although the spike glycoproteins were removed, three non-glycoproteins, corresponding in mobility to proteins 3, 5 and 6, remain in the enzyme-treated particles. Co-electrophoresis of a control virus preparation labelled with [3H]amino acids and enzyme-treated virus labelled with [14C]amino acids (Fig. 5) shows that the electrophoretic mobilities of the proteins remaining in the enzyme-treated virus correspond precisely with those of the control virus proteins. This indicates that the proteins 3, 5 and 6 which remain in the enzyme-treated particles have not been altered by the protease, probably because they are protected by the lipid in the virus membrane. Protein 3 is the subunit of the nucleocapsid (Mountcastle et al. 1970), and although the precise location in the virus envelope of proteins 5 and 6 has not yet been determined, it appears that protein 6 with a mol. wt of about 41,000 is the major protein in the smooth-surfaced membrane which surrounds the spikeless particles. Proteins with similar mol. wts (38,000 to 41,000) which contain no carbohydrate have also been found in Newcastle disease and Sendai virus particles (Mountcastle et al. 1971), indicating that a protein in this size range is a common feature of paramyxovirus envelopes.

The present demonstration that the two SV5 glycoproteins are spike proteins and previous
studies indicating that the spikes of influenza (Compans et al. 1970; Schulze, 1970) and Sindbis virus (Compans, 1971) are glycoproteins, suggest that spikes composed of glycoproteins are a characteristic feature of enveloped viruses in general. This conclusion is also supported by preliminary observations with Sendai virus (W. E. Mountcastle, R. W. Compans & R. W. Choppin, unpublished results) and vesicular stomatitis virus (J. McSharry, R. W. Compans & P. W. Choppin, unpublished results) which suggest that, in these two

Fig. 4. (a) Polyacrylamide gel electrophoresis of the polypeptides of untreated SV 5 virus particles labelled with both [3H]glucosamine (○—○) and [3H]amino acid mixture (●—●). The glycoproteins, 2 and 4, are labelled with glucosamine. Protein 1, which is a very minor component, is not seen in the gel. (b) SV 5 virus particles after protease treatment. The two glycoproteins have been removed, and proteins 3, 5 and 6 remain.
viruses, the surface spikes also consist of glycoproteins. The spike proteins of all of these viruses are incorporated into the plasma membrane of the infected cell before virus maturation, and the presence of carbohydrate bound to these proteins may be a requirement for this mode of assembly. It has also been found that spikeless virus particles clump together, suggesting that one function of the carbohydrate in the spikes is to provide a hydrophilic surface which permits the virus to be freely dispersed in an aqueous environment (Compans et al. 1970; Compans, 1971).

Fig. 5. Co-electrophoresis of the polypeptides of untreated SV 5 virus particles labelled with [3H]amino acid mixture and protease-treated particles labelled with [14C]amino acid mixture. The electrophoretic mobility and the relative amounts of proteins 3, 5 and 6, which remain after protease treatment, were unaltered by the enzyme.

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