A Novel Mechanism for LSECtin Binding to Ebola Virus Surface Glycoprotein through Truncated Glycans∗§

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LSECtin is a member of the C-type lectin family of glycan-binding receptors that is expressed on sinusoidal endothelial cells of the liver and lymph nodes. To compare the sugar and pathogen binding properties of LSECtin with those of related but more extensively characterized receptors, such as DC-SIGN, a soluble fragment of LSECtin consisting of the C-terminal carbohydrate-recognition domain has been expressed in bacteria. A biotin-tagged version of the protein was also generated and complexed with streptavidin to create tetramers. These forms of the carbohydrate-recognition domain were used to probe a glycoprotein ligand. LSECtin binds with high selectivity to glycoprotein ligands. LSECtin binds with high selectivity to glycoproteins terminating in GlcNAcβ1–2Man. The inhibition constant for this disaccharide is 3.5 μM, making it one of the best low molecular weight ligands known for any C-type lectin. As a result of the selective binding of this disaccharide unit, the receptor recognizes glycoproteins with a truncated complex and hybrid N-linked glycans on glycoproteins. Glycan analysis of the surface glycoprotein of Ebola virus reveals the presence of such truncated glycans, explaining the ability of LSECtin to facilitate infection by Ebola virus. High mannose glycans are also present on the viral glycoprotein, which explains why DC-SIGN also binds to this virus. Thus, multiple receptors interact with surface glycoproteins of enveloped viruses that bear different types of relatively poorly processed glycans.

Mammalian glycan binding receptors interact with endogenous glycans on cells and secreted glycoproteins and with sugar structures on the surfaces of pathogenic microorganisms (1, 2). Endocytosis mediated by sugar-specific receptors results in clearance of glycoproteins from circulation, so these receptors can participate in normal turnover of serum glycoproteins and in the scavenging of glycoproteins released as a result of damage to tissues (3, 4). Glycan-binding receptors on cells of the immune system bind to glycans on the outer surfaces of viruses, bacteria, fungi, or parasites and direct uptake of the pathogens, leading to their destruction and facilitating presentation of pathogen fragments to the immune system (5–7). Glycan-binding receptors are particularly common on the surfaces of cells in the immune system, such as macrophages and dendritic cells, and in the liver (8). In both cases the distinct but overlapping roles of the different receptors remain poorly defined.

LSECtin is a recently described member of the C-type family of receptors that is found predominantly on sinusoidal endothelial cells of the liver and lymph nodes, suggesting that it plays a specific role in these cells (9). LSECtin has also been reported to be expressed on peripheral blood and thymic dendritic cells (10). It is most closely related in sequence to the receptors DC-SIGN and DC-SIGNR (also known as L-SIGN) and shares with these receptors the ability to bind mannose, N-acetylglucosamine, and related sugars. Both scavenging and pathogen-recognition functions for LSECtin can be envisioned, but its ligand-binding properties and its interactions with pathogens have not been extensively studied.

DC-SIGN and DC-SIGNR have been implicated in enhanced infection by enveloped viruses. For example, DC-SIGN on dendritic cells has been shown to present human immunodeficiency virus to T lymphocytes, dramatically increasing the efficiency of infection (11, 12). In an analogous manner, DC-SIGN and DC-SIGNR on liver sinusoidal endothelial cells enhance infection of hepatocytes by hepatitis C virus in culture (13). LSECtin shares with these receptors the ability to bind to surface glycoproteins of enveloped viruses. Interaction of LSECtin with the surface glycoproteins of severe acute respiratory syndrome coronavirus and Ebola virus has recently been described, and LSECtin-mediated infection of cells Ebola virus has been demonstrated (14). DC-SIGN, which shows a similar cellular distribution to LSECtin, also binds to Ebola virus, so there is

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4 The abbreviations used are: DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin; DC-SIGNR, DC-SIGN-related; CRD, carbohydrate-recognition domain; BSA, bovine serum albumin; MALDI, matrix-assisted laser desorption; TOF, time of flight.
**LSECtin Binding to Truncated Glycans on Ebola Virus**

potential overlap in their functions (15–17). However, there are also clear differences in the way that these receptors interact with viral glycoproteins, because LSECtin does not interact with lentiviral particles harboring the surface proteins of human immunodeficiency virus or hepatitis C virus, whereas DC-SIGN and DC-SIGNR do (10).

To clarify the differential interactions of LSECtin and the other, better characterized receptors, the binding specificity of LSECtin has been investigated by probing a glycan array and by employing other assays for sugar binding activity. It is demonstrated that the most effective glycan ligands for LSECtin contain the disaccharide sequence GlcNAc1–2Man. Analysis of the Ebola virus surface glycoprotein shows the presence of both this target structure and high mannose oligosaccharides, which would account for the ability of LSECtin as well as DC-SIGN and DC-SIGNR to bind to the viral glycoprotein.

**EXPERIMENTAL PROCEDURES**

**Expression of the Carbohydrate-recognition Domain from LSECtin**—A cDNA for LSECtin was isolated by polymerase chain reaction amplification from a human liver cDNA library from BD Biosciences. Synthetic oligonucleotides from Invitrogen were combined with restriction fragments from the cDNA to insert the region encoding the carbohydrate-recognition domain (CRD) into a modified version of the vector ompA2 (18). The codons for the bacterial ompA signal sequence were followed immediately by the cDNA sequence beginning at residue 160 of LSECtin, which encodes the sequence NSC at the N-terminal end of the CRD. For expression of biotin-tagged protein, the C-terminal end of the cDNA was similarly modified by insertion of synthetic oligonucleotides so that the expression vector codes for a protein in which the C-terminal cysteine residue of LSECtin is followed by the sequence GLN-

**Formation of Streptavidin Complexes**—Streptavidin obtained from Sigma was iodinated by the chloramine T method (22) using Na125I from PerkinElmer Life Sciences. Alexa 488-labeled streptavidin was purchased from the Molecular Probes division of Invitrogen. Approximately 250 μg of biotin-tagged CRD in 6 ml of eluting buffer was adjusted to 25 mM CaCl2 and incubated overnight at 4 °C with 150 μg of labeled or unlabeled streptavidin. The complex was re-applied to a 1-ml column of fucose-Sepharose, which was rinsed with 5 ml of loading buffer and eluted with 0.5-ml aliquots of eluting buffer.

**Generation of Polyclonal Antibody**—A sample of 1 mg of purified CRD from LSECtin without a biotin tag was provided to Eurogentec (Seraing, Belgium) for immunization of rabbits following their standard protocol.

**Expression of LSECtin in Fibroblasts**—The full-length cDNA for LSECtin was inserted in to a retroviral expression vector that was used to generate pseudoviruses that were in turn used to infect Rat-6 fibroblasts following exactly the protocols previously described for other glycan-binding receptors (23, 24). Cells were harvested from two 225-cm² flasks by scraping in phosphate-buffered saline and were pelleted by spinning at 2000 × g for 2 min. The pellet was suspended in 5 ml of eluting buffer containing 0.5 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride, 150 mM aprotinin, 1 mM E-64 protease inhibitor, and 1 mM leupeptin from Calbiochem and sonicated for 5 s at low power. Additions were made to bring the suspension to final concentrations of 1% Triton X-100 and 25 mM CaCl2 followed by a further 5-s sonication and incubation for 15 min on ice. The sample was centrifuged at 150,000 × g for 20 min, and the supernatant was applied to a 1-ml column of fucose-Sepharose, which was rinsed with 4 ml of loading buffer containing 0.1% Triton X-100 and eluted with 0.5-ml aliquots of eluting buffer containing 0.1% Triton X-100. Fractions were precipitated by the addition of half a volume of 30% trichloroacetic acid and incubation for 10 min on ice. Precipitated protein was collected by centrifugation for 5 min at 18,000 × g, and the pellet was washed twice with 0.5 ml of ethanol (1:1) and dried for 5 min under vacuum. The samples were dissolved directly in sample buffer and run on SDS-polyacrylamide gels that were blotted onto nitrocellulose (25). Blots were blocked for 30 min with 5% bovine serum albumin (BSA) in Tris-buffered saline, incubated for 90 min with primary antiserum at 1:500 dilution in the same buffer, washed with Tris-buffered saline, incubated with 20 μg/ml alkaline phosphatase-conjugated protein A from Calbiochem in Tris-buffered saline containing 5% BSA, and finally washed again with Tris-buffered saline before incubation with 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium phosphate substrate from Calbiochem.

**Screening for Glycan Ligands**—The glycan array of the Consortium for Functional Glycomics was screened in buffer containing 150 mM NaCl, 2 mM CaCl2, 2 mM MgCl2, 20 mM Tris-Cl, pH 7.4, 0.05% Tween 20, and 1% BSA. Glycoprotein blotting was performed as previously described (26). For competition binding assays (27) and pH dependence assays (28), polystyrene wells were coated with CRD from LSECtin at a concentration of 50 μg/ml. Oligosaccharides were obtained from Dextra Laboratories (Reading, UK).
Other Analytical Procedures—SDS-polyacrylamide gels were performed by the method of Laemmli (29). Protein concentrations were determined by the method of Bradford (30).

Modeling of the LSECtin Binding Site—The structure of rat mannose-binding protein A in complex with a mannose-containing ligand, Protein Data Bank code 2msb, was modified by changing side chains to their LSECtin equivalents using Insight II software from Accelrys (Cambridge, UK). The GlcNAcβ1–2Man disaccharide was abstracted from chain A in entry 1fc1, and the mannose residue was superimposed on the mannose in the primary binding site of mannose-binding protein. The model figure was created using Molscript (31).

Analysis of LSECtin Binding to Cells Expressing Ebola Glycoprotein—To assess binding of soluble LSECtin to Ebola glycoprotein on the cell surface, 293T cells were transiently transfected with the expression plasmid pcDNA3.1 encoding full-length Ebola glycoprotein (32) or control vector without a cDNA insert. At 48 h post-transfection, the cells were harvested, washed, resuspended in ice-cold phosphate-buffered saline containing 3% fetal calf serum and 0.1% NaN₃, and incubated with the indicated amounts of soluble, tetrameric Alexa 488-labeled LSECtin in the presence or absence of EDTA. Thereafter, the cells were again washed and analyzed using a FACSCalibur flow cytometer (BD Biosciences).

Production of Soluble Ebola Virus Glycoprotein—Soluble Ebola virus glycoprotein was produced by transient transfection of 293T cells with plasmid pAB61-ZEROV-GP-Fc, which encodes the GP1 subunit of Ebola glycoprotein fused to the Fc portion of human IgG, as described previously (13). The supernatant of the transfected cells was collected and passed through a HiTrap protein A column from GE Healthcare. Bound protein was eluted with 0.1 M glycine, pH 3.0, mixed with 1 M Tris-HCl, pH 9.0, and stored at −80 °C. The purity of the eluted samples was analyzed by SDS-gel electrophoresis and Coomassie Blue staining.

Solid Phase Assay of LSECtin Binding to Ebola Glycoprotein—Streptavidin-coated wells from Perbio Sciences (Tattenhall, Cheshire, UK) were washed with loading buffer and incubated overnight with 100 μg/ml biotin-tagged CRD in loading buffer containing 0.1% BSA. Wells were washed twice with loading buffer and incubated for 6 h at room temperature with Ebola glycoprotein-Fc fusion protein at various dilutions. After three washes with loading buffer, wells were incubated for 30 min at room temperature with 0.1 μg/ml alkaline phosphatase-conjugated protein A in loading buffer containing 0.1% BSA. After five further washes with loading buffer, wells were incubated for 15 min at room temperature with 100 μl of a 1 mg/ml solution of p-nitrophenylphosphate in 0.1 M glycine, pH 10.4, containing 1 mM MgCl₂ and 1 mM ZnCl₂. The reaction was stopped by addition of 50 μl of 1.5 N NaOH, and the absorbance at 405 nm was read on a Wallac 1420 plate reader.

Analysis of Glycans on Ebola Glycoprotein—Approximately 100 μg of Ebola glycoprotein-Fc fusion protein was precipitated with trichloroacetic acid as described above. The pellet was rehydrated in 50 mM ammonium bicarbonate, pH 8.4, and digested at 37 °C overnight by the addition of tosylphenyl chloromethyl ketone-trypsin at 1 mg/ml. N- and O-linked glycans were released and purified as described previously (33).Permethyl ketone-trypsin at 1 mg/ml. Diges
ing the wash. This behavior, reflecting relatively weak binding, is characteristic of other C-type CRDs expressed in isolation (23). Similar results were obtained using a fucose-Sepharose affinity column (Fig. 2), consistent with the previous demonstration that fucose binds to LSECtin with nearly the same affinity as mannose (9). The need for an extended column with very high densities of monosaccharide ligands to retain LSECtin probably explains why binding to mannose-containing columns was not detected in some recent experiments (10).

To enhance the affinity of the protein for binding studies, a multivalent form of the CRD was created by appending a biotinylation sequence at the C-terminal end of the CRD. Expression of the CRD in bacteria also expressing the birA gene, which encodes biotin ligase, results in efficient biotinylation of the single lysine residue in the C-terminal extension (20). Complexes containing four CRDs can then be formed by allowing the tagged protein to interact with streptavidin. This approach has successfully been used to enhance the affinity of other C-type CRDs (34). The biotinylated protein was purified in the same way as the untagged CRD. After incubation with streptavidin, the complex was efficiently repurified on a smaller affinity column because of the enhanced affinity resulting from tetramerization (Fig. 2). The biotinylated monomeric and tetrameric forms of the protein were used in subsequent studies of the interaction of LSECtin with target ligands.

Previous studies have suggested that the extracellular domain of LSECtin assembles into forms that are larger than monomers, but a unique oligomeric structure has not been defined. The sequence of the neck region conforms very closely to the heptad repeat pattern of aliphatic hydrophobic amino acid side chains expected for an amphipathic α-helix that would be able to form coiled-coil structures (Fig. 1) (35). This domain also contains three cysteine residues, two of which are positioned at the hydrophobic positions that would be expected to face inwards in such a coiled coil. Although previous studies have suggested that the receptor does exist as disulfide-linked oligomers, these experiments were not performed on material selected for activity, and thus, the results may have been confounded by the presence of misfolded biosynthetic intermediates with heterogeneous disulfide-bond arrangements (9). To examine the possibility that the cysteine residues in the neck link polypeptides in the native receptor, the intact protein was expressed in rat fibroblasts, and functional receptor was purified by affinity chromatography. Based on the results with the monomeric and tetrameric forms of the CRDs, it was expected that an oligomeric receptor would bind efficiently to fucose-Sepharose, and blotting with an antibody to the CRD revealed that the protein is retained on the column and can be eluted with EDTA (Fig. 3). This active material was run on gels in the presence and absence of reducing agent, revealing that it exists as a disulfide-linked dimer. Thus, binding results obtained with the streptavidin-complexed CRD can be taken as an indication

FIGURE 2. SDS-polyacrylamide gel electrophoresis of fractions from the affinity purification of biotin-tagged CRD from LSECtin expressed in bacteria. A and B, elution of 10-mL columns of mannose- and fucose-Sepharose with buffer containing EDTA. Fractions of 2 mL were collected. C, the complex of biotin-tagged CRD with streptavidin was repurified by affinity chromatography on a 1-mL column. Elution fractions of 0.5 mL were collected. In all cases aliquots of 20 μL were run on 17.5% gels that were stained with Coomassie Blue. The expected molecular mass of the expressed CRD is 15,000 Da.
of the type of affinity enhancement that would be expected for the native oligomeric protein.

**Binding of LSECtin to Truncated Complex Glycans**—The binding specificity of LSECtin was initially investigated using the glycan array developed by the Consortium for Functional Glycomics (36). Fluorescently labeled streptavidin-CRD complex was created by incubating the biotin-tagged CRD with streptavidin that was prelabeled with Alex-488. The purified complex was then used to probe the array at multiple concentrations (Fig. 4). At low concentrations highly selective binding to a limited set of glycans was obtained. The common feature of the glycans with the highest signals is the presence of one or more terminal GlcNAc\(\beta1\rightarrow2\)Man sequences. However, some binding was also observed for a bi-antennary complex glycan in which both branches bear galactose residues linked \(\beta1\rightarrow4\) to the terminal GlcNAc residue and to a core trimannose structure. At higher concentrations of labeled probe, binding to an additional set of glycans with terminal \(\alpha1\rightarrow2\)-linked fucose residues was observed. There is some specificity to the interaction, because binding to glycans containing \(\alpha1\rightarrow3\) and \(\alpha1\rightarrow4\)-linked fucose was not detected. Glycans with fucose linked \(\alpha1\rightarrow6\) to the core are not present on the array. Although these results suggest that the receptor can bind to a second type of glycan, the binding occurs only at high concentrations of CRD presented in the form of a tetramer, indicating that the interaction would be weak. These results demonstrate the value of probing the glycan array at multiple concentrations of CRD and emphasize that preferential binding to potentially physiologically relevant ligands is revealed at lower concentrations of the labeled probe, whereas high concentrations may result in binding to low affinity ligands.

The glycan array results were confirmed using a competition assay to compare the binding of oligosaccharides to immobilized CRD from LSECtin. In this assay, which employs \(^{125}\)I-labeled Man-BSA as a reporter ligand at concentrations well below the \(K_D\) the observed inhibition constants provide a good guide to the relative affinities of competing low molecular weight ligands. As predicted from the array results, the disaccharide GlcNAc\(\beta1\rightarrow2\)Man is the highest affinity ligand (Fig. 5). The branched trimannose oligosaccharide, which forms the core of the complex glycans and, thus, is also common to many of the ligands identified on the glycan array, binds only 2-fold better than mannose, reflecting the presence of two terminal mannose residues. It is unclear why this trisaccharide, when linked to a chitobiose core, is a ligand in the glycan arrays screened at low receptor concentrations. The presence of two GlcNAc\(\beta1\rightarrow2\)Man branches on a single glycan also does not enhance the affinity per terminal residue. The Fuca1\(\rightarrow2\)Gal disaccharide shows only very slightly enhanced affinity compared with a simple monosaccharide, consistent with the conclusion...
that the affinity for this type of ligand is much lower than for the preferred GlcNAcβ1–2Man disaccharide as suggested by the glycan array results.

The specificity of the binding of LSECtin is unusual for C-type CRDs. Many receptors that contain C-type CRDs bind to broad categories of glycans that share simple features, such as terminal galactose or mannose residues, although a few receptors, such as the selectins and the scavenger receptor C-type lectin, bind much more restricted sets of glycans. In the latter cases, the best ligands typically contain branched terminal structures, such as the sialylated or unsialylated versions of the Lewisx and Lewisa trisaccharides, and binding is mediated by primary interactions with one terminal sugar residue and secondary interactions with the other terminal sugar residue. In contrast, LSECtin binds a simple linear disaccharide with micromolar affinity.

The affinity for GlcNAcβ1–2Man is also unusually high for such a simple ligand binding to a C-type CRD (37), so it is interesting to consider how this affinity may arise. The binding characteristics of multiple C-type CRDs have been studied in molecular detail, and a common feature of binding is coordination of vicinal 3- and 4-hydroxyl groups to a conserved Ca2⁺ in the primary binding site (5, 37). Based on this feature and the fact that free mannose and GlcNAc have similar affinities for LSECtin (9), there are two ways in which LSECtin might interact with the GlcNAcβ1–2Man ligand, in which the 3- and 4-hydroxyl groups of either the GlcNAc or the mannose residue are coordinated to Ca2⁺ the primary binding site. The fact that ligands in which the GlcNAc residue is substituted with α-4-linked galactose can still bind, albeit with reduced affinity, strongly suggests that binding is through the mannose residue because the presence of galactose would mean that there would be no free 4-hydroxyl groups on the substituted GlcNAc residues available for coordination. With mannose in the primary binding site, the enhanced affinity could result from secondary contacts with the GlcNAc residue.

To explore the possible secondary interactions of a disaccharide ligand with LSECtin, some of the features of the binding site of LSECtin were modeled. Among C-type CRDs for which structures in complex with sugar ligands have been determined, the CRD of LSECtin is most closely related to the CRD of DC-SIGN in overall sequence. However, in the area around the Ca2⁺ and sugar binding site, the sequence is more like serum mannose-binding protein, so this latter protein was used as a basis for the modeling studies (Fig. 6). When the disaccharide is modeled into the binding site with mannose ligated to Ca2⁺, potential interactions of the GlcNAc residue (orange) with backbone and side chain atoms are highlighted with green shading.
mal, plant, and viral sugar binding proteins (37, 38). In other C-type CRDs, interactions of aromatic residues with an N-acetyl substituent at the 2 position on a sugar ligated in the primary binding site through hydroxyl groups at the 3 and 4 positions accounts for increased selectivity of this site (39, 40), whereas in LSECtin the interaction would form a component of the secondary binding site. When the GlcNAc residue was placed in the primary binding site, the mannose residue did not appear to make secondary interactions that would account for the enhanced affinity of the disaccharide.

Taken together, the results from the glycan array, competition, and modeling studies are consistent with identification of terminal GlcNAcβ1–2Man disaccharides as the preferred ligands for LSECtin. The selective binding of LSECtin to such truncated glycans was further confirmed by using biotinylated CRD from LSECtin complexed with 125I-labeled streptavidin to probe blots of glycoproteins with different glycan structures (Fig. 7). The results confirm that glycoproteins with exposed terminal GlcNAcβ1–2Man disaccharides are the best ligands. Examples include partially degraded glycoproteins, such as asialo-orosomucoid, and glycoproteins that naturally bear significant amounts of truncated hybrid structures, such as chicken ovalbumin. Weaker binding to asialo-orosomucoid is consistent with the observed weaker binding to the galactose-terminated glycans. Thus, these studies identify glycoproteins with truncated complex N-linked glycans as potential target ligands for LSECtin. Such ligands could arise either from incomplete processing in the biosynthetic pathway or through limited degradation by exoglycosidases.

Effect of pH on Binding of LSECtin to Glycan Ligands—There is evidence that LSECtin can participate in the uptake of ligands, such as viruses (14). Other receptors on liver sinusoidal endothelial cells, including the mannose receptor, mediate clearance of glycoproteins, particularly those released during tissue damage (4). Although GlcNAcβ1–4Man is not a common terminal sequence on secreted glycoproteins, glycoproteins with truncated glycans might be released from an intracellular compartment following cell lysis, and rapid clearance of GlcNAc-terminated glycoproteins from human circulation has been described, although the site of clearance was not identified (41). In the case of related receptors containing C-type CRDs, such as DC-SIGN, the ability to mediate degradation of ligands is correlated with their ability to release ligands at endosomal pH (42). With the availability of the purified ligand binding domain of the receptor, it was possible to test the effect of pH on ligand binding using the solid phase binding assay (Fig. 8). Surprisingly, binding of the 125I-labeled Man-BSA reporter ligand increases rather than decreases below neutral pH. In this respect it much more closely resembles DC-SIGNR than DC-SIGN (42). It is interesting that both LSECtin and DC-SIGNR are expressed in endothelial cells, and it is possible that they participate in specialized forms of endocytosis in these cells rather than the general clathrin-mediated uptake and recycling through acid endosomal compartments.

LSECtin Can Bind to Ebola Virus Glycoprotein Bearing Unusual Terminal Glycans—Based on the finding that the binding specificity of LSECtin is very different to the specificity of DC-SIGN and DC-SIGNR, it was of interest to examine the molecular details of the interaction of LSECtin with target glycoproteins on Ebola virus. There is a single surface glycoprotein on this virus, which is the product of an edited mRNA (43). Ebola glycoprotein is synthesized as a type 1 transmembrane protein of ∼150 kDa that bears both N- and O-linked glycans and forms trimers on the surfaces of infected cells and virions (44). It is cleaved into two disulfide-linked polypeptides, GP1 and GP2, by proteases in the secretory pathway.

The fluorescently labeled complex of streptavidin with the CRD from LSECtin was used to probe cells transfected with Ebola glycoprotein from the Zaire strain of the virus. Fluorescence-activated cell sorting at varying concentrations of labeled receptor revealed concentration-dependent binding (Fig. 9A). Controls in which either non-transfected cells were probed or transfected cells were probed in the presence of EDTA confirmed that the binding is specific (Fig. 9B). Further direct evi-
Ebola glycoprotein, the amino acid sequence of the protein core suggests that the GP1 portion would be modified with up to 15 N-linked glycans and possibly as many as 80 O-linked glycans (46). Release of N- and O-linked glycans from the Ebola glycoprotein-Fc fusion protein followed by mass spectrometry confirmed the presence of both types of glycans. Masses of many of the N-linked glycans suggest that they would be expected to have exposed terminal GlcNAc residues.

The masses of the O-linked glycans released from Ebola glycoprotein are consistent with the presence of typical mucin-type core 1 and core 2 structures (Galβ1–3GalNAc1–Ser/Thr and Galβ1–3(GlcNAcβ1–6)GalNAc1–Ser/Thr), but only a small proportion contain sialic acid or fucose residues or have N-acetyllactosamine extensions (data not shown). None of the O-linked oligosaccharides that were detected correspond to high affinity ligands for LSECtin, indicating that the primary interaction between Ebola glycoprotein and LSECtin is likely to be through GlcNAc-terminated N-linked glycans.

The N-glycan analysis also revealed the presence of high mannose oligosaccharides, including Man₉ and Man₉ structures. Man₉ and Man₉ oligosaccharides are bound with high affinity by DC-SIGN and DC-SIGNR (42), so the glycan analysis is consistent with the ability of Ebola glycoprotein to interact with these receptors as well as LSECtin (14, 16). Analysis of glycans on the spike glycoprotein of severe acute respiratory syndrome coronavirus revealed that, as in the case of Ebola virus, both high mannose glycans and complex glycans with terminal GlcNAc residues are present (47). This analysis, combined with the demonstration of LSECtin specificity presented here explains the ability of the severe acute respiratory syndrome spike protein to interact with LSECtin as well as DC-SIGN and DC-SIGNR (14, 48, 49).
The presence of glycans with 8 to 9 mannose residues is a common feature of surface glycoproteins of enveloped viruses (17, 50, 51). These glycans represent relatively unprocessed forms of N-linked oligosaccharides, because they have not been subjected to the trimming reactions that are required to generate smaller oligomannose and complex glycans. The data presented here indicate that a substantial proportion of the N-linked glycans of Ebola virus have been processed to hybrid and complex forms, but fully processed N-linked glycans with terminal sialic acid residue are relatively uncommon. Thus, although they have reached a later stage in the processing pathway, these glycans are still truncated. In a similar way, only a small proportion of the O-linked glycans released from Ebola glycoprotein bear sialic acid residues. Therefore, the O-linked glycans also show reduced processing at the terminal stages of biosynthesis. Limited processing might reflect poor accessibility of glycans to processing glycosidases in the endoplasmic reticulum and Golgi apparatus, due perhaps to their dense packing on the glycoproteins, or it might be caused by the high level of viral glycoprotein biosynthesis in infected cells, which could result in overloading of the processing machinery. Alternatively, the replication of virus might result in diminished expression of the galactosyltransferases and sialyltransferases that perform the terminal glycosylation steps in glycoprotein biosynthesis or the trafficking of the virus may bypass compartments in which terminal glycosylation normally occurs. In any case, the presence of various types of incompletely processed glycans may facilitate the interaction of enveloped viruses with multiple cell surface receptors.

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CONCLUSIONS

LSECtin is encoded in the same gene cluster as DC-SIGN and DC-SIGNR, and the CRDs of these three receptors are closely related in overall sequences and share the common features of mannose and GlcNAc binding receptors. However, the data presented here demonstrate that they bind very distinct sets of glycan ligands. Thus, although LSECtin and DC-SIGNR are both expressed in sinusoidal endothelial cells, they would be expected to bind different sets of physiological and pathological ligands. The specificity data combined with the analysis of glycans on the Ebola virus glycoprotein explain the fact that human immunodeficiency virus and hepatitis C virus bind exclusively to DC-SIGN and DC-SIGNR through high mannose oligosaccharides, whereas Ebola virus and severe acute respiratory syndrome coronavirus bind to both these receptors and to LSECtin because of the presence of both high mannose and truncated complex glycans. The results emphasize that the presence of various types of incompletely matured glycans may facilitate the interaction of enveloped viruses with multiple cell surface receptors.

FIGURE 10. Mass spectrometry of glycans from Ebola glycoprotein. Glycans released with protein N-glycanase were permethylated and subjected to MALDI-TOF mass spectrometry. Structures of labeled peaks, except the high mannose oligosaccharides, were further characterized by MALDI-TOF-TOF tandem mass spectrometry. The symbols are defined in Fig. 4.
**LSECtin Binding to Truncated Glycans on Ebola Virus**

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