COMPARATIVE MOLECULAR WEIGHT ESTIMATES OF MEASLES AND SUBACUTE SCLEROSING PANENCEPHALITIS VIRUS STRUCTURAL POLYPEPTIDES BY SIMULTANEOUS ELECTROPHORESIS IN ACRYLAMIDE GEL SLABS

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Summary. Structural polypeptides of a measles virus strain isolated from a case of subacute sclerosing panencephalitis (SSPE) were compared with those of the Edmonston strain by electrophoresis in acrylamide gel slabs containing sodium dodecyl sulfate. The smallest polypeptide, which appears to be the membrane protein, of the SSPE strain migrated more slowly than the corresponding measles polypeptide, indicating molecular weights of 40,000 and 38,000 daltons, respectively. The five other proteins successfully compared showed identical mobilities. A greater concentration of one of these (66,000 daltons) was seen in SSPE virions.

The successful isolation of strains of measles virus from cases of subacute sclerosing panencephalitis (SSPE) (1, 2) prompted inquiry into the possible differences and similarities between these viruses and those isolated from acute measles infections. Differences in growth patterns and host range have been reported (3), and recent studies of Yeh provided evidence by RNA-RNA hybridization for regions of non-homology between complementary RNA from a strain of measles virus and RNA from a strain of SSPE virus (4).

We approached this problem by examining the polypeptides of measles virus in acrylamide gels with the intent of comparing the patterns of conventional measles virus strains with those of strains isolated from cases of SSPE. This report deals with a comparison of the structural polypeptides of the Edmonston strain of measles virus with those of SSPE (1), a strain kindly provided by Dr. Horta-Barbosa (5). Both viruses were passaged at least 5 times in Vero cells in our laboratory before analysis. A differ-

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ence was noted with respect to one major and one minor polypeptide.

The molecular weights assigned to the various polypeptides are based on the means of multiple determinations in tube gels of 6 different preparations of the measles isolate. SSPE proteins were also examined in tube gels, but the comparative sizes of the various polypeptides are based on parallel electrophoresis of multiple samples of both strains in gel slabs.

The high pH discontinuous buffer system of Maizel (6) was used with 0.1% sodium dodecyl sulfate, a 7.5% resolving gel, and a 4% stacker gel of the same acrylamide composition adjusted to pH 6.8. We used as migration standards the H and L chains of human IgG globulin, bovine serum albumin, and lysozyme. Samples were solubilized in 1% sodium dodecyl sulfate and 1% 2-mercaptoethanol, heated at 100°C for 2 min and layered on the gels which were then subjected to electrophoresis at constant current. Gel slabs were prepared and run in a chamber purchased from Gibson Development Co., Chicago, Illinois. The gels were stained with Coomassie brilliant blue, and the distances migrated were measured directly on each gel in relation to the marker dye, bromphenol blue.

Preparations examined included whole virions purified from clarified culture fluids that were concentrated by ultrafiltration with Amicon membranes followed by rate-zonal sedimentation on dextran 10 gradients (5-20% w/w) in an SW 41 rotor for 23 min at 11,000 rpm. Fractions collected from the lower half of the tube were layered on a sucrose (10-40% w/w)-Ficoll (3-12% w/w)-D_2O gradient and centrifuged for 3 hr at 40,000 rpm. Examination in the electron microscope revealed large, mostly intact virions which banded at a density of about 1.22 g/ml. Nucleocapsids isolated by sedimenting virions through a zone of 10% Nonidet P40, as described previously (7), yielded relatively clean preparations banding at a density of 1.28 g/ml. A third group of subviral particles, banding at a density of 1.25-1.26 g/ml, sometimes resulted from incomplete detergent stripping, and in the electron microscope they resembled the projectionless particles obtained by protease treatment of parainfluenza viruses (8).

Table 1. Comparative molecular weight estimates of measles and SSPE virus structural polypeptides by simultaneous electrophoresis in acrylamide gel slabs.

Measles	SSPE	Measles Hall & Martin (10)	Probable Nature of Polypeptide
79,000-89,000	identical	76,000	———
69,000	identical	69,000[1]	large glycoprotein
66,000[2]	identical[2]		———
56,000	identical	60,000[3]	nucleocapsid protein
54,000	?[4]	53,000[1]	small glycoprotein
48,000	?[4]	51,000	———
38,000[5]	40,000[5]	46,000	membrane protein
45,000	identical		nucleocapsid subunit

[1]Glycosylated
[2]Minor component detected in 5/6 SSPE and 2/9 measles preparations
[3]Nucleocapsid protein
[4]Resolution unclear or bands too faint
[5]Measles: mean 38,300, n=9. SSPE: mean 39,700, n=6.

Three major bands were regularly seen with whole measles virion preparations. These have been underlined in Table 1 and they have estimated molecular weights of 69,000, 56,000, and 38,000 daltons. A single band of highly variable mobility was also seen in the region indicating a size range of 79,000-89,000 daltons. Migration of this band in slab gels was identical for all samples tested in a single electrophoresis. Other minor bands also were seen with molecular weights of 66,000 (see footnote, Table 1), 54,000, 48,000 and 45,000 daltons.

Nucleocapsid preparations contained the 56,000 and 45,000 dalton polypeptides, although contamination with lesser amounts of the other poly-

peptides was sometimes noted. Based on the studies of Mountcastle et al (9) we suspect that the 45,000 dalton protein is a subunit derived from the 56,000 dalton protein by proteolytic enzyme activity either before or during the purification procedures. This will require experimental confirmation.

The projectionless subviral particles contained significant amounts of the 38,000 dalton protein in addition to the two nucleocapsid-associated proteins and were conspicuous in lacking polypeptides with molecular weights above 56,000 daltons.

Our data are in good agreement with those of Hall and Martin (10), as shown in Table 1. On the basis of our combined data and by analogy with the polypeptide analyses of other paramyxoviruses (11), we tentatively conclude that the 69,000 dalton and larger proteins represent components of the envelope spikes, that the nucleocapsid protein is found both as an intact 56,000 dalton protein and as a 45,000 dalton subunit, and that the membrane protein is the 38,000 dalton component.

When the measles and SSPE isolates were compared, the most tangible difference noted was in the polypeptide that we think is the membrane protein. This protein migrated more slowly in the case of the SSPE strain, indicating a molecular weight of 40,000 daltons. Whether or not an alteration in this protein could affect virus maturation can only be conjectured at this time, but these results suggest a focus for further study.

We also found higher concentrations of the 66,000 dalton polypeptide in SSPE virions. Recently Scheid and Choppin described a protein of $\sim$ 65,000 daltons which appeared to be a precursor related to the hemolytic and fusing activities of NDV virions (12). It is tempting to speculate that we are picking up a difference in measles and SSPE virions that may be associated with these activities. However, even if it could be shown that the polypeptide in question is related to the measles hemo-

lysin, conclusions based on concentration differences with these very fragile viruses are always tenuous. Many more strains from each disease syndrome will have to be compared before we can begin to assess the significance of these preliminary observations.

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