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PRELIMINARY STUDIES OF THE MEMBRANE-ASSOCIATED PORTION OF THE HEMAGGLUTININ

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Like other 'intrinsic' membrane proteins the hemagglutinin is amphipathic. The results of comparative analyses of intact hemagglutinin molecules and preparations which lack the membrane associated hydrophobic region indicate that the portion of the molecule associated with the lipid bilayer is near the COOH-terminus of the smaller polypeptide component, HA_2 (Skehel and Waterfield, 1975).

In this communication two approaches to the detection and isolation of the membrane associated fragment are reported which are based on the hydrophobic characteristics expected of this portion of the molecule.

The isolation procedure is an application of the observations of Helenius and Simons (1977) that the electrophoretic mobilities of membrane proteins which bind detergent micelles are influenced by the charge of the micelle and may be manipulated by varying the type of detergent. In these experiments the cationic detergent cetyltrimethylammonium bromide and the nonionic detergent polyoxethylene 10-lauryl ether, Brij 36T were used. There were two sources of hemagglutinin protein – purified virus particles and plasma membranes from virus-infected chick embryo fibroblasts. Similar results were obtained using either source and the latter, in which the hemagglutinin polypeptide component was uncleaved biosynthetic precursor HA_0, was used in most experiments since the protein could be conveniently labelled in tissue culture using ^{35}S-methionine. The purification procedure used in the isolation of the radioactive hemagglutinin molecules from infected cell plasma membranes will be described in detail elsewhere but briefly it involved dissolution of the membranes in Brij 36T followed by sucrose density gradient centrifugation in solutions containing detergent. When such preparations in 0.5% Brij 36T were made with

respect to CTAB and electrophorezed at pH 8.6 in 1% agarose gels containing the detergents in the same proportions, they migrated slightly towards the cathode (Figure 1).

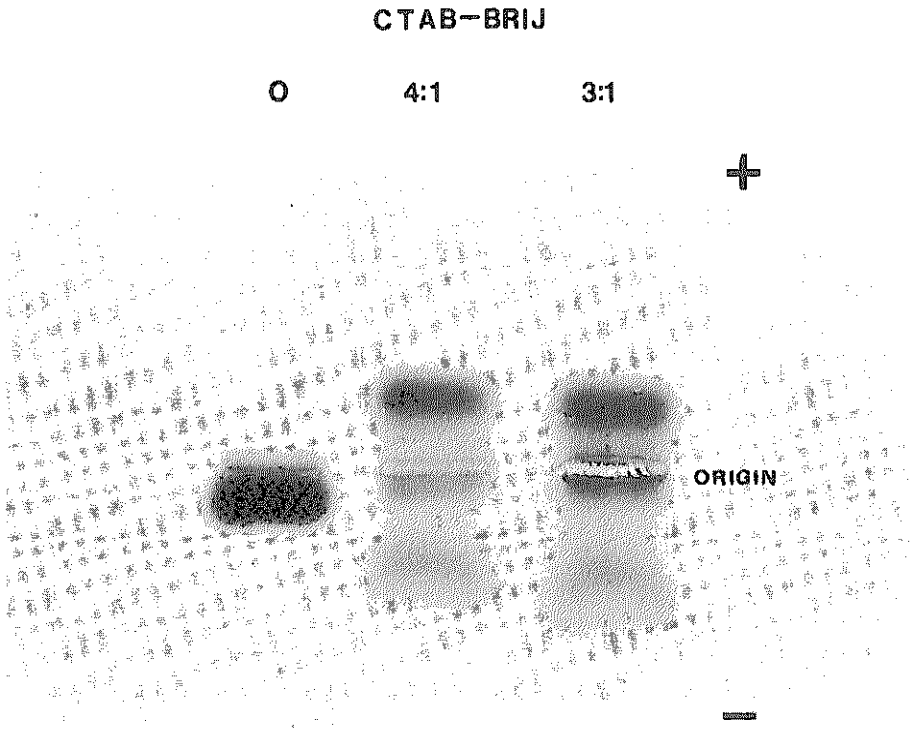

Figure 1

'Charge-shift' electrophoresis in agarose gels of the hemagglutinin before and after digestion with bromelain. Lane 0 undigested HA_0 migrates as a cation. Lanes 4:1 and 3:1 (referring to HA:bromelain ratio).
Bromelain digests of HA_0 at 37° for 3 hr. Electrophoresis was at 60 volts for 40 min. The anionic material has been identified as BHA on agarose and by SDS-polyacrylamide gel electrophoresis of bands cut from agarose gels. The light band near the origin is undigested HA.
The cationic band which presumably binds the positively charged detergent micelles contains peptides of molecular weight 5000-7000 as judged by the results of analyses on SDS-polyacrylamide gels.

Bromelain digests of HA_0 are resolved into three components:

1. Near the origin, undigested HA_0 migrates as a cation.
2. The dominant component is an anion identified, by SDS polyacrylamide gel electrophoresis, as BHA – soluble bromelain released HA.
3. A cationic and probably, therefore, a detergent micelle-binding component. SDS-polyacrylamide gel electrophoretic analyses of this component indicate that its molecular weight is between 5000 and 7000. A similar low molecular weight component is found in CTAB-Brij/Agarose electrophoresis of bromelain-digested virus particles which have been pelleted and washed following digestion to remove soluble digestion products. These peptides which are probably derived from the hydrophobic region of the hemagglutinin molecule are the objects of further analyses.

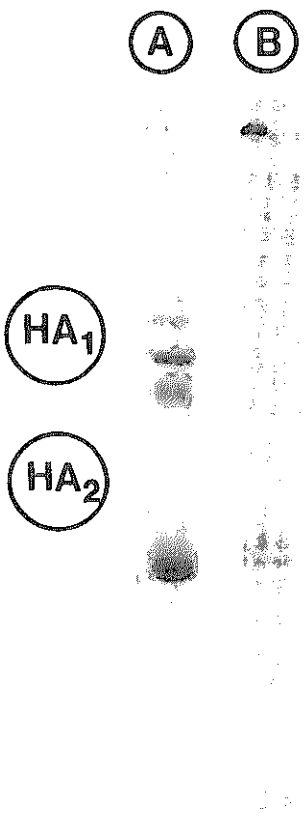

Figure 2

SDS-Polyacrylamide gel analysis of ^{125}I-nitrene labelled influenza virus. A. Virus polypeptides stained with Coomassie blue. B. Autoradiogram of A.

As stated above, for convenience the glycoprotein used in these studies was HA_0 labelled *in vivo* using ^{35}S-methionine. An alternative procedure which may be generally useful for labelling the lipid-associated regions of membrane proteins has also been investigated. Aryl azides have been utilized as photo-activateable reagents in several biological systems. In the present studies a diiodohexanoyl-derivative of 2-nitro, 4-azido phenyl tyramine prepared by M. Hebden and J. Knott, which partitioned preferentially before activation into the lipid bilayer of virus particles was used to label the lipid and lipid-associated virus protein molecules. The results of the procedure can be assessed following separation of the SDS-dissociated virus polypeptides by polyacrylamide gel electrophoresis (Figure 2). The smaller polypeptide of the hemagglutinin, HA_2, was clearly the most highly labelled virus component and since, as mentioned above, the COOH-terminal portion of HA_2 has been implicated as the lipid associated part of the molecule it appears likely that this region is specifically labelled.

An important practical implication of this result is of course that the hemagglutinin of large quantities of egg-grown virus can be labelled *in vitro* and preparation of the hydrophobic 'tails' from the labelled molecules should facilitate their detailed analysis.

REFERENCES

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