Glycoconjugate Journal (1994) **11**:133–139

Glycosylation of glycoprotein 55 encoded by the anaemia-inducing strain of Friend spleen focus-forming virus

JÜRGEN VÖLKER, HILDEGARD GEYER and RUDOLF GEYER*
Biochemisches Institut am Klinikum der Universität, Friedrichstr. 24, D-35392 Giessen, Germany

Received 14 December 1993

Normal rat kidney cells, non-productively infected with the anaemia-inducing variant of Friend spleen focus-forming virus (F-SFFV$_A$), were metabolically labelled with [2-^{3}H]mannose. The primary translation product of the viral envelope gene (*env*), representing a glycoprotein with an apparent molecular M_r of 55 000 (gp55), was isolated from cell lysates by immunoaffinity chromatography and purified by preparative SDS/PAGE. Radiolabelled oligosaccharides, released from tryptic glycopeptides by treatment with endo-β-*N*-acetylglucosaminidase H, were characterized chromatographically, by enzymic digestion and by acetolysis. The results revealed that F-SFFV$_A$ gp55 obtained from this source carried predominantly oligomannose type sugar chains with five to nine mannoses. As a characteristic feature, glycans with seven to nine mannoses contained, in part, an additional glucose residue. Although the amount of glucosylated species found was higher in F-SFFV$_A$ gp55 (about 25% of total endo-H-sensitive oligosaccharides) than in gp55 of the corresponding polycythaemia-inducing variant (F-SFFV$_P$, 16.3%), the overall glycosylation pattern of the F-SFFV$_A$ *env* product closely resembled that of F-SFFV$_P$ gp55 [Strube *et al.* (1988) *J Biol Chem* **263**:3762–71]. Hence, our results demonstrate that the different intracellular processing and transport of the primary F-SFFV$_A$ *env* product cannot be attributed to aberrant trimming of its oligomannose type glycans.

Keywords: Friend spleen focus-forming virus, glycoprotein, oligosaccharide processing, SFFV

Abbreviations: endo H, endo-β-*N*-acetylglucosaminidase H from *Streptomyces griseus*; *env*, envelope gene; Env protein, translation product of *env*; F-SFFV, Friend spleen focus-forming virus; F-SFFV$_A$, anaemia-inducing variant of F-SFFV; F-SFFV$_P$, polycythaemia-inducing variant of F-SFFV; Hex, hexose; NRK, normal rat kidney; PNGase F, peptide-N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase F from *Flavobacterium meningosepticum*.

Introduction

Friend spleen focus-forming virus (F-SFFV) is a highly pathogenic, replication-defective retrovirus inducing an acute erythroleukaemia in mice with is either associated with a polycythaemia (F-SFFV$_P$) or a slight anaemia (F-SFFV$_A$; for reviews see [1–3]). In both cases, the presence and expression of the viral envelope gene (*env*) was shown to be essential and sufficient for induction of the disease [4–9]. The precise mechanism by which SFFV causes erythroleukaemia is unknown. Available evidence, however, indicates that the F-SFFV$_P$ *env* product interacts with the cellular erythropoietin receptor [10–12] leading to an erythropoietin-independent proliferation of the cells [13, 14].

F-SFFV *env* encodes a glycoprotein with five (F-SFFV$_P$) or four (F-SFFV$_A$) potential *N*-glycosylation sites [15–18]. The primary gene products formed have apparent M_rs of about 55 000 (gp55) and mostly accumulate intracellularly. Small proportions, however, are further processed in their carbohydrate moieties yielding secondary products with M_rs of about 65 000 (gp65; F-SFFV$_P$) or 60 000 (gp60; F-SFFV$_A$), both of which are found in the culture supernatant of erythroleukaemia cells and/or fibroblasts [19–21]. It is noteworthy that only F-SFFV$_P$-infected cells expose detectable amounts of this secondary *env* product at their plasma membrane [22]. It has been suggested that the surface expression of gp65 may be a prerequisite for the induction of the polycythaemic phenotype of the disease [23].

Using F-SFFV$_P$ glycosylation mutants, we demonstrated that the destruction of potential *N*-glycosylation sites in the C-terminal domain of the Env protein significantly altered viral pathogenicity [24]. In agreement with data reported by others [25], our studies further revealed that the primary *env* products of pathogenic mutants were, in part, further

* To whom correspondence should be addressed.

processed in transfected Rat-1 cells, whereas those of apathogenic mutants were not [26], indicating that intracellular transport and maturation of the *env* product in this model cell line correlated with the *in vivo* pathogenicity of the respective mutant. Hence, carbohydrates may play an important role in maintaining the active conformation of the protein.

As mentioned above, F-SFFV$_A$-infected fibroblasts do not contain detectable amounts of the secondary *env* product (gp60) at their cell surface. Since the intracellular transfer of F-SFFV$_P$ Env protein to the plasma membrane has been found to depend on proper trimming of the oligomannose type glycans of gp55 [20], the different routeing of F-SFFV$_A$ gp55 might be due to aberrant processing of its sugar chains. So far detailed carbohydrate analyses have, however, only been carried out in the case of F-SFFV$_P$ gp55 [27]. In the present study we have, therefore, studied the glycosylation of F-SFFV$_A$ gp55 expressed in normal rat kidney (NRK) cells. The results obtained are compared with our previous data on the carbohydrate structure of the F-SFFV$_P$ encoded Env protein synthesized in the same host cell line.

Materials and methods

Materials

Aspergillus oryzae mannosidase, specific for the α(1-2)-linkage, was generously provided by Dr H. Yamaguchi (University of Osaka, Japan). The structure and origin of the oligosaccharide standards used for chromatographic comparison and column calibration have been described in detail elsewhere [27, 28]. Culture supernatants from hybridoma 7C10 cells [29] containing rat monoclonal IgG directed against the aminoterminal domain of F-SFFV Env-proteins as well as normal rat kidney (NRK) cells nonproductively infected with the anaemia-inducing strain of Friend spleen focus-forming virus (F-SFFV$_A$/NRK) were kindly supplied by Dr L. Wolff (National Cancer Institute, Bethesda, MD, USA).

Cell culture

Virus-infected NRK cells were maintained in roller bottles (850 cm^2) at 37 °C in Dulbecco's modified Eagle's medium (DMEM; Gibco, Germany) containing 4.5 g glucose l^{-1} supplemented with 10% (by vol.) fetal calf serum (Biochrom, Germany).

Radiolabelling of cells and cell lysis

Metabolic labelling of cells with D-[2-^{3}H]mannose in the presence of 1 g glucose per liter of medium as well as cell lysis was performed as described [27].

Purification of monoclonal antibodies

Rat monoclonal IgG was isolated from the culture medium of hybridoma 7C10 cells as described by Burchiel [30]. After filtration through a 0.45 µm filter (Millipore, Germany), aliquots of 500 µl were subjected to anion-exchange HPLC using a DEAE 5PW-column (Millipore). Separation was achieved by isocratic elution with 20 mM Tris-HCl buffer, pH 7.7 for 5 min, followed by a linear gradient of 0–0.5 M NaCl in the same buffer within 20 min at a flow rate of 1 ml min^{-1}. Fractions (500 µl) were checked for the presence of IgG by SDS/PAGE under reducing conditions [31]. Those containing IgG were pooled and dialysed against aqueous 0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.3, prior to coupling to cyanogen bromide-activated Sepharose 4B (Pharmacia, Germany).

Isolation and purification of gp55

Radiolabelled gp55 was isolated from cell lysates by immunoaffinity chromatography and purified by preparative SDS/PAGE as described [27] except that purified rat monoclonal antibodies were used instead of rabbit antiserum for preparation of the immunoaffinity column.

Isolation of oligosaccharides

Radiolabelled gp55 glycans were released by sequential treatment of tryptic glycopeptides with endo-β-*N*-acetylglucosaminidase H (endo H) from *Streptomyces griseus* and peptide-N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase F (PNGase F) from *Flavobacterium meningosepticum* (both from Boehringer, Germany), separated from residual peptide material by gel filtration or reversed-phase HPLC, reduced and desalted as described earlier [32].

Chromatographic procedures

Identification of radiolabelled monosaccharide constituents by HPLC using Aminex HPX-87H and HPX-87P columns and fractionation of glycans by HPLC using a LiChrosorb-Diol column were carried out as described in detail previously [27, 32]. After addition of a scintillation cocktail (Roth, Germany), aliquots of fractions obtained were monitored for radioactivity by liquid scintillation counting.

Enzymatic digestions

Oligosaccharide alditols were digested with α-mannosidase from jack beans (Sigma, Germany) or with α(1-2)-linkage-specific mannosidase from *Aspergillus oryzae* as detailed elsewhere [27, 33].

Acetolysis

Acetolysis of oligomannosidic glycans as well as de-*O*-acetylation, reduction and chromatographic identification of the reaction products were as detailed elsewhere [34].

Results

Isolation and characterization of F-SFFV$_A$ gp55

F-SFFV$_A$-infected NRK cells were cultivated in roller bottles in Dulbecco's MEM and metabolically labelled with

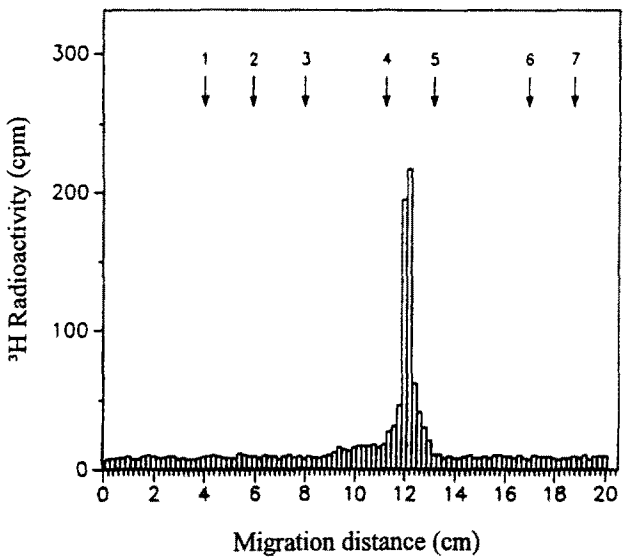


Figure 1. Analytical SDS-polyacrylamide gel electrophoresis of purified F-SFFV$_A$ gp55, metabolically labelled with [2 ^{3}H]mannose. An aliquot of purified gp55 was applied to a slab gel (200 × 140 × 2.5 mm). After separation, horizontal strips of 2 mm were excised. Numbers (1–7) with arrows indicate migration positions of standard proteins: (1) α_2-macroglobulin, 180 kDa; (2) β-galactosidase, 116 kDa; (3) phosphofructokinase, 84 kDa; (4) pyruvate kinase, 58 kDa; (5) fumarase, 48 kDa; (6) lactate dehydrogenase, 36 kDa; (7) triose phosphate isomerase, 26 kDa.

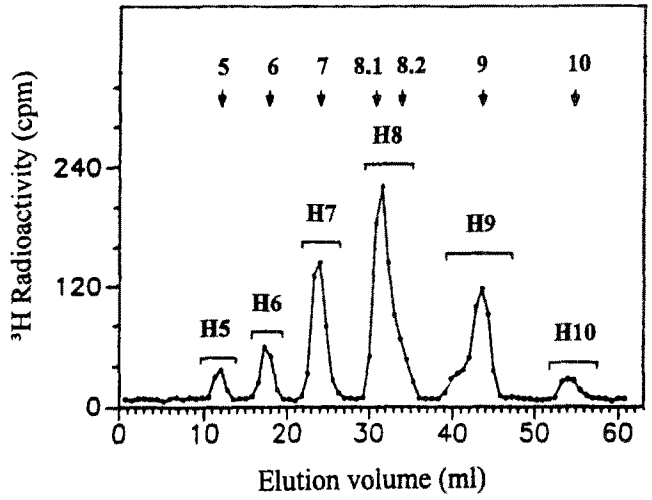


Figure 2. Separation of endo-H-sensitive glycans from F-SFFV$_A$ gp55. Oligosaccharide alditols obtained from tryptic gp55 glycopeptides, metabolically labelled with [2-^{3}H]mannose, were fractionated by HPLC using a LiChrosorb-Diol column (5 µm; 4 × 150 mm) and acetonitrile:water (74:26) as eluant. Fractions (0.75 ml) were collected at a flow rate of 0.5 ml min^{-1} and monitored for radioactivity. Numbers with arrows indicate the elution volumes of oligomannosidic oligosaccharide alditol standards: Man$_{5-9}$GlcNAcOH (5, 6, 7, 8.2 and 9), GlcMan$_7$GlcNAcOH (8.1) and GlcMan$_9$GlcNAcOH (10). H5–H10, oligosaccharide fractions pooled as indicated by horizontal brackets..

[2-^{3}H]mannose in the presence of glucose. After cell lysis, gp55 was isolated by immunoaffinity chromatography using monoclonal antibodies directed against the amino-terminal region of F-SFFV Env-proteins [29] and purified by preparative SDS/PAGE. A homogeneous preparation of radiolabelled gp55 with 7 × 10^4 cpm was thus obtained (Fig. 1). For determination of radiolabelled monosaccharide constituents, an aliquot of gp55 was hydrolysed and the radioactive monosaccharides were identified by co-chromatography with monosaccharide standards (data not shown). The results revealed that [^{3}H]mannose-labelled gp55 contained predominantly radiolabelled mannose in addition to small amounts of fucose and galactose indicating that [2-^{3}H]mannose has been converted to some extent into other sugars during metabolic labelling of the cells.

Liberation and fractionation of gp55 glycans

After digestion with trypsin, glycopeptides were treated with endo H. Oligosaccharides released were separated from residual glycopeptides by gel filtration. After 90% of total radioactivity was thus liberated from gp55. Endo-H-resistant glycans, which could be recovered by treatment of resistant glycopeptides with PNGase F, were not further studied due to the small amounts of radioactivity. Endo-H-sensitive oligosaccharides were reduced and the resulting alditols were fractionated by HPLC on a LiChrosorb-Diol column (Fig. 2) yielding the fractions H5–H10 (4.9, 8.1, 19.8, 34.7, 20.1 and 2.4% of total radioactivity).

Characterization of glycans

Oligosaccharide alditol fractions H5–H10 were characterized by gel filtration and HPLC in conjunction with authentic oligosaccharide standards, by acetolysis (H7–H9) and by digestion with α-mannosidase from jack beans and α-1,2-mannosidase from *A. oryzae* (see Fig. 3 and Table 1). Reaction products were analysed by Bio-Gel P-4 chromatography and their hydrodynamic volumes were estimated by co-chromatography with glucose oligomers [35, 36]. After degradation with the jack bean enzyme, which cleaves all α-linked mannoses irrespective of their linkage positions, fractions H5–H7 yielded two radioactive fragments in proportions approaching 1:4, 1:5 or 1:6, respectively, which co-eluted with authentic ManGlcNAcOH (3.2 glucose units) and mannose (0.9 glucose units). Treatment of the same glycan fractions with α-1,2-mannosidase resulted in the liberation of zero (H5), one (H6) and two (H7) mannose residues and the concurrent formation of a radioactive fragment eluting at 6.8 glucose units, i.e., the elution position of authentic Man$_5$GlcNAcOH. Acetolysis fragments obtained in the case of H7 indicated the presence of two different isomers lacking outer mannose residues in different positions (data not shown). Clearly, these fractions comprised solely oligomannosidic oligosaccharide alditols containing one β- and four to six α-linked mannose residues, zero to two of which were α-1,2-linked (cf. Table 2).

In the case of fraction H8, however, only part of the glycans could be converted into the ManGlcNAcOH

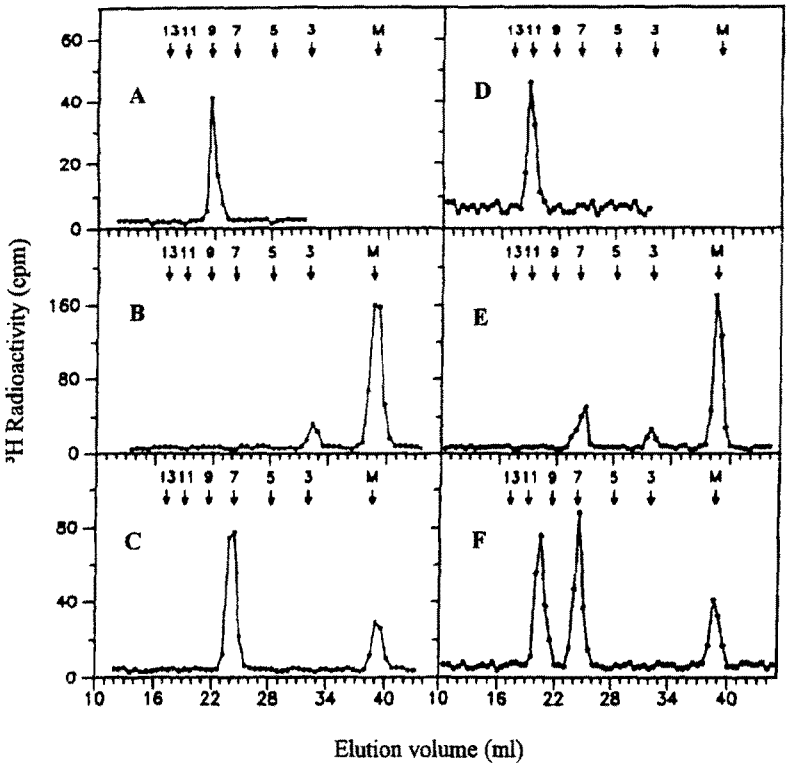


Figure 3. Degradation of oligosaccharides with α-mannosidases. Radiolabelled oligosaccharide alditol fractions H7 and H9 (cf. Fig. 2) and degradation products obtained thereof by treatment with α-mannosidase from jack beans or α-1,2-mannosidase from *A. oryzae* were analysed by gel filtration. **A** and **D**, size determination of fraction H7 and H9 glycans; **B** and **E**, analysis of reaction products obtained after digestion with α-mannosidase; **C** and **F**, radioactivity profiles of fragments produced by degradation with α-1,2-mannosidase. Samples were chromatographed on a Bio-Gel P-4 column (–400 mesh, 6 × 2000 mm) at hydrostatic pressure (2.5×10^4 Pa) using aqueous sodium azide (0.03%) as eluant. Fractions (0.4 ml) were collected at a flow rate of 1.2 ml h^{-1} and monitored for radioactivity. Column calibration with glucose oligomers (3–13 glucose residues) and mannose (M) is shown by arrows.

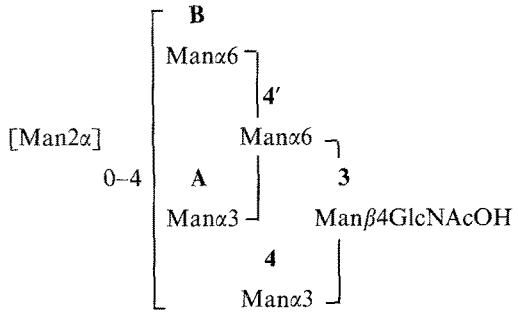
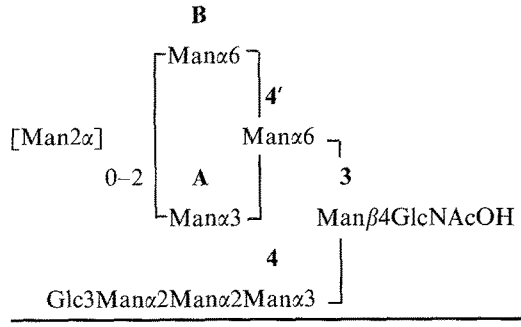
Table 1. Degradation of endo-H-sensitive oligosaccharide alditols from F-SFFV$_A$ gp55 with α-mannosidases. Oligosaccharide alditol fractions H5–H10 (see Fig. 2) isolated from gp55, metabolically labelled with [2-^{3}H]mannose, were digested with α-mannosidase from jack beans (I) or α-1,2-mannosidase from *A. oryzae* (II). Products were analysed by gel filtration using a calibrated Bio-Gel P-4 column (cf., for example, Fig. 3). The results are given in percentage [^{3}H]radioactivity found in the products obtained.

Hexose units of fragments	*Co-eluting standard oligosaccharide*	*H5*			*H6*			*H7*			*H8*			*H9*			*H10*		
		SM	*I*	*II*	*SM*	*I*	*II*	*SM*	*I*	*II*	*SM*	*I*	*II*	*SM*	*I*	*II*	*SM*	*I*	*II*
12	$GlcMan_9GlcNAcOH$																100	–	–
10.8	$Man_9GlcNAcOH$													100	–	–	–	–	–
9.8	$Man_8GlcNAcOH$										100	–	33	–	–	40	–	–	79
8.9	$Man_7GlcNAcOH$							100	–	–	–	–	–	–	–	–	–	–	–
7.8	$Man_6GlcNAcOH$				100	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6.8	$Man_5GlcNAcOH$	100	–	100	–	–	85	–	–	73	–	19	47	–	22	40	–	51	–
3.2	ManGlcNAcOH	–	20	–	–	16	–	–	14	–	–	8	–	–	8	–	–	–	–
0.9	Man	–	80	–	–	84	15	–	86	27	–	73	20	–	70	20	–	49	21

SM, starting material; Man, mannose.

fragment by jack bean α-mannosidase-catalysed release of seven mannose residues (see Table 1). Remaining sugar chains lost only three mannose residues and led to a fragment eluting at about 7 glucose units, i.e., the position of glycans comprising five hexoses and one reduced *N*-acetylglucosamine ($Hex_5GlcNAcOH$). Digestion with α-1,2-mannosidase confirmed these results in so far as fragments co-eluting with $Man_5GlcNAcOH$ and mannose, indicating the release of three α-1,2-linked mannoses, were obtained in addition to species which

Table 2. Condensed structural representation of oligosaccharide alditols released from F-SFFV$_A$ gp55 by endo H. The general architecture of the glycans, the anomeric structure of Man-**3** as well as the linkage positions of mannose residues **3**, **4**, **4'**, **A**, **B** and terminal glucose are proposed in accordance with previous data on the structure of corresponding glycans from F-SFFV$_P$ gp55 expressed in the same host cell line [27]. The designation of sugar residues is taken from van Halbeek [37]. Relative amounts of glucosylated and non-glucosylated species were roughly estimated from the distribution of the radioactivity considering the number of mannose residues present in corresponding fragments after enzymic treatment.

Condensed structure	*Relative amount (%)*
	75
	25

mannoses, were obtained in addition to species which were not affected. From these results, the elution position of the oligosaccharides in HPLC and previous data [27], it can be assumed that fraction H8 represents a mixture of oligomannosidic species with eight mannose residues (Man$_8$GlcNAcOH) and glucosylated glycans carrying seven mannoses (GlcMan$_7$GlcNAcOH).

Fraction H9 similarly contained a mixture of oligomannosidic and glucosylated glycans. After incubation with jack bean α-mannosidase, radioactive fragments co-eluting with Hex$_5$GlcNAcOH or ManGlcNAcOH were obtained in addition to free mannose, indicating the liberation of four or eight mannose residues, respectively (Fig. 3E). Analogous treatment with α-1,2-mannosidase demonstrated that one or four of these mannosyl residues were α-1,2-linked (see Fig. 3F). Acetolysis yielded, *inter alia*, fragments with the compositions Hex$_4$GlcNAcOH or Hex$_5$GlcNAcOH (data not shown). Since this type of mild acid hydrolysis leads to a preferential cleavage of α-1,6-linkages, this result demonstrates that the lower α-1,3-linked branches of some glycans contained an additional hexose residue. Therefore, fraction H9 is proposed to contain the common oligomannosidic Man$_9$GlcNAcOH species and GlcMan$_8$GlcNAcOH glycans.

Glycans of fraction H10 co-eluted with the standard oligosaccharide GlcMan$_9$GlcNAcOH. Upon digestion with jack bean α-mannosidase or α-1,2-specific mannosidase, five or two mannose residues were released with concurrent formation of fragments eluting at 6.8 or 9.8 glucose units, respectively (Table 1). Obviously, H10 glycans represented glucosylated Man$_9$GlcNAcOH species carrying the glucose residue in terminal position at the lower α-1,3-linked branch (Table 2).

Assuming that [^{3}H]mannose radioactivity was equally distributed in all structures, molar ratios of the different species were roughly estimated from the distribution of radioactivity after normalization on the number of mannose residues present in fragments produced by enzymic treatment. The results indicate that about one quarter of the endo-H-sensitive glycans carried an additional glucose residue (Table 2).

Discussion

The envelope genes of F-SFFV$_P$ and F-SFFV$_A$ encode glycoproteins with five and four potential *N*-glycosylation sites, respectively. Since the C-terminal site of the F-SFFV$_P$ Env protein is considered not to be glycosylated [3], expression of both genes in rat fibroblasts leads to primary translation products with similar apparent molecular masses of about 55 000 Da (gp55), which are, however, processed in different ways. Whereas a small proportion of F-SFFV$_P$ gp55 is further modified in its carbohydrate side chains yielding a secondary gene product (gp65), which readily appears on the cell surface, cell-associated secondary *env* products of F-SFFV$_A$ can hardly be detected [22]. On the other hand, culture supernatants of F-SFFV$_A$- and F-SFFV$_P$-infected cells have been reported to contain secondary *env* products of both viruses with apparent M_rs of 60 000 (gp60) and 65 000 (gp65), respectively [19]. Since the proper glycosylation and oligosaccharide trimming have been shown to influence intracellular processing and transport of F-SFFV$_P$ Env proteins [20, 26], we have now analysed the glycosylation pattern of F-SFFV$_A$ gp55.

The results revealed that F-SFFV$_A$ gp55, expressed in NRK cells, carried predominantly oligomannose type sugar chains with five to nine mannose residues, part of which contained an additional glucose. Although glycans with five mannose residues as well as GlcMan$_8$GlcNAcOH species were not detected in the corresponding analysis

of F-SFFV$_P$ gp55 expressed in the same cell line [27], the overall glycosylation pattern of F-SFFV$_A$ gp55 was rather similar. Nevertheless, the oligosaccharide profiles at individual *N*-glycosylation sites may still be different in both glycoproteins. Possibly, glucosylated glycans representing about one quarter of total endo-H-sensitive glycans are selectively attached to one of the four *N*-glycosylation sites present. If so, almost all primary F-SFFV$_A$ *env* products would carry at least one glucosylated oligosaccharide chain. Since glucosylated glycans do not only reflect early trimming intermediates of oligosaccharide processing, but may also be formed by post-translational reglucosylation of malfolded proteins [38, 39] leading to a retardation in the rough endoplasmic reticulum [40, 41], a site-specific location of this type of glycans may influence the intracellular processing and transport of the corresponding glycoprotein. Clearly, isolation and characterization of individual glycosylation sites of F-SFFV$_A$ gp55 are required in order to decide whether differences in Env protein trafficking are due to different oligosaccharide trimming. In this context, it would also be interesting to analyse the endo-H-resistant glycans representing about 10% of the total radioactivity. The question of whether the Env protein of F-SFFV$_A$ differs from that of F-SFFV$_P$ in its ability to acquire O-linked sugar chains could not be addressed, since this type of glycans is not detectable when [2-^{3}H]mannose is used for metabolic labelling.

The released secondary *env* product of F-SFFV$_A$ (gp60) has a lower apparent M_r than that of F-SFFV$_P$ (gp65) [19, 22], although both glycoproteins are supposed to carry the same number of oligosaccharide side-chains. The reason for this discrepancy, however, is not understood. Possibly, gp60 carries predominantly truncated complex type *N*-glycans and/or lacks O-linked sugar chains which have been reported to occur in F-SFFV$_P$ gp65 [20, 21, 26]. Comparative carbohydrate structure analyses of the released, secondary Env proteins of F-SFFV$_A$ and F-SFFV$_P$ will help to trace the intracellular route of these two glycoproteins.

Acknowledgements

We thank U. Planck, H.-H. Schott, H. Thiele and P. Weber for cultivation of F-SFFV$_A$-infected NRK cells and S. Stirm for helpful discussions. This work was supported by the *Deutsche Forschungsgemeinschaft* (Sonderforschungsbereich 272).

References

1. Ruscetti S, Wolff L (1984) *Curr Top Microbiol Immunol* **112**:21–44.
2. Ostertag W, Stocking C, Johnson GR, Kluge N, Kollek R, Franz T, Hess N (1987) *Adv Cancer Res* **48**: 193–355.
3. Kabat D (1989) *Curr Top Microbiol Immunol* **148**:1–42.
4. Linemeyer DL, Ruscetti SK, Scolnick EM, Evans LH, Duesberg PH (1981) *Proc Natl Acad Sci USA* **78**:1401–5.
5. Linemeyer DL, Menke JG, Ruscetti SK, Evans LH, Scolnick EM (1982) *J Virol* **43**:223–33.
6. Machida CA, Bestwick RK, Boswell BA, Kabat D (1985) *Virology* **144**:158–72.
7. Wolff L, Ruscetti S (1985) *Science* **228**:1549–52.
8. Wolff L, Ruscetti S (1988) *J Virol* **62**:2158–63.
9. Li JP, Bestwick RK, Machida C, Kabat D (1986) *J Virol* **57**:534–38.
10. Li JP, D'Andrea AD, Lodish HF, Baltimore D (1990) *Nature* **343**:762–64.
11. Yoshimura A, D'Andrea AD, Lodish HF (1990) *Proc Natl Acad Sci USA* **87**:4139–43.
12. Casadevall N, Lacombe C, Muller O, Gisselbrecht S, Mayeux P (1991) *J Biol Chem* **266**:16015–20.
13. Hoatlin ME, Kozak SL, Lilly F, Chakraborti A, Kozak CA, Kabat D (1990) *Proc Natl Acad Sci USA* **87**:9985–89.
14. Ruscetti SK, Janesch NJ, Chakraborti A, Sawyer ST, Hankins WD (1990) *J Virol* **63**:1057–62.
15. Amanuma H, Katori A, Obata M, Sagata N, Ikawa Y (1983) *Proc Natl Acad Sci USA* **80**:3913–17.
16. Clark SP, Mak TW (1983) *Proc Natl Acad Sci USA* **80**:5037-41.
17. Wolff L, Scolnick E, Ruscetti S (1983) *Proc Natl Acad Sci USA* **80**:4718–22.
18. Wolff L, Kaminchik J, Hankins WD, Ruscetti SK (1985) *J Virol* **53**:570–78.
19. Pinter A, Honnen WJ (1985) *Virology* **143**:646–50.
20. Pinter A, Honnen WJ (1989) *Virology* **173**:136–43.
21. Gliniak BC, Kabat D (1989) *J Virol* **63**:3561–68.
22. Ruscetti, SK, Feild JA, Scolnick EM (1981) *Nature* **294**:663–65.
23. Li JP, Bestwick RK, Spiro C, Kabat D (1987) *J Virol* **61**:2782–92.
24. Rau S, Geyer R, Friedrich RW (1993) *J Gen Virol* **74**:699–705.
25. Wang Y, Kayman SC, Li JP, Pinter A (1993) *J Virol* **67**:1322–27.
26. Freis A, Rau S, Friedrich RW, Geyer R (1993) *Glycobiology* **3**:465–73.
27. Strube KH, Schott HH, Geyer R (1988) *J Biol Chem* **263**:3762–71.
28. Pfeiffer G, Schmidt M, Strube KH, Geyer R (1989) *Eur J Biochem* **186**:273–86.
29. Wolff L, Koller R, Ruscetti S (1982) *J Virol* **43**: 472–81.
30. Burchiel SW (1986) *Methods Enzymol* **121**:596–615.
31. Laemmli UK (1970) *Nature* **227**:680–85.
32. Geyer R, Geyer H (1993) *Methods Molec Biol* **14**: 131–42.
33. Geyer H, Will C, Feldmann H, Klenk HD, Geyer R (1992) *Glycobiology* **2**:299–312.
34. Geyer R, Geyer H (1994) *Methods Enzymol* **230**:86–108.
35. Yamashita K, Mizuochi T, Kobata A (182) *Methods Enzymol* **83**:105–26.

36. Kobata A, Yamashita K, Takasaki S (1987) *Methods Enzymol* **138**:84–94.
37. Van Halbeek H (1993) *Methods Molec Biol* **17**:115–48.
38. Parodi AJ, Mendelzon DH, Lederkremer GZ, Martin-Barrientos J (1984) *J Biol Chem* **259**:6351–57.
39. Suh K, Bergmann JE, Gabel CA (1989) *J Cell Biol* **108**:811–19.
40. Lodish HF, Kong N (1984) *J Cell Biol* **98**:1720–29.
41. Schlesinger S, Malfer C, Schlesinger MJ (1984) *J Biol Chem* **259**:7597–601.