GLYCOProTEIN E1 OF CORONAVIRUS A59:
A NEW TYPE OF VIRAL GLYCOProTEIN

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INTRODUCTION

Glycosylation of viral glycoproteins as a co- and posttranslational event has been studied in a large number of viral systems (for review see Klenk and Rott, 1980). From these data a general picture can be drawn:

In a first step the nascent polypeptide chain extending into the lumen of the rough endoplasmic reticulum (RER) is glycosylated by an en bloc transfer of the oligosaccharide from a dolichol-linked intermediate Dol-P-P-(GlcNAc)₂ Man₉ Glc₃. This glycosylation step requires a tripeptide-sequence (H₂N·Asn·X·Ser(Thr)·COOH) and the resulting carbohydrate-protein linkage is of the N-glycosidic type between N-acetylglucosamine and asparagine. During transport of the glycoprotein to smooth membranes the carbohydrate side chains become trimmed by specific glycosidases. After sequential removal of the glucoses, a varying number of mannose residues may be cleaved off to yield the generally heterogeneous "mannose rich" side chains, also found in glycoproteins of mature virions.

If trimming of the side chains proceeds to a Man₉(GlcNAc)₂-Asn-species, re-addition of N-acetylglucosamine, galactose, fucose, and neuraminic acid will occur to form various "complex type" side chains. This presumably takes place in the Golgi. From here the viral glycoproteins are transported to the plasma membrane where they are sequestered into budding virus particles.

In this paper we describe a new type of viral glycoprotein having a carbohydrate composition and characteristics hitherto unknown for viral glycoproteins.
MATERIALS AND METHODS

Virus and Cells

The A59 strain of murine coronavirus was grown in the 17 clone 1 line of spontaneously transformed Balb C 3T3 cells. Both the virus and the cell line were kindly provided by Dr. L. S. Sturman. Virus was radiolabeled by the addition of radioisotopes to the growth medium, reinforced Eagle's medium containing 10% fetal calf serum, after the 1 hr adsorption period. Virus was harvested 30 hrs after infection, collected by centrifugation (2 hrs, 53,700 xg) and purified by isopycnic centrifugation on a 30 to 50% (w/w) sucrose gradient.

SDS-polyacrylamide gel electrophoresis

Initially cylindrical gels containing 10% polyacrylamide were employed essentially as described by Laemmli (1970).

For preparative isolation of radiolabeled glycoprotein E1 samples were prepared according to Sturman et al. (1980). Glycoprotein was recovered from crushed gels after freezing and thawing by elution with 0.1% SDS, filtration through a 0.45 μm Millipore filter units and precipitation in 90% aqueous acetone at 0°C. Preparations were then exhaustively dialysed against distilled water and lyophilized.

Isolation of E1 glycopeptides

Radiolabeled glycoprotein was digested with pre-digested pronase at 50°C in 1.0 M-Tris-chloride containing 10^-4 M CaCl2 at pH 8.0 for 48 hrs with a second addition of protease after 24 hrs. Insoluble degradation products were removed by centrifugation and the supernatant was extracted with chloroform/methanol (2/1). The upper phase containing essentially all the radioactive label was desalted by gel filtration on a Biogel P2 column (1x35 cm).

E1 glycopeptides were further fractionated by affinity chromatography on WGA-Sepharose 6B as described by Krusius and Finne (1978).

Release of carbohydrate by alkali-borohydride-treatment

Glucosamine labeled E1 was subjected to β-elimination conditions according to Carlson (1968). In short, lyophilized E1 was incubated in a solution containing 0.05 M NaOH and 1.0 M NaBH4 at 45°C for 10 hrs. After cooling to room temperature excessive borohydrate was destroyed by the addition of glacial acetic acid to pH 5.0 and boric acid was chased by repeated evaporation with methanol. Aliquots were spotted onto Whatman 3 MM paper sheets and subjected to high voltage paper electrophoresis in pyridine-acetic acid-water (4/10/86) at pH