Glycosylation and Intracellular Transport of Membrane Glycoproteins Encoded by Murine Leukemia Viruses

INHIBITION BY AMINO ACID ANALOGUES AND BY TUNICAMYCIN*

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Addition of asparagine-linked oligosaccharides to nascent murine leukemia virus (MuLV)-encoded membrane glycoproteins was inhibited either completely by tunicamycin or specifically at Asn-X-Thr glycosylation sites by incorporation of the threonine analogue \(\beta\)-hydroxynorvaline. In conditions of partial analogue substitution, a series of subglycosylated components is formed which are related by a constant apparent \(M\), difference when assayed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The total number of asparagine-linked oligosaccharides is then estimated by dividing the measured apparent \(M\), of one oligosaccharide into the total apparent \(M\), difference between the complete glycoprotein and the polypeptide chain that is synthesized in cells incubated with tunicamycin. Correct results were obtained using glycoproteins with known numbers of oligosaccharides. Our analyses indicate that the gp70 membrane envelope glycoproteins of certain ecotropic MuLVs contain seven oligosaccharides, whereas the Glc\(\alpha\)X antigen-containing variant gp70 contains one fewer Asn-X-Thr-linked oligosaccharide. The membrane glycoprotein encoded by the gag gene of Friend MuLV contains only one asparagine-linked oligosaccharide. Similarly, the gp55 membrane glycoprotein encoded by Friend erythroleukemia virus contains four asparagine-linked oligosaccharides. Pulse-chase and cell surface iodination analyses indicate that MuLV membrane envelope glycoprotein processing by partial proteolysis and transport to the cell surface can be efficiently blocked by structural perturbations caused by incorporation of different amino acids or by loss of oligosaccharides. Our data also suggest that loss of oligosaccharides may expose new antigenic sites in viral membrane glycoproteins and increase their susceptibility to intracellular proteolysis.

Asparagine-linked oligosaccharides in glycoproteins occur in the tripeptide sequences Asn-X-Ser(Thr), where X represents any amino acid except asparatic acid (1-3). Furthermore, this sequence serves as an acceptor site for transfer of a preformed lipid-linked oligosaccharide with the structure Glc\(\alpha\)Man\(\alpha\)Man\(\alpha\)GlcNAcGln\(\alpha\) (4-9). Following transfer of this oligosaccharide onto nascent polypeptide chains in the rough endoplasmic reticulum, oligosaccharide processing ensues by removal of Glc and some Man residues to produce high mannose core-type oligosaccharides (10, 11). Therefore, newly attached asparagine-linked oligosaccharides have a transiently existing common size and structure (4-11).

Recently, Hortin and Boime (3) reported that incorporation of the threonine analogue \(\beta\)-hydroxynorvaline into nascent secretory proteins in a cell-free system causes inhibition of oligosaccharide attachment at the Asn-X-Thr sites. Furthermore, \(\beta\)-hydroxynorvaline or the leucine analogue \(\beta\)-hydroxyleucine inhibits removal of the signal or leader peptide from newly synthesized pre-prolactin polypeptide chains (12). We have now used these analogues with whole cells and have found that \(\beta\)-hydroxynorvaline can be used to estimate the numbers of oligosaccharides in a variety of membrane glycoproteins encoded by murine retroviruses. Although neither of these amino acid analogues inhibits the removal of a leader peptide from the nascent viral-encoded membrane glycoproteins, both analogues appear to block the processing of newly synthesized MuLV' gp70 precursor glycoproteins. Furthermore, processing can also be reduced in the absence of amino acid analogues by inhibiting glycosylation with tunicamycin.

**EXPERIMENTAL PROCEDURES**

**Cells and Viruses**—NRK cells and nonproducer cells (NRK clone 1) infected with F-SFFV were kindly provided by Edward Scolnick (National Institutes of Health, Bethesda, MD). The Friend MuLV clone F12 and the F-SFFV-infected NRK clone 1 have been previously described (13, 14). Both Friend MuLV clone F12 and Moloney MuLV (M) were grown in NRK cells. Gs is a type-specific antigen found in the gp70 molecules encoded by some Gross-type ecotropic viruses (15) and is expressed on the surfaces of thymocytes of certain mouse strains (17, 18). The Gs and Gs' viruses infected into NIH 3T3 fibroblasts (19) were kindly provided by Marsha Rosner, Nancy Hopkins, and Phillips Robbins (Massachusetts Institute of Technology, Cambridge). The infected cell lines were maintained as described previously (13, 14).

**Radioactive Labeling and Extraction of Cells**—Cells were preincubated for 10 min in methionine-free minimal essential medium (Gibco Laboratories) which contained 10% dialyzed fetal calf serum. For analogue experiments, the preincubation media contained either (i) normal amino acids, (ii) 5 mM \(\beta\)-hydroxynorvaline instead of threonine, or (iii) 5 mM \(\beta\)-hydroxyleucine instead of leucine. These media were then removed and were replaced with identical media supplemented with 50 \(\mu\)Ci/ml of \(\text{L-}^{35}\)S]methionine. After incubation for various times, the labeling media were removed, and cell extracts were prepared as described elsewhere (13).

**Other Procedures**—All other procedures have been previously described. These include the iodination of cell surfaces with \(\text{I}^{125}\) (20),

*The abbreviations used are: MuLV, murine leukemia virus; SFFV, spleen focus-forming virus; NRK, normal rat kidney fibroblasts; env, the MuLV gene encoding the envelope glycoprotein gp70; gag, the MuLV gene encoding the viral core proteins including p30; F- and R-SFFV, the Friend and Rauscher strains of SFFV; F-MuLV, the Friend strain of MuLV.

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immunoprecipitation of proteins from labeled cell extracts with monoclonal antiserum to Rauscher MuLV gp70 or to p30 (13), electrophoresis of immunoprecipitated proteins in polyacrylamide gels in the presence of sodium dodecyl sulfate (13, 14), fluorographic detection of radioactive protein components on the dried gels (13, 14), and the quantitative estimation of radioactive components by densitometry, scanning of appropriately exposed autoradiographic films (20). Apparent $M_r$ estimates were done using standard curves made by plotting log $M_r$ versus distance migrated. The $^{14}C$-protein standards (New England Nuclear) were employed in all gels and had $M_r$ = 92,500, 69,000, 46,000, 30,000, and 12,300. Tunicamycin was used to inhibit glycosylation at Asn-X-Ser(Thr) sites of glycoproteins (20). Optimal drug concentrations to obtain complete inhibition of glycosylation were 0.6 $\mu$g/ml with NRK cells and 25 $\mu$g/ml with NIH-3T3 cells. Cell lines were treated with tunicamycin both prior to (3 h) and during labeling (30 min) with $L-[^{35}S]$methionine.

RESULTS

Effects of $\beta$-Hydroxynorvaline and $\beta$-Hydroxyleucine on Synthesis of Virus-encoded Glycoproteins—Electrophoretic analysis of virus-encoded membrane glycoproteins synthesized in the presence or absence of the threonine analogue $\beta$-hydroxynorvaline and of the leucine analogue $\beta$-hydroxyleucine is shown in Fig. 1. Both analogues are incorporated into polyepptides without inhibition of net $L-[^{35}S]$methionine incorporation into cellular proteins. The leucine analogue causes a slight increase in apparent $M_r$ (lanes 1 and 4) compared with the unsubstituted glycoproteins (lanes 2 and 5). We believe that this size increase is caused by the extra bulk of the leucine analogue and by its effects on protein folding rather than by retention of a leader or signal peptide, because cytoplasmically synthesized proteins which lack leader peptides also have a larger size when formed in the presence of $\beta$-hydroxyleucine. On the contrary, the threonine analogue causes formation of a series of discrete smaller components (lanes 3 and 6). Interestingly, only one smaller component is formed with the gp55 molecule (13, 21, 22, 31) encoded by Friend spleen focus-forming virus (lane 3), whereas three smaller components are produced with gPr90$^{\mathrm{CHO}}$ encoded by F-MuLV (lane 6). Therefore, the number of smaller components depend upon the glycoprotein.

Evidence concerning the effects of different concentrations of $\beta$-hydroxynorvaline on the F-MuLV-encoded glycoproteins is shown in Fig. 2. Clearly, the smaller derivatives (−2 and −3) of gPr90$^{\mathrm{CHO}}$ are produced in higher proportions when the $\beta$-hydroxynorvaline concentration is increased (e.g. compare lanes 2, 3, 4, and 6). However, even in the highest concentrations employed, components smaller than −3 were not detected. Furthermore, when these same radioactive cell extracts were precipitated with antiserum made to the MuLV core protein p30, a more complex protein pattern was obtained (lanes 7–12). This pattern occurs because the F-MuLV gag gene codes for two distinct primary translation products: a Pr65$^{\mathrm{CHO}}$ polyprotein that is formed in the cytoplasm and is proteolytically cleaved to produce viral core proteins including p30, and a gPr80$^{\mathrm{CHO}}$ membrane glycoprotein that is formed in the rough endoplasmic reticulum and is processed by oligosaccharide modification in the Golgi apparatus to produce a plasma membrane glycoprotein gp93$^{\mathrm{CHO}}$ (15, 23–25). The effects of the glycosylation inhibitor tunicamycin (lanes 7 and 10) clearly show that gPr80$^{\mathrm{CHO}}$ is a glycoprotein. Furthermore, in contrast to its effects on gPr90$^{\mathrm{CHO}}$, $\beta$-hydroxynorvaline does not cause heterogeneity of gPr80$^{\mathrm{CHO}}$ or of other gag gene products. Therefore, certain plasma membrane glycoproteins can be synthesized in the presence of $\beta$-hydroxynorvaline without forming any smaller derivatives.

The Smaller Components Formed in $\beta$-Hydroxynorvaline Are Deficient in Asn-linked Oligosaccharides—Conceivably,
the polypeptide chain synthesized in tunicamycin.

It is essential to realize that we are not implying that this measured apparent time. As shown in Table 11 summarizes results obtained using this method to estimate the total number of oligosaccharides in glycoproteins. Furthermore, the number of subglycosylated components synthesized in the presence of β-hydroxynorvaline should provide at least a minimum estimate of the number of Asn-X-Thr oligosaccharides in the glycoprotein. Thus, gpPr90″ encoded by F-MuLV must have at least three Asn-X-Thr-linked oligosaccharides, whereas gpPr90″ may have zero.

Table I shows a representative analysis of the gpPr90″ and gp55 glycoprotein components formed in the presence and absence of β-hydroxynorvaline and of tunicamycin. The results suggest that gp55 contains four asparagine-linked oligosaccharides, whereas gpPr90″ contains seven.

Oligosaccharide Analyses of Different Glycoproteins—Table II summarizes results obtained using this method to estimate the oligosaccharides of different retrovirus-encoded glycoproteins. The results suggest that several different ecotropic MuLV envelope glycoproteins contain six or seven asparagine-linked oligosaccharides. The gp55 glycoprotein encoded by F-SFFV contains four oligosaccharides, whereas gpPr90″ encoded by Rauscher MuLV contains seven to eight. Our average oligosaccharide estimates for these glycoproteins differ from the estimates of Rosner et al. (19) by less than 10%. This degree of error is consistent with the reproducibility of our measurements of individual glycoproteins (see Table II).

Effects of Amino Acid Analogues and of Oligosaccharides on gpPr90″ Processing—Both β-hydroxynorvaline and β-hydroxyeucine have a profound effect on the processing of gpPr90″ molecules. As shown in Fig. 3, L-[35S]methionine-labeled gpPr90″ synthesized in the absence of these analogues is processed during a nonradioactive chase period to form the normal product glycoprotein gp70 (lanes 4–6). However, components related to gp70 are not formed in the presence of either amino acid analogue (lanes 1–3 and 7–9). In the case of β-hydroxyeucine, which does not inhibit glycosylation, this failure in processing can be ascribed to an alteration in the structure of the polypeptide chain.

Nevertheless, two lines of evidence suggest that extensive loss of oligosaccharides from gpPr90″ can also block its processing. First, as shown in Fig. 4, the unglycosylated gp70 = 70,000 ene gene product synthesized in F-MuLV-infected cells in the presence of tunicamycin (lanes 6–8) does not appear to be processed by partial proteolysis to form a gp70-related fragment. Such a fragment would have an expected Mₐ = 55,000 (29). Secondly, the unglycosylated Mₐ = 70,000 protein synthesized in cells preincubated with tunicamycin is apparently also blocked from transport to the cell surface as indicated by lactoperoxidase-catalyzed iodination using 125I (Fig. 5). Thus, after tunicamycin is added to the medium of the

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Glycoprotein</th>
<th>Electrophoretic component</th>
<th>Apparent M₀</th>
<th>Apparent Mₒ/oligosaccharide</th>
<th>Total apparent Mₒ of all oligosaccharides</th>
<th>No. of asparagine-linked oligosaccharides</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F-MuLV gpPr90″</td>
<td>Control</td>
<td>88,000</td>
<td>2,430</td>
<td>18,000</td>
<td>7.4</td>
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<tr>
<td></td>
<td></td>
<td>−1</td>
<td>85,500</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>−2</td>
<td>83,200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>−3</td>
<td>80,700</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tunicamycin</td>
<td>70,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>F-SFFV gp55</td>
<td>Control</td>
<td>56,000</td>
<td>3,100</td>
<td>11,800</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
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<td>−1</td>
<td>52,900</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Tunicamycin</td>
<td>44,200</td>
<td></td>
<td></td>
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</tbody>
</table>

* Measurements of apparent Mₒ/oligosaccharide differ in different experiments analyzed on distinct gels and also depend upon the labeling time. It is essential to realize that we are not implying that this measured apparent Mₒ contribution of one oligosaccharide is the true Mₒ of the oligosaccharide.

The total apparent Mₒ of all oligosaccharides is the difference between the apparent Mₒ of the control glycoprotein and the apparent Mₒ of the polypeptide chain synthesized in tunicamycin.

<table>
<thead>
<tr>
<th>Glycoprotein</th>
<th>No. of Asn-X-Thr susceptible sites</th>
<th>Total number of asparagine-linked oligosaccharides</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-MuLV gpPr90″</td>
<td>3</td>
<td>7.1, 7.4, 6.7, 6.8</td>
</tr>
<tr>
<td>gpPr90″</td>
<td>0</td>
<td>0.85</td>
</tr>
<tr>
<td>Moloney MuLV gpPr90″</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td>Gxk gpPr90″</td>
<td>2</td>
<td>6.2, 5.9</td>
</tr>
<tr>
<td>GXL gpPr90″</td>
<td>3</td>
<td>7.2, 7.7</td>
</tr>
<tr>
<td>F-SFFV gp55</td>
<td>1</td>
<td>3.8, 3.95</td>
</tr>
</tbody>
</table>

* Independent measurements of the same glycoprotein are all recorded in the order performed. The Gxk″ and GXL gpPr90″ molecules were analyzed in parallel in two independent experiments. The F-SFFV gp55 glycoprotein was analyzed twice, whereas F-MuLV gpPr90″ was analyzed three times. The gpPr90″ samples were analyzed in parallel on the same gel with gpPr90″ calibration controls (see Fig. 2).
Abnormal processing of gPr90 containing the threonine analogue \( \beta \)-hydroxyxynorvaline or the leucine analogue \( \beta \)-hydroxyxyleucine. The samples were from cells labeled with \( \text{L-\text{[\( \text{\textsuperscript{35}} \text{S} \text{]}} \text{methionine}} \) for 15 min (lanes 1, 4, and 7), for 30 min (lanes 2, 5, and 8), or for a 30-min pulse followed by a 90-min chase with unlabeled methionine (lanes 3, 6, and 9). The F12 NRK cells were incubated with the leucine analogue (lanes 1–3), with normal amino acids (lanes 4–6), or with the threonine analogue (lanes 7–9). The labeled cell extracts were precipitated using antiserum to gp70 before dissolving the precipitates in electrophoresis buffer.

Inhibition of Glycosylation by \( \beta \)-Hydroxyxynorvaline—Although glycosylation of proteins at Asn-X-Thr sequences could conceivably be completely blocked by \( \beta \)-hydroxyxynorvaline substitution, several observations suggest that glycosylation at these sites may be only partially inhibited or even unaffected in certain cases. First, it is known that Asn-X-Ser(Thr) sites in glycoproteins frequently lack oligosaccharides or are only partially glycosylated, indicating that structural characteristics in addition to the tripeptide sequence are necessary for glycosylation (32, 33). Second, Hortin and Boime (3) obtained evidence that the extent of glycosylation inhibition at Asn-X-Thr sites might be lower than the extent of analogue substitution. Our results support this idea because nucleic acid sequence studies indicate that the Moloney MuLV \( \text{env} \) gene product contains seven possible glycosylation sites, six with an Asn-X-Thr sequence (29). Although our cultured cells, the quantity of preformed fully glycosylated gp70 on their surfaces begins to decrease, presumably due to shedding and degradation (30). However, there is no corresponding increase in any unglycosylated gp70-related component(s) detectable on the cell surface.

DISCUSSION

Inhibition of Glycosylation by \( \beta \)-Hydroxyxynorvaline—Although glycosylation of proteins at Asn-X-Thr sequences could conceivably be completely blocked by \( \beta \)-hydroxyxynorvaline substitution, several observations suggest that glycosylation at these sites may be only partially inhibited or even unaffected in certain cases. First, it is known that Asn-X-Ser(Thr) sites in glycoproteins frequently lack oligosaccharides or are only partially glycosylated, indicating that structural characteristics in addition to the tripeptide sequence are necessary for glycosylation (32, 33). Second, Hortin and Boime (3) obtained evidence that the extent of glycosylation inhibition at Asn-X-Thr sites might be lower than the extent of analogue substitution. Our results support this idea because nucleic acid sequence studies indicate that the Moloney MuLV \( \text{env} \) gene product contains seven possible glycosylation sites, six with an Asn-X-Thr sequence (29). Although our results suggest that six of these sites are glycosylated intracellularly, only three subglycosylated components are formed when the Moloney MuLV glycoprotein precursor is synthesized in the presence of \( \beta \)-hydroxyxynorvaline (Table II).

Several other methods have recently been developed for counting asparagine-linked oligosaccharides in newly synthesized glycoproteins (19, 34, 35). These methods involve enumerating components resolved by polyacrylamide gel electrophoresis after incubating cells with various quantities of tunicamycin (35), or 2-deoxy-D-glucose (34), or after digestion of immunoprecipitated glycoproteins for varying times with endoglycosidase H (19). Although the latter methods offer the advantage that they enable direct counting of oligosaccharides attached at both Asn-X-Ser and Asn-X-Thr sites, we also believe that our method has several advantages. In our experience, extraneous or confusing minor components are often produced in cells incubated with 2-deoxy-D-glucose or tunicamycin, as is evident in some of the published data on gels obtained using these techniques (e.g. Ref. 34 and 35) (see also Fig. 4). In addition, partially glycosylated derivatives are not always present in significant amounts, even when the complete and fully unglycosylated components are both being produced (e.g. Figs. 2 and 4). Our interpretation is that the concentration of lipid-linked oligosaccharides may not limit glycosylation until they are almost depleted, and that different cells or regions of the endoplasmic reticulum may become depleted at different times after adding these drugs. Furthermore, the endoglycosidase H method requires that the resuspended immunoprecipitated glycoprotein be digested at all oligosaccharide sites with a substantial efficiency, a condition that may sometimes be difficult to achieve or even to ascertain. Although our technique requires extrapolation in order to determine the total number of oligosaccharides, it gives reproducible estimates which agree closely with results obtained by other methods. In addition, it provides at least a minimum estimate of the Asn-X-Thr sites in glycoproteins. For example, our results support the conclusion (19, 28) that the G\textsubscript{X}– antigen-containing gp70 contains one fewer Asn-X-Thr-linked oligosaccharide than the closely related gp70 which lacks this antigen (see Table II). Even glycoproteins such as gPr80 of F-MuLV which may lack Asn-X-Thr-linked oligosaccharides can be analyzed if the labeling and electrophoresis are performed in parallel on another glycoprotein which contains as Asn-X-Thr oligosaccharide (see Table II).
The Processing of Retrovirus-encoded Plasma Membrane Glycoproteins—Our observations suggest that the processing of gPr90enu encoded by F-MuLV can be inhibited by alterations of its structure caused by amino acid analogue substitution (Fig. 3) or by removal of its oligosaccharides (Figs. 4 and 5). Specifically, partial proteolysis of gPr90enu synthesized in the absence of analogues yields gp70 plus p15E, whereas analogue-containing glycoproteins appear to be relatively resistant to partial proteolysis (Fig. 3). Furthermore, the unglycosylated M, = 70,000 env gene product synthesized in F-MuLV-infected cells is the presence of tunicamycin is neither cleaved by partial proteolysis (Fig. 4) nor transported to the cell surface (Fig. 5). Similarly, a variety of recent observations suggest that partial proteolysis of gPr90enu can be inhibited by env gene mutations (20, 30), by env gene recombination that may be involved in the formation of dual tropic MuLVs (37, 38), and by a cellular mutation presumed to alter the microenvironment of the rough endoplasmic reticulum (24). Therefore, partial proteolysis of this glycoprotein seems to be highly dependent upon its native structure.

It has recently been shown that gPr90enu encoded by F-MuLV accumulates in a substantial pool intracellularly and that its partial proteolysis either directly precedes or coincides with its transfer to the Golgi apparatus (30). However, partial proteolysis is not a prerequisite for glycoprotein transport through the latter organelle because glycoproteins encoded by dual tropic MuLVs or by an env gene mutant of MuLV can reach plasma membranes without cleavage (36, 37). In other cases, however, uncleaved env gene products accumulate intracellularly (20, 24, 39) (Figs. 4 and 5). Failure of partial proteolysis, therefore, does not provide unambiguous evidence concerning the intracellular site of env glycoprotein accumulation.

The role of oligosaccharides in glycoprotein structure, function, and intracellular transport is not well understood. For example, recent evidence has indicated that 6-phosphomannose residues in oligosaccharides may serve as "signals" for transport into lysosomes (40-42). However, many plasma membrane and secretory glycoproteins can reach the cell surface in the absence of any oligosaccharides (43-45). Nevertheless, the intracellular transport of glycoproteins depends upon their structural characteristics and can be blocked or inhibited by abnormalities caused by mutation (e.g. Refs. 20 and 39), environmental modification (24), amino acid analogues (Fig. 3), or in some cases by removal of oligosaccharides (Fig. 4) (43-45). Furthermore, different plasma membrane glycoproteins are transported intracellularly at different rates (30). This evidence suggests that structural features of the polypeptide chain are essential for intracellular transport from the rough endoplasmic reticulum to plasma membranes and that the effect of any structural perturbation depends upon the specific stability characteristics of the protein.

Our results suggest that the oligosaccharides of MuLV-encoded gPr90enu glycoproteins may perform several different functions. First, the oligosaccharides may be necessary for the folding of gPr90enu into a configuration which can be cleaved by partial proteolysis and transported to the cell surface (Figs. 4 and 5). Second, our evidence indicates that oligosaccharide addition may play an important role in protecting these glycoproteins from proteolysis in the membranous subcellular organelles. The unglycosylated M, = 70,000 env gene product synthesized in F-MuLV-infected cells in the presence of tunicamycin is removed from the cells with a half-life of approximately 20 min (see Fig. 4). Presumably, this loss must occur by intracellular degradation because this protein is apparently not transported to the cell surface (Fig. 5). On the contrary, the glycosylated gPr90enu derivatives synthesized in the presence of amino acid analogues appear to be relatively stable (Fig. 3). Similarly, the gPr90enu encoded by a temperature-sensitive MuLV mutant is stable at an intracellular site at the nonpermissive temperature (20). In addition, tunicamycin dramatically reduces the intracellular stability of a glycoprotein encoded by the env gene of a MuLV mutant (36). Third, the fact that the Gx2 antigen-containing gp70 contains one fewer oligosaccharide than the closely related gp70 which lacks this antigen (Table II) (19, 28) is consistent with the hypothesis that oligosaccharides may mask the expression of potentially important tumor antigens and allow viral membrane glycoproteins to escape elimination by the host immune system.

REFERENCES

Analysis of MuLV Oligosaccharides