MEMBRANE GLYCOPROTEIN SYNTHESIS: CLEAVAGE OF THE SIGNAL SEQUENCE IN THE ABSENCE OF GLYCOSYLATION

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1. Introduction

The transmembrane glycoprotein G of vesicular stomatitis virus (VSV) has provided a model for understanding the sequence of events involved in the biogenesis of integral membrane glycoproteins [1]. Studies in vitro on the biogenesis of G protein showed that glycosylation and insertion into microsomal membrane vesicles were a co-translational event [2–5] which also involved the proteolytic cleavage of an NH_2-terminal signal peptide containing 16 amino acids of which ~70% are hydrophobic [6,7]. In the VSV-infected cell the non-glycosylated G protein (G_{TM}) synthesized in the presence of the glycosylation inhibitor tunicamycin (TM) [8] was unable to migrate to the plasma membrane [9]. The non-glycosylated G protein had altered solubility and aggregated at an elevated temperature presumably due to an altered conformation [9–12]. In vitro studies have shown that the non-glycosylated protein could be inserted into microsomal membrane vesicles [13,14] but it was not established if the hydrophobic signal peptide was cleaved in the absence of glycosylation. Thus, the observed increase in aggregation of G_{TM} could be due both to the presence of the hydrophobic signal peptide and to an altered conformation.

Here, we show that the non-glycosylated G_{TM} possesses the same NH_2-terminal sequence as the glycosylated G indicating that the signal peptide was cleaved in the absence of glycosylation.

Abbreviations: VSV, vesicular stomatitis virus; G, fully glycosylated viral glycoproteins (M_r 69 000); G_{TM}, non-glycosylated G (M_r 63 000) synthesized in the presence of tunicamycin; TM, tunicamycin; G_2, partially glycosylated G (M_r 67 000) synthesized in vitro in the presence of microsomal membrane; G_1, non-glycosylated primary translation (M_r 63 000) of G mRNA synthesized in vitro in the absence of membrane; L, N, NS and M, non-glycosylated VS viral proteins; M_r, relative molecular mass

2. Experimental

Plaque-purified VSV (Indiana HR-LT) and BHK-21 and HeLa cells were grown as in [2,3]. BHK-21 cells in monolayer were treated with TM (Lilly Co. Ltd, Indianapolis IN) at 2 μg/ml for 1 h and were infected with VSV at m.o.i. 30–50. After incubation in the presence of TM for 4.5 h, infected cells were labelled either with [^{35}S]methionine (50 μCi/ml) or a single [^{3}H]amino acid (100 μg/ml) for 20 min. At the end of the labelling the cells were washed and suspended in a homogenizing buffer (0.01 M Tris (pH 7.5)–0.02 M KCl–0.001 M $MgCl_2$) and disrupted in a Dounce homogenizer. A post-nuclear supernatant fraction (S4) was obtained by centrifuging the extract at 4000 × *g* for 5 min. The labelled virus-specific proteins in the S4 extract were separated on a 10% acrylamide slab gel containing 0.1% SDS [2,3]. The radioactive proteins were extracted and isolated from the slab gel as in [16]. The G protein samples labelled with [^{35}S]methionine and the individual [^{3}H]amino acids were combined and analyzed for partial NH_2-terminal sequence. Sequential Edman degradation in a Beckman 890C sequencer was performed as in [6].

In vitro protein synthesis using VSV-specific mRNA and ribosomal extracts containing microsomal membranes from HeLa cells were done as in [2,3,6]. A ribosomal extract deficient in endogenous oligosaccharide donor present in the microsomal membrane was prepared from HeLa cells grown for 4 h in the presence of TM at 5 μg/ml.

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3. Results and discussion

In agreement with [13,14] we found that the non-glycosylated protein G_{TM} synthesized in the presence of TM has a decreased $M_r \sim 63\,000$ compared to M_r 69 000 for G protein (fig.1(c,a)) and G_{TM} was inserted in the microsomal membrane vesicle as assayed by protection from proteolytic digestion (fig.1(d)). A polypeptide of ~63 000 M_r was also synthesized in vitro when VSV-mRNA was translated in a ribosomal extract containing microsomal membranes from HeLa cells grown in the presence of TM (fig.1(e)) in comparison to the partially glycosylated precursor G_2 (M_r 67 000) synthesized in vitro in an extract derived from cells not treated with TM (fig.1(f)) [2–4].

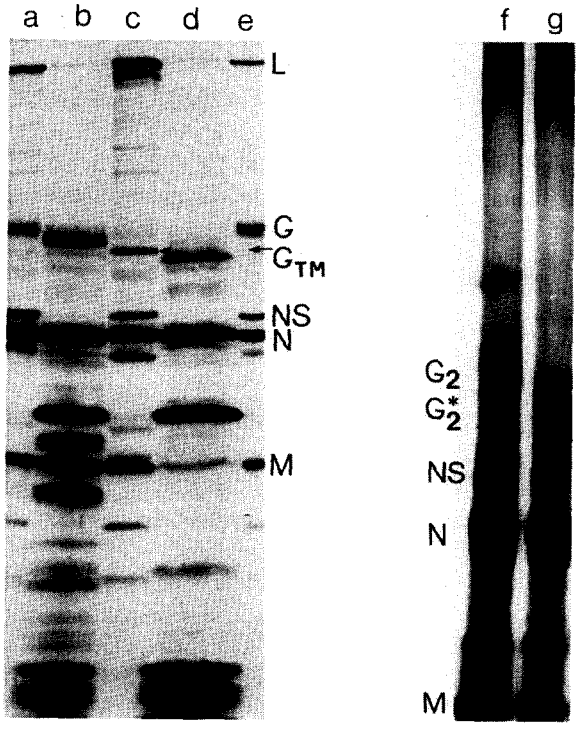

Fig.1. Synthesis of non-glycosylated G_{TM}. The autoradiogram shows [^{35}S]methionine-labelled intracellular VSV proteins (L, G, NS, N and M): (a) control extract; (b) control extract + 50 μg/ml trypsin; (c) extract from TM-treated cells; (d) extracts from TM-treated cells + 50 μg/ml trypsin; (e) [^{35}S]methionine-labelled VSV marker; (f,g) from a separate gel showing in vitro synthesized proteins by HeLa cell extracts from control cells and TM-treated cells, respectively. G_2 represents an in vitro synthesized partially glycosylated form of G and G_2* represents G_2 with its COOH-terminus digested by protease treatment [3].

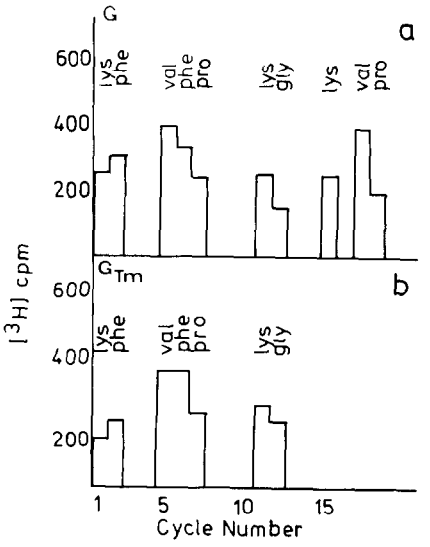

Fig.2. Partial NH-terminal sequence analysis of G and G_{TM}. G and G_{TM} labelled with [^{35}S]Met (500 Ci/mmol), [^{3}H]Leu (30 Ci/mmol), [^{3}H]Lys (30 Ci/mmol), [^{3}H]Phe (50 Ci/mmol), [^{3}H]Pro (30 Ci/mmol), [^{3}H]Val (60 Ci/mmol) and [^{3}H]Gly (12 Ci/mmol), was isolated and sequenced by automatic Edman degradation [6]. The radioactivity recovered in the fractions was identified by thin-layer chromatography on silica gel plates [6] and was plotted against cycle of degradation. The yield of radioactive lysine in the first cycle of degradation was lower than the lysine released in cycle 11 and 14. Similar yields of non-radioactive lysine was also observed during sequencing of non-radioactive G protein (unpublished).

To determine if G_{TM} contains the signal peptide we isolated G_{TM} from VSV-infected cells labelled with [^{35}S]methionine and [^{3}H]leucine, lysine, valine, proline and phenylalanine in the presence of TM and analyzed the NH_2-terminal sequence by automatic Edman degradation. For comparison G protein labelled with the same amino acids but in the absence of TM was isolated from VSV-infected cells and its NH_2-terminal sequence determined. The partial NH_2-terminal

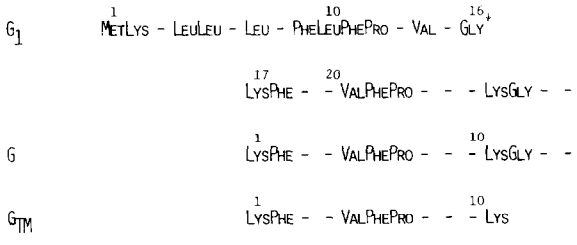

Fig.3. Comparison of the partial NH_2-terminal amino acid sequence of in vitro synthesized G_1 [6,15] and in vitro synthesized G and G_{TM}.

sequence of G_{TM} and G were identical (fig.2). A comparison of the NH_2-terminal sequences of G_{TM}, G and the non-glycosylated primary translation product G_1 [6,5] (fig.3) shows that the signal peptide of 16 amino acid present in G_1 is absent in both G_{TM} and G establishing that the signal peptide is correctly cleaved in the absence of glycosylation.

The altered physical properties of non-glycosylated G presumably due to a different conformation of the polypeptide is thus, not due to the presence of the hydrophobic signal peptide or an incorrect cleavage of the signal peptide.

Acknowledgements

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