A Single Glycosylation Site Within the Receptor-Binding Domain of the Avian Sarcoma/Leukosis Virus Glycoprotein Is Critical for Receptor Binding

Sue E. Delos, Michael J. Burdick, and Judith M. White

INTRODUCTION

Avian sarcoma/leukosis virus (ASLV), an alpharetrovirus, is a major pathogen in poultry, causing sarcomas and leukemias. As do other enveloped viruses, ASLV enters target cells by a membrane fusion event. Fusion is mediated by the single-surface glycoprotein, Env. Env is initially synthesized as a single polypeptide chain which forms trimers in the ER and is subsequently cleaved in the Golgi into two subunits, SU (gp85), responsible for receptor binding, and TM (gp37), the fusion-mediating subunit (Einfeld and Hunter, 1988). The cleavage event, which places Env in a metastable state, appears to be needed for membrane fusion (Gilbert et al., 1995). By analogy to other fusion proteins harboring two heptad repeat coiled coil domains, the ASLV TM domain appears to follow a common fusion mechanism in which the final fusion active form is thought to involve a six helix bundle. The role of SU in the fusion process is less well understood. Avian retroviral fusion has been studied in the most detail for ASLV, subtype A (ASLV-A). Binding of a single receptor, Tva, to the single viral glycoprotein, EnvA, induces fusion-relevant conformational changes in EnvA that activate it for fusion (Damico et al., 1998; Gilbert et al., 1996; Hernandez et al., 1997). In contrast, many receptor-mediated virus-cell fusion systems require multiple receptors and/or multiple viral glycoproteins. Thus, the ASLV system offers a tractable model for studying the role of SU in receptor-mediated virus-cell fusion.

N-linked glycosylation is important for glycoprotein function. Retroviral glycoproteins are heavily glycosylated, particularly in their SU domains. A primary function of N-linked glycosylation is to ensure proper folding of the glycoprotein (Ellgaard et al., 1999; Helenius and Aebi, 2001). N-linked glycosylation is also important for glycoprotein stability (Fenouillet et al., 1999; Imperiali and O’Connor, 1999). N-linked glycosylation has been exploited by many viral glycoproteins for immune evasion (Bolmstedt et al., 1996; Kinsey et al., 1996; Reed et al., 1997; Reitter et al., 1998; Schonning et al., 1996; Sjolander et al., 1996; Willey et al., 1996). In addition, it can alter protein–protein interactions, either directly or by altering protein structure (see, for example, Rudd et al., 1999).

The ASLV-A glycoprotein, EnvA, contains 11 potential glycosylation sites within the receptor-binding domain. To determine their role in EnvA function, we mutated

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each of these sites singly and in combination and examined the ability of the resulting EnvAs to be expressed, processed, incorporated into virions and to promote infection of target cells expressing the ASLV-A receptor, Tva. We also examined the ability of each mutant EnvA to bind Tva and a monoclonal antibody which inhibits receptor binding. We found two sites which are important for protein folding and one site which is essential for receptor binding.

RESULTS

Predicted N-linked glycosylation sites in the SU domains of ASLV Env

As shown in Fig. 1A, there are 11 predicted N-linked glycosylation sites in the receptor-binding subunit (SU) of our EnvA isolate. These sites are numbered 1 through 11 in Fig. 1A. The majority of these sites are near, but not within, variable regions of the protein thought to be involved in receptor binding. A comparison of ASLV Env SU sequences showed that there is a high degree of conservation of glycosylation sites. Of the 11 sites in SU, all but sites 1 and 5 are conserved among all ASLV Env sequences examined (Fig. 1B). Furthermore, site 5 is missing only in subtype D and is likely compensated for by the adjacent 5′ site not present in EnvA. For those SUs harboring glycosylation sites 5 and 5′, it is unlikely that both are utilized simultaneously due to steric interference among the glycosylation enzymes (Wu et al., 1995). Additional glycosylation sites 5″ and 10″ are present in most SU sequences but missing in EnvA, RAV-0-A 1, and subtype C SUs. Thus, 10 of 14 possible sites are conserved among all ASLV Env sequences of subtypes A–E examined to date.

Generation of glycosylation deletion mutant EnvAs

The recognition sequon for N-linked glycosylation is NXS/T, where X can be any residue except proline (Kasturi et al., 1997; Shakin-Eshleman et al., 1998). It has been observed that deletion of glycosylation by substitution at N is more often deleterious to protein structure than substitution of S/T with A (Lee et al., 1992; Ohgimoto et al., 1998). We therefore mutated the S or T in each of the 11 potential N-linked glycosylation sequons within the SU subunit of EnvA to A. The mutant EnvAs are designated EnvAΔN-g followed by the number(s) of the site(s) deleted.

Functional analysis of single glycosylation deletion mutant EnvAs

We first examined the expression and processing of each mutant EnvA after transient transfection of 293T cells (Delos et al., 2000). As seen in Fig. 2A, most single glycosylation site mutations were well tolerated. The mutant EnvAs were expressed as well as wild-type EnvA and processed into mature forms. However deletion of either glycosylation site 2 or 6 (EnvAΔN-g2 and EnvAΔN-g6, respectively) resulted in protein that was not processed (no gp37 band observed in Fig. 2A, lanes “2” and “6”). In addition, EnvA was poorly processed when glycosylation site 10 was mutated (EnvAΔN-g10) (Fig. 2A, lane “10”).

We next examined the ability of each mutant EnvA to be incorporated into MLV pseudotyped virions. For those mutant EnvAs which were processed normally, the processed form of EnvA (see Fig. 3A) was incorporated into virions. For EnvAΔN-g10, although processing was significantly impaired, a reasonable amount of the pro-
cessed form of EnvAΔN-g10 was incorporated into virions. This phenomenon has been seen previously (Delos et al., 2000) and is likely due to preferential display of processed EnvA at the cell surface, the site of virion assembly. For the unprocessed mutant EnvAs, EnvAΔN-g2 and EnvAΔN-g6, no gp37 was incorporated into virions.

FIG. 2. Expression and processing of glycosylation deletion mutant EnvAs. 293T cells were transfected with 10 μg pCB6-EnvA, induced with 10 mM sodium butyrate 30 h post transfection, and harvested 48 h post transfection. Cells were lysed in lysis buffer [1% NP-40, 20 mM HEPES, pH 7.3, 130 mM NaCl], resolved by SDS–PAGE, transferred to nitrocellulose, and probed with an anti-Ngp37 antibody which recognizes both the TM subunit, gp37, and the uncleaved precursor protein, pr95. The numbers above each lane indicate the glycosylation site(s) which has been mutated in the respective EnvA. WTA, wild-type EnvA; Acl, a cleavage defective mutant EnvA (Gilbert et al., 1995).

FIG. 3. Incorporation of glycosylation deletion mutant EnvAs into MLV pseudotyped virions. ALSV-MLV pseudotyped virus was prepared by the three-plasmid transfection method (Landau and Littman, 1992). 293T cells were transfected with 10 μg each pHIT 60 (MLV gag-pol), pHIT111 (lacZ), and pCB6-EnvA, induced with 10 mM sodium butyrate 30 h posttransfection, and harvested 48 h posttransfection. Virions were concentrated from cell supernatants by pelleting through 20% sucrose, resuspended in sample buffer, resolved by SDS–PAGE, transferred to nitrocellulose, and probed with the anti-Ngp37 antibody. An immunoblotting band, at approximately 70 kDa, is denoted with a filled arrowhead. Numbering is as for Fig. 2.
In some cases (EnvΔN-g2, EnvΔN-g3, EnvΔN-g4, and EnvΔN-g10), unprocessed material was incorporated into virions (Fig. 3A, the pr95 band), a characteristic we also observe for the cleavage defective mutant, Acl. In these instances, unprocessed EnvA is found at the cell surface.

We next examined the ability of each mutant EnvA, in the context of an MLV pseudotyped virion, to infect target cells expressing the receptor for EnvA, Tva. Figure 4 shows the results of this analysis. For mutant EnvAs that were well processed and readily incorporated into virions, approximate wild-type titers were observed. Consistent with their lack of incorporation into virions (Fig. 3), mutants EnvΔN-g2 and EnvΔN-g6 did not support infection. Infectivity for EnvΔN-g10 was reduced by more than 1000-fold. The results of these studies are summarized in Table 1.

### Binding of receptor and a receptor-blocking mAb

To further define the defect in mutant EnvΔN-g10, we examined the ability of mutant EnvAs to associate with their receptor, Tva, using a co-immunoprecipitation assay. For this assay, we employed a soluble fragment of the receptor, s47, which has been shown to associate with a soluble form of EnvA and induce conformational changes that initiate fusion (Damico et al., 1998; Hernández et al., 1997). All single-mutant EnvAs except EnvΔN-g10 were able to bind s47 (Fig. 5). For EnvΔN-g10, little or no co-immunoprecipitation of s47 was observed, even after prolonged exposures (data not shown).

An mAb against the SU subunit of EnvA has recently been prepared which prevents s47 binding to EnvA (C. Ochsenbauer-Jambor, S. E. Delos, J. M. White, and E. Hunter, in press). We analyzed our mutant EnvAs for reactivity with this mAb. We observed a significant defect

![Image](image-url)

**FIG. 4.** Infectivity of glycosylation deletion mutant EnvAs. Aliquots from culture supernatants containing MLV pseudotyped virus, prepared as for Fig. 3, were serially diluted and titered on PG950 cells (NIH3T3 cells stably expressing the ASLV-A receptor, Tva). Forty-eight hours postinfection, cells were fixed, stained for β-galactosidase activity, and blue cells counted. Numbering is as for Fig. 2.

### TABLE 1

Summary of Results for EnvA Glycosylation Deletion Mutants

<table>
<thead>
<tr>
<th>EnvA Processing</th>
<th>Virion incorporation of gp37</th>
<th>Infectivity (% WTA)</th>
<th>Receptor binding</th>
<th>Antibody binding</th>
</tr>
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<tr>
<td>WTA</td>
<td>+++++</td>
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<td>+++</td>
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<td>1</td>
<td>+++</td>
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<td>246</td>
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Note. The results from the immunoblots from respective experiments were qualitatively evaluated for this table: ++++, greater than or near wild-type activity; ++, +, and +/-, decreasing levels of activity; –, no activity observed. N.D., not done.

### In some cases (EnvΔN-g2, EnvΔN-g3, EnvΔN-g4, and EnvΔN-g10), unprocessed material was incorpo-
in mAb binding to EnvAΔN-g10, but not to any of the other mutant EnvAs (Fig. 6). These results have been confirmed by a FACS-based binding assay (C. Ochsenbauer-Jambor, S. E. Delos, J. M. White, and E. Hunter, in press; S. E. Delos, data not shown).

Functional analysis of multiple glycosylation deletion mutant EnvAs

We also prepared selected double and triple glycosylation deletion mutant EnvAs. In choosing which mutant

FIG. 5. Receptor-binding activity of glycosylation deletion mutant EnvAs. Cell lysates expressing mutant EnvAs were prepared as for Fig. 2 and immunoprecipitated using an antibody against the cytoplasmic tail of EnvA. The beads with bound EnvA were then incubated with a biotinylated fragment of the ASLV-A receptor, s47, for 30 min in the cold. The beads were then washed extensively and boiled in sample buffer, and the released proteins resolved by SDS–PAGE, transferred to nitrocellulose, and probed with HRP-conjugated streptavidin to detect the biotinylated s47. + or − denotes whether or not s47 has been added to the given immunoprecipitate. Numbering is as for Fig. 2.

FIG. 6. Binding of a function blocking antibody to the glycosylation deletion mutant EnvAs. Glycosylation deletion mutant EnvAs were expressed and immunoprecipitated as described in the legend to Fig. 5. The beads with bound EnvA were then incubated with a monoclonal antibody against the SU domain of EnvA, which blocks receptor binding (C. Ochsenbauer-Jambor, S. E. Delos, J. M. White, and E. Hunter, unpublished observations), for 30 min in the cold. The beads were then washed and boiled in sample buffer, and the bound proteins resolved by SDS–PAGE and transferred to nitrocellulose and probed with HRP-conjugated anti-mouse antibody. Numbering is as for Fig. 2.
EnvAs to make, we asked whether we could delete, in combination, those glycosylation sites which supported near wild-type EnvA function when deleted singly. For the triple glycosylation deletion mutations, particular interest was placed on those glycosylation sites close to the defined host-range determining sequences, sites 5, 7, 8, and 9 (Fig. 1). As seen in Figs. 2B, 3B, 4, 5B, and 6B, and summarized in Table 1, each of the double glycosylation deletion mutants were well tolerated for all EnvA functions examined. The results were more variable for triple glycosylation deletion mutants. For EnvAΔN-g7,8,9 and EnvAΔN-g7,9,11, processing appeared normal (Fig. 2B), but only EnvAΔN-g7,9,11 was incorporated, as processed EnvA, into virions (Fig. 3B). Therefore, as expected, EnvAΔN-g7,8,9, EnvAΔN-g5,7,9, and EnvAΔN-g5,8,9 were severely impaired in their ability to support infection, but EnvAΔN-g7,9,11 exhibited wild-type levels of infectivity.

Each of the double and triple glycosylation deletion mutant EnvAs were able to bind s47 (Fig. 5B) and the receptor inhibiting mAb (Fig. 6B and data not shown). However, EnvAΔN-g5,7,9 and EnvAΔN-g5,8,9 appeared to exhibit decreased affinity for s47 (Fig. 5B). These results identify one combination of triple N-linked glycosylation deletions (EnvAΔN-g7,9,11), which is well tolerated by EnvA, and others (EnvAΔN-g5,7,9 and EnvAΔN-g5,7,8), which are not.

**DISCUSSION**

To determine the role of N-linked glycosylation in EnvA SU function, we mutated each of the potential N-linked glycosylation sites in the SU domain individually and in selected combinations and examined the ability of the resulting EnvAs to be expressed, processed, and incorporated into virions, and to support infection of target cells expressing the ASLV-A receptor, Tva. The ability of each mutant EnvA to bind Tva and a monoclonal antibody which inhibits receptor binding was also examined. Most single mutations were well tolerated, exhibiting near wild-type function in all of the assays employed. All double mutants exhibited near wild-type activity, whereas most triple deletion mutants were severely impaired. In the course of this analysis, we identified two sites essential for EnvA structure, and, most importantly, a single glycosylation site essential for receptor binding and ASLV-A entry into target cells.

**Relationship with other ASLV EnvS**

The glycosylation pattern in the SU domains of the various ASLV subtypes retains a high degree of conservation (Fig. 1B). Of the 11 potential glycosylation sites present in EnvA SU, all but two sites, 1 and 5, are conserved among all ASLV Env sequences examined, and one of these, 5, is likely compensated for by an adjacent site, 5’, not present in EnvA. Deletion of the other site, 1, had no apparent effect on EnvA function. Three additional glycosylation sites, 5’, 5”, and 10’, are present in some SU domains but not in EnvA. As EnvA is fully functional, these are likely nonessential. In summary, 9 (circled site numbers in Fig. 1B) of 14 possible N-linked glycosylation sites are conserved among all ASLV Env sequences of subtypes A–E examined to date. This conservation of sequence notwithstanding, all but 3 of the 11 sites within the EnvA SU were found to be individually dispensable for the EnvA functions we examined.

An important potential role of N-linked glycosylation of viral glycoproteins is immune evasion. Indeed, much of the mutational variation seen among and within HIV clades under severe immune pressure results in altered glycosylation patterns (Kinsey et al., 1996; McNearney et al., 1992; Meyers et al., 1996; Overbaugh and Rudensey, 1992; Simmonds et al., 1991; Willey et al., 1996). It has been difficult to isolate antibodies against the ASLV Env SU subunits. Perhaps the ASLV Env has evolved a structure which is impervious to immune pressure so that further evolution to escape antibody-induced neutralization is selected against.

**Role of glycosylation in folding**

A primary function of glycosylation is to aid in proper folding of the glycoprotein (Helenius and Aebi, 2001). We found two glycosylation sites, 2 and 6, whose deletion resulted in severely misfolded protein which was not processed (Fig. 2), was poorly expressed at the cell surface (S. E. Delos, unpublished results), and was not incorporated into virions (Fig. 3); hence the resulting virions were noninfectious. The importance of glycosylation at site 6 for EnvA structure may be due to its central location between hr-1 and hr-2, regions important for receptor choice and affinity (Dorner et al., 1985; Holmen et al., 1996; Overbaugh and Rudensey, 1992). Thus, glycosylation at site 6 likely plays a critical role in directing correct folding of the central region of the SU subunit. Why site 2 appears to be equally important is less clear. However, it has been reported that glycoproteins are most sensitive to removal of glycosylation sites near their N-termini, as these sites first engage the ER protein folding machinery to initiate the “correct” folding process (Doms et al., 1985; Hebert et al., 1997; Helenius and Aebi, 2001). Site 2 is the most N-terminal glycosylation site conserved among all ASLV Env (Fig. 1B). Our data do not directly distinguish utilization of individual glycosylation sites, but since EnvAΔN-g1 exhibits wild-type function, site 2 may be the first glycosylation site used. Our data suggest that either the sequences harboring sites 2 and 6, the sugar chains, or both are important for the structure of EnvA. An alternative possibility is that substitution of A for the third position S or T in the glycosylation sequon alters critical structure by, for example, eliminating a critical hydrogen bond.

Another reason sites 2 and 6 are important for folding may be that the N to be modified is immediately followed
by a cysteine. Thus, glycosylation at these sites may be required for presentation of the adjacent cysteine for proper disulfide bond formation. In contrast to sites 2 and 6, site 9, which is also adjacent to a cysteine, appears to be unnecessary for proper folding. In this case, however, the presence of two additional glycosylation sites (7 and 8) within the same cysteine-bound sequence may compensate for the mutation at site 9 in controlling proper orientation of its adjacent cysteine. Glycosylation of at least one of these sites appears to be necessary for Env structure (processed EnvAN\_g7,8,9 was not incorporated into virions and these virions were unable to support infection of target cells), but apparently any two of the three sites can be eliminated (EnvAN\_g7,9 and EnvAN\_g8,9 exhibited near wild-type function). It has been reported that when multiple glycosylation sites are present in close proximity, they are not all used simultaneously and that mutation at one site can be compensated for by utilization of another (Wu et al., 1995).

The effect of triple glycosylation deletions on EnvA structure depended upon the combination of sites mutated. As stated above, mutation of the 7,8,9 triplet (EnvAN\_g7,8,9) was not tolerated. Similarly, combining mutation of site 5 with mutations of either 7 and 9 (EnvAN\_g5,7,8) or 8 and 9 (EnvAN\_g5,8,9) resulted in severely defective EnvA. In contrast, combining mutations at sites 7 and 9 with a mutation at site 11 (EnvAN\_g7,9,11) had no significant effect upon EnvA function. Thus, it is not deletion of three glycosylation sites per se that results in defective EnvA. Rather, in addition to the requirement for at least one glycosylation site of the 7,8,9 trio, there must be some important cross-talk between the sugar backbones of and/or the sequences surrounding sites 5 and the 7,8,9 segment for proper EnvA structure. Apparently, site 11 is not part of this cross-talk network. Since s47 binding is decreased for both EnvAN\_g5,7,9 and EnvAN\_g5,8,9 (Fig. 5), combinations of mutations in sequences flanking the hr-1 and hr-2 regions apparently affect structure at the receptor-binding site as well as more global EnvA structure.

Processing of EnvA into its SU and TM subunits is critical for EnvA function. N-linked glycosylation deletion mutants, which were defective in EnvA function, were not properly processed, and, in general, improperly processed EnvA was poorly incorporated into virions. Similar results have been observed for EnvAs harboring mutations in the fusion peptide sequence within TM (Delos et al., 2000). The exception to this observation is EnvAN\_g7,8,9, which appears, on the basis of the strong gp37 band, to be processed (Fig. 2), yet little, if any, processed EnvA is incorporated into virions (Fig. 3). This may therefore represent the first case of a mutant EnvA which is processed but not incorporated into virions. This result suggests there may be additional, as yet unknown, requirements for Env incorporation into virions.

Deletion of glycosylation sites often results in less stable protein (Luo et al., 1993; Reitter and Desrosiers, 1998; Wu et al., 1995). It is interesting to note that for many of the glycosylation deletion mutant EnvAs, a band at approximately 70 kDa is observed in blots of virion preparations. This band, not present for WTA (Fig. 3), is reminiscent of the TM\_e band observed on immunoblots of EnvA isolated from virions after receptor-induced inactivation (P. Bates, unpublished results; Mothes et al., 2000). Perhaps EnvAs lacking glycosylation at these sites have decreased stability in their native, metastable states and are (partially) inactivated during either virion incorporation or isolation. Nevertheless, for those EnvAs which retained some gp37 in isolated virions, the observed infectivity was near wild-type (Fig. 4), suggesting that the presence of only a small amount of active EnvA is sufficient to mediate infection. A similar result was obtained when the cleavage-negative, infection-defective mutant EnvA, Acl, was coexpressed with EnvA in MLV pseudotype experiments (S. E. Delos, unpublished results).

A specific role for glycosylation site 10

The most striking result from our study is the identification of a single glycosylation site, 10, whose deletion abolished receptor binding. On a linear map, glycosylation site 10 is removed from the host-range determining sequences in contrast to glycosylation sites 5, 7, 8, and 9, each of whose individual mutation was well tolerated (Fig. 1). A similar loss or gain of receptor binding has been observed for HIV upon deletion or addition of specific single glycosylation sites (Dumonceaux et al., 2001; Kolchinsky et al., 2001; Losman et al., 2001; Ly and Stamatatos, 2000; Nakayama et al., 1998; Ogert et al., 2001; Pollakis et al., 2001). In particular, loss of a glycosylation site at the base of the V1/V2 loop rendered the ADA variant HIV virus CD4 independent (Kolchinsky et al., 2001). This was attributed to alteration of the V1/V2 loop orientation in the absence of the glycan, which rendered the CCR5 binding region accessible without prior CD4 binding. Perhaps a similar alteration of loop orientation occurs in EnvAN\_g10, in this case abolishing the receptor-binding site. The simplicity of this mutation may provide the opportunity to understand the structural requirements for receptor binding. As discussed above for the sites 2 and 6 deletion mutants, it is possible that the A substituted for S rather than the glycan itself is important for EnvA structure. It is important to note that while our data identify site 10 as important for structure at the receptor-binding site, we have no evidence of a direct interaction between s47 and either the glycan or the S within the glycosylation sequon. For HIV, comparable glycosylation mutations in different strains do not always have equivalent effects on Env structure and virus infectivity (Ogert et al., 2001). It will be interesting to see if glycosylation site 10 is important for other ASLV SUs to bind their respective receptors or is uniquely important for EnvA.
MATERIALS AND METHODS

Sequence comparison

Sequences for the SU domains of several Env proteins of ASLV subtypes A–E were obtained from GenBank (RAV0-A1, AF257657; Schmidt-Ruppin A, S83510; type C, NC001408; Prague C, V01197; Schmidt-Ruppin B, AF052428; Schmidt-Ruppin D, D10652; and RAV 0-E, M12172). These sequences were aligned using ClustalW option within MacVector (Oxford Molecular Group, Inc.). Sequences are presented from the first residue after cleavage of the ER translocation signal sequence and are numbered starting with this residue.

Reagents for expression and detection of EnvA

MVA, an avian-tropic vaccinia virus encoding T7 polymerase, was a gift from Dr. Bernard Moss (National Institutes of Health). 293T and PG950 cells have been obtained (Gilbert et al., 1995). PG950 cells express the ASLV-A receptor, Tva. Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% supplemented calf serum (Hy-Clone, Ogden, UT) and 500 mg Geneticin per liter, supplemented with 1× glucose, 1× pyruvate, and 1× penicillin/streptomycin (Gibco-BRL). pCB6 plasmids encoding EnvA and the EnvA cleavage-negative mutant, Acl, have been described (Gilbert et al., 1994; Hernandez and White, 1998). Protocols and plasmids for the purification of s47, a functional recombinant fragment of the ASLV-A receptor, were gifts from Dr. D. Peters and Dr. D. Agard, University of California at San Francisco. s47 was biotinylated as previously described (Delos et al., 2000).

Mutagenesis

Mutations to convert the serine or threonine in each N-X-S/T sequon within SU to alanine were introduced into the pCB6/EnvA plasmid using the Quick-Change mutagenesis kit (Stratagene) according to the manufacturers instructions. The resulting EnvA genes were sequenced (DNA Sequencing Core, Biomolecular Research Facility, University of Virginia School of Medicine) to verify the presence of desired mutation and the absence of spurious mutations.

Protein expression, pseudotype virus production, and infectivity

EnvA expression, incorporation into MLV pseudotyped virions, and the infectivity assay were performed as previously described (Delos-pro).

s47 and 8C5.4 binding to EnvA

To assay for s47 binding to EnvA, 100 μl of lysate from cells expressing a given EnvA was precleared with pre-immune serum bound to protein A beads, immunoprecipitated with the anti-A tail antibody, washed twice with lysis buffer, and then incubated for 30 min at 4°C with 1 μl of biotinylated s47. After additional washing, samples were boiled in SDS–PAGE sample buffer, resolved by SDS–PAGE, transferred to nitrocellulose, and probed with horseradish peroxidase conjugated streptavidin. The assay for binding of the monoclonal antibody, 8C5.4, to EnvA was similar except that 1 μl of antibody was substituted for the s47 and the immunoblot was probed with horseradish peroxidase conjugated anti-mouse antibody.

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REFERENCES


GLYCOSYLATION DELETIONS IN THE ASLV-A SU DOMAIN


