

Determination of Measles Virus Protein Molecular Weights on High Percentage, Highly Cross-Linked SDS Polyacrylamide Gels

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A recent examination of measles virus mRNA molecules has shown that the nucleocapsid and haemagglutinin messengers are of the size expected from a consideration of their protein products. However, the mRNA for membrane protein is approximately 50% larger than the size required. The molecular weight of matrix protein has been determined by SDS-polyacrylamide gel electrophoresis, and this procedure can lead to an underestimation of the true size of hydrophobic molecules which show increased SDS binding. It is therefore appropriate to examine the molecular weight determination of this protein to exclude such an artefactual discrepancy in mRNA and protein sizes. We report here that measles virus membrane protein does not show such anomalous behaviour and confirm that the size discrepancy is a true phenomenon.

Six virus-specific structural polypeptides are recognised in mature measles virus particles. These proteins are designated; the large protein (L); the haemagglutinin (H); the phosphoprotein (P); the nucleocapsid protein (N); the fusion protein which is cleaved into F₁ and F₂, and the membrane protein M. There is widespread agreement on the size range of these proteins [1]. Recently the application of recombinant DNA technology has permitted the cloning of parts of several measles virus-specified mRNA molecules [2, 3]. Northern blotting has then provided an estimation of the sizes of the intact virus mRNAs. The nucleocapsid and haemagglutinin proteins are specified by mRNAs of similar size, 1735 and 1680 bases respectively, whilst the membrane protein mRNA is 1515 nucleotides in length [2, 3]. Rozenblatt and co-workers have calculated the coding capacities of these molecules and it was apparent that N and H protein mRNAs were approximately the size expected from the molecular weights of the unmodified proteins produced in *in vitro* translation experiments. However,

this was not so when the membrane protein mRNA was considered. Even allowing for a poly-A tail of 100 residues, these workers derived a predicted molecular weight of 59 K for the product of this mRNA. The M protein is produced as a 36–37 K molecular weight protein in *in vitro* translation experiments [4–7], and is labelled in infected cells by short-pulse labelling procedures. This information excludes any post-translational precursor processing mechanism in the production of M protein.

Bayreuther *et al.* [8] have presented data concerning the electrophoretic migration of another membrane-associated protein, the lactose permease of *E. coli*. This hydrophobic protein shows an anomalously high binding capacity for SDS resulting in an increased charge-to-mass ratio of the complex. Consequently electrophoretic migration is assisted and molecular weight determinations underestimate the size of the protein. A similar effect, but opposite in direction, has also been reported for some glycoproteins which show decreased SDS binding [9]. Hall and co-workers [10] have isolated measles virus M protein and found it to be rich in hydrophobic aminoacids. These workers also obtained some evidence for complex formation with the non-ionic detergent "triton", which lead to a mobility shift towards a higher molecular weight. It is therefore possible that anomalous SDS binding could also occur. Consequently it is appropriate to reexamine the molecular weight determination of this peptide.

The electrophoretic migration of an SDS-protein complex is affected both by the amount of SDS bound (*i.e.* charge) and by the molecular sieving effect of the gel mesh. The latter is governed by the cross-linking within the gel, and Bayreuther *et al.* [8] have shown that the sieving effect can predominate in high percentage polyacrylamide gels with a low acrylamide/bisacrylamide ratio. In the case of lactose permease, an apparent molecular weight of 30 K determined by electrophoresis on a 10% polyacrylamide gel (acrylamide:bisacrylamide=37.5:1) was increased by over 50% to 46.5 K if electrophoresis was performed on a 20% gel of the same acrylamide:bisacrylamide ratio. Since the true molecular weight of this protein was known from DNA sequence to be 46.5 K [11], this procedure for molecular weight determination effectively overcomes errors due to increased SDS binding. The measles virus M protein molecular weight has been determined from SDS gels as 37–40 K. Exact in-

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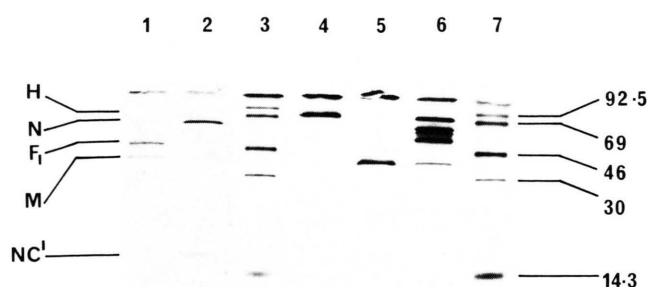


Fig. 1. Measles virus (Edmonston)-infected vero cells were labelled with [35 S]methionine (Amersham Buchler, 200 μ Ci/ml) for 2 h from 18–20 h p.i. Cell lysates were then prepared for immunoprecipitation as described by Stephenson and ter Meulen [20]. Protein-A conjugated to sepharose CL4B (pharmacia) was pre-armed with rabbit-antimouse immunoglobulins (Dako) before use with monoclonal antibodies. Lysates were then immunoprecipitated with rabbit hyperimmune anti-measles serum (track 6) or with monoclonal antibodies specific for F (track 1); N (track 2); H (track 4) or M (track 5). [14]Methylated protein molecular weight markers were obtained from Amersham Buchler and electrophoresed in tracks 3 and 7. These comprised: Myosin, 200 K; Phosphorylase b, 92.5 K; BSA, 69 K; Ovalbumin, 46 K; Carbonic Anhydrase, 30 K and Lysozyme 14.3 K. All samples were denatured and reduced by boiling with dithiothreitol and SDS before loading. Samples were analysed on discontinuous gels [21]. Stacking gels contained: 3.5% Acrylamide, 0.15% Bis, 0.14 M Tris pH 6.5, 0.1% SDS. Separating gels contained 20% Acrylamide, 0.53% Bis, 0.375 M Tris pH 8.7, 0.1% SDS, electrophoresis was performed at 120 V.

formation on gel composition is difficult to obtain, but most workers have used highly cross-linked low percentage acrylamide gels (7.5%–10%; acrylamide:bisacrylamide 30–39:1), [12–14] or higher percentage gels with lower proportions of cross-linker (15–25%; acrylamide:bisacrylamide = 55–100:1) [7, 10, 15, 16]. Other reports do not give this information [17, 18]. There is one report [19] of a molecular weight determination using a highly crosslinked, intermediate-strength gel system (15%; acrylamide:bisacrylamide = 37.5:1). However, this could still result in a 30% underestimation of M protein molecular weight [8].

In order to exclude this possibility we have determined the molecular weights of measles virus H, N, F and M proteins using a highly cross-linked (acrylamide:bisacrylamide = 37.5:1), 20% polyacrylamide gel. A typical experiment is shown in Figure 1. The molecular weight markers showed a linear relationship between migration and the logarithm of molecular weight over the range

30–90 K. The 200 K molecular weight marker and virus L protein did not enter this gel. Measles virus polypeptides were identified by immunoprecipitation using monoclonal antibodies specific for these molecules.

The nucleocapsid protein is very susceptible to proteolytic degradation, the major product of this process is termed NC and has a molecular weight of 47 K [20]. The monoclonal antibody used in this work precipitates only the 60 K parental N protein, and although NC is present and runs as the major band immediately above F₁ (compare tracks 1 and 6, Figure 1), it is not detected by this antibody. This suggests that the site against which this monoclonal antibody is directed is removed during the degradation. A small polypeptide, NC' (15.8 K) is precipitated and presumably corresponds to the product of this cleavage. The small cleavage product of the fusion protein (F₂) could not be detected either by monoclonal antibody or hyperimmune serum, presumably because F was present in too small amounts. Long exposures were necessary to demonstrate the small amounts of F and NC'. Consequently non-specifically precipitated background proteins were more noticeable in these two tracks but their amounts were always equivalent to those observed in negative controls. The identification of H and M was straight forward.

Molecular weights determined from analysis on this type of gel and from 10% SDS polyacrylamide gels were not found to be significantly different, Table I shows the average of six determinations. It can thus be concluded that the measles virus membrane protein does not show anomalous SDS bind-

Table I. Molecular weights determined in our laboratory for the major measles-virus (Edmonston) specific polypeptides. The L protein did not enter the high percentage gel, and the NC' molecule was electrophoresed out of the 10% gel. The P protein is present in too low an amount for conclusive identification in the absence of specific monoclonal antibodies. NC was not well resolved from F₁ in the higher percentage gel.

Protein	10% PAGE	20% PAGE
L	190	—
H	78	76
N	58	61
NC	47	—
F ₁	45	44
M	39	39
NC'	—	15.8

ing and consequently the disparity in mRNA and protein size must represent a real phenomenon. In general, with the notable exception of the coronaviruses [22], most RNA virus mRNAs are related to the size of their product. Exceptions being spliced mRNAs which might contain information functionless in the processed product, or proteins formed by the processing of precursors. Measles is a cytoplasmic virus and splicing mechanisms are therefore unlikely. Post-translational processing mechanisms can also be excluded although a co-translational cleavage could still occur. Selection of membrane protein mRNA and its translation *in vitro* leads to the production of only one protein [3]. However, this work has utilised immune precipitation. A small peptide, produced as above, might not be

incorporated into virus particles, and not therefore recognised by antiserum. Alternatively the excess information in the mRNA could be non-coding and perhaps serve some regulatory or structural function. However, the reason for the disparity in protein and mRNA size, confirmed in this report, is at present unknown and must await sequence determination.

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