Proposed three-dimensional model for the attachment protein G of respiratory syncytial virus

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Protein G of respiratory syncytial virus (RSV) is an envelope glycoprotein that is structurally very different from its counterparts (haemagglutinin-neuraminidase and haemagglutinin) in other paramyxoviruses. In this study, we put forward a model for this unique viral envelope protein. We propose that protein G of RSV contains several independently folding regions, with the ectodomain consisting of a conserved central hydrophobic region located between two polymeric mucin-like regions. The central conserved region is probably the only relatively fixed and folded part of the ectodomain of RSV-G. This central conserved region contains four conserved cysteine residues which can form two disulphide bridges. Analysis of the proteolytic digestion products of a peptide corresponding to the central conserved region showed that one of the three theoretically possible combinations of disulphide connections could be eliminated. The final disulphide bridge assignment was established by affinity measurements with peptide variants in which different disulphide connections were formed. Additionally, peptide binding studies were used to map the binding site, at the amino acid level, of a monoclonal antibody directed against the central conserved region. These studies indicated the level of surface exposure of the amino acid side-chains. The surface exposure agreed with the structural model. The proteolytic digestion, the peptide binding studies and the affinity measurements with structural peptide variants support a structural model with disulphide connections that correspond to a structural motif called a cystine noose. This model provides a structural explanation for the location and molecular details of important antigenic sites.

Introduction

Infections with human respiratory syncytial virus (HRSV) and bovine (B)RSV are a major cause of respiratory tract disease in humans and cattle respectively. These viruses are classified within the genus Pneumovirus of the family Paramyxoviridae. The pneumoviruses contain two envelope glycoproteins, the fusion protein (F) and the attachment protein (G). The pneumoviruses are classified as a separate genus because of differences in the diameter of the nucleocapsid (Kingsbury et al., 1978) and differences in viral RNA and protein structure (Collins, 1991). In particular, the attachment protein G is unique for RSV because it shares neither sequence nor structural homology with attachment proteins of other paramyxoviruses or morbilliviruses (haemagglutinin-neuraminidase and haemagglutinin, respectively) (Satake et al., 1985; Wertz et al., 1985). The only resemblance to G proteins of other pneumoviruses is the presence of regions with a high Ser and Thr content. RSV-G is highly variable between HRSV subgroups (53% amino acid identity) (Johnson et al., 1987), and between HRSV and bovine RSV (30% amino acid identity) (Lerch et al., 1990). In contrast to the attachment proteins of other paramyxoviruses, RSV-G is shorter and lacks haemagglutination and neuraminidase activity. RSV-G exists as an anchored type II membrane protein and as a smaller soluble form that is secreted into the medium (Hendricks et al., 1987; Roberts et al., 1994).

RSV-G may be at least a dimer in the viral membrane (Lambert, 1988; Collins & Mottet, 1992), but the exact number of monomers in the homo-oligomer is not known. HRSV-G contains about 60% carbohydrate by weight: approximately 20% is N-linked carbohydrate and 80% is O-linked carbohydrate, attached via the unusually high number of hydroxy-amino acids in the protein (Lambert, 1988; Wertz et al., 1989). Johnson et al. (1987) suggested that RSV-G was homologous to

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mucins because of the high Ser and Thr content of protein G. The C-terminal part of HRSH-G is an important antigenic region (Rueda et al., 1991; Garcia-Barreno et al., 1990; Cane & Pringle, 1995) and according to peptide binding studies and analysis of escape mutants, a conserved peptide in the central conserved region (residues 174–188) is immunodominant (Norrby et al., 1987; Åkerlind-Stopner et al., 1990; Rueda et al., 1994). This same peptide induced protection against HRSH infection in mice and an antibody directed against this peptide conferred passive protection against challenge (Trudel et al., 1991).

In this study, we propose a modular structure for RSH-G. The central conserved region is predicted to be the main globular protein module which is probably involved in protein–protein interaction. We present a three-dimensional (3D) model of the central conserved region of RSH-G based on proteolytic digestion analysis, on detailed knowledge of the antigenic surface and on affinity measurements with two peptide variants with different disulphide links. The structural model may guide functional studies.

Methods

Peptide synthesis. Three peptides were synthesized corresponding to the central conserved region of BRSV-G, strain 391-2 (Lerch et al., 1990).


* An analogue with acetamidomethyl groups attached to Cys\(^{172}\) and Cys\(^{182}\).

* An analogue with acetamidomethyl groups attached to Cys\(^{172}\) and Cys\(^{186}\).

Synthesis was performed according to standard procedures on an Applied Biosystems 430A synthesizer using Fastmoc chemistry (Fields et al., 1991). A peptide with both disulphide bridges was obtained as follows. \(\beta\)-Mercaptoethanol reduced, deprotected peptide was slowly oxidized by dialysing for 3 days against 1\% NH\(_4\)HCO\(_3\), which was renewed frequently. To generate a peptide in which the Cys\(^{172}\)-Cys\(^{182}\), Cys\(^{178}\)-Cys\(^{186}\) [1–3, 2–4] disulphide bridges were deliberately created, the peptide analogue with acetamidomethyl groups attached to Cys\(^{173}\) and Cys\(^{182}\) was oxidized in two steps. First, the disulphide bridge between Cys\(^{176}\) and Cys\(^{186}\) was formed by stirring the peptide for 2 days in 1\% NH\(_4\)HCO\(_3\), until it was completely oxidized. Next, the acetamidomethyl groups were cleaved from the two remaining non-bonded Cys residues by stirring at room temperature with a tenfold molar excess of I\(_2\) in slightly acidic conditions. Under these oxidizing conditions, the second disulphide bridge is formed instantaneously (Maeder et al., 1992). After 25 min, the excess I\(_2\) was titrated with Na\(_2\)S\(_2\)O\(_3\) (158 mg/ml). A peptide with the [1–4, 2–3] disulphide bridges was made similarly, with the exception that the first disulphide bridge (2–3) was completely formed after 18 h. A peptide variant was produced in which all four free –SH groups were blocked by the addition of a fivefold molar excess of 2-iodoacetamide to the reduced peptide. Peptides were purified by preparative HPLC on a Waters Delapack C18 reversed-phase column with an acetonitrile-water gradient. The peptide was further purified by FPLC on a Pharmacia Mono-Q HR 5/5 anion-exchange column with a NaCl gradient.

Pepscreen analysis. Peptides were synthesized on functionalized polyethylene rods and tested for their reactivity with polyclonal antisera and a monoclonal antibody (MAb) in an ELISA according to established procedures (Geysen et al., 1984). To test whether antibody binding was dependent on the presence of a cystine bridge, pepscreen analysis was performed with both oxidized and reduced peptides. Peptides on the rods were oxidized by incubation in 1\% NH\(_4\)HCO\(_3\) for 2 days and exposure to air for 1 day. Wash buffer, precoat buffer and diluted test serum were saturated with air. Pepscreen under reducing conditions was performed as follows. Peptides on the rods were reduced in a 70 °C water-bath containing \(\beta\)-mercaptoethanol (Geysen et al., 1984). Rods were washed with N\(_2\)-saturated buffer. Precoating buffer and diluted test serum for this test were saturated with N\(_2\). The antibody incubation was performed under an N\(_2\) atmosphere.

The following peptides were synthesized. All 186 overlapping dodecapeptides of the ectodomain of BRSV-G, strain 391-2, and a set of 240 analogues for the dodecapeptide STCEGNLACLSL in which each amino acid was consecutively replaced by the other 19 naturally occurring amino acids.

ELISA. Antibody reactivity was measured in an indirect ELISA (Westenbrink et al., 1985) using microtitre plates coated with the peptides described above ('Peptide synthesis') (150 ng/well). Peptides were coated in 0.05 \(\mu\)l carbonate buffer, pH 9.6, at 4 °C overnight. To obtain an estimate of the relative affinities of several peptides with MAB 20, the half-maximal binding values were determined. Ascites fluid of MAB 20 (diluted 1:40000) was incubated overnight in an uncoated microtitre plate at 20 °C with serial dilutions of BRSV-G peptide. Subsequently, the solution was transferred to a microtitre plate coated with peptide, and the unbound antibodies were measured using the indirect ELISA as described (Frieret et al., 1985).

Sera and monoclonal antibody. Bovine serum samples were obtained from cattle from several farms with a history of BRSV infection. MAB 20 was produced as described previously (Wensvoort et al., 1986). BALB/c mice were immunized intraperitoneally with 100 \(\mu\)g of purified BRSV (Lelystad strain) mixed with Freund's complete adjuvant. The specificity of MAB 20 was determined using an immunoperoxidase monolayer assay (IPMA) as described (Wensvoort et al., 1986). For IPMA, Vero cells infected with BRSV (strain Lelystad) were used.

Proteolytic digestion. Fifty \(\mu\)g of oxidized BRSV-G 32-residue synthetic peptide (see 'Peptide synthesis') was incubated with 1 U of endopeptidase Glu-C (EC 3.4.21.19) type XVII-B (Sigma) for 2 h. Additionally, oxidized digested peptide and reduced (excess of dithiothreitol) digested peptide were analysed using analytical HPLC and amino acid analysis for identification of possible digestion products.

Results

Global structure

Detailed analysis of the primary structure of RSH-G allowed a dissection of the protein into several modules. Hydroxy-amino acids like Ser and Thr are clustered together with Pro in two discrete regions of the protein. These regions, enriched in Ser, Thr and Pro, are common
motifs that are heavily O-glycosylated and probably adopt a stiff and extended conformation (Jentoft, 1990). Because such regions are the major constituent of mucins, the regions are called mucin-like. By definition, the amino acid sequences in mucin-like regions comprise 25–40% of Ser or Thr residues (Jentoft, 1990). The number and distribution of hydroxy-amino acids in RSV-G suggest that the first mucin-like region in RSV-G (approximately 37% Ser, Thr) extends from the transmembrane region to the conserved double Pro motif at positions 155 and 156, and the second mucin-like region (approximately 38% Ser, Thr) extends from the conserved Pro194 to the C terminus. Based on this analysis, the protein can be dissected into a cytoplasmic region, a transmembrane region, an elongated mucin-like region, a central conserved hydrophobic globular region and a second elongated mucin-like region (Fig. 1).

Disulphide bridge assignment by proteolytic digestion

Four completely conserved Cys residues are present in the central conserved region of all natural isolates of RSV-G. Theoretically, four Cys residues [1, 2, 3, 4] may be connected via disulphide bridges in three different ways. By digesting the synthetic BRSV-G peptide with endoproteinase Glu-C, which cleaves the C-terminal peptide bond of Ghu177 (between Cys 2 and Cys 3), it is possible to distinguish between connection [1–2, 3–4] and connections [1–4, 2–3] or [1–3, 2–4]. When the products of the endoproteinase Glu-C digested synthetic peptide were analysed by HPLC analysis (Fig. 2a), the chromatogram showed only one peak at 38 min. An identical chromatogram was obtained for the undigested synthetic peptide (data not shown). No fragments were observed

Fig. 1. Schematic representation of the primary structure of RSV-G. ●, Cys residue; hatched box, transmembrane region (TM); shaded box, positively charged region. The arrows indicate mucin-like regions that are enriched in Ser, Thr and Pro (37–38% Ser and Thr content). The general variability in G (Cane et al., 1991; Mallipeddi & Samal, 1993; Sullender et al., 1991) is indicated at the bottom of the figure.

Fig. 2. HPLC analysis of endoproteinase Glu-C digested BRSV-G peptide in the oxidized (a) and reduced (b) form. Amino acid analysis was used for the identification of the peaks. The peptide fragments (thick lines) are shown schematically, with possible cystine connections.
Affinity measurements with structural variants

A peptide was synthesized corresponding to the central conserved region of BRSV-G in which the [1-3, 2-4] disulphide bridges were formed, and a peptide was synthesized in which the [1-4, 2-3] disulphide bridges were formed (see Methods). To obtain an estimate of the relative affinities of both HPLC-purified peptides with MAb 20, the half-maximal binding values were determined. The peptide with [1-4, 2-3] disulphide bridges had a relative affinity of 0.7 nM and the peptide with the [1-3, 2-4] disulphide bridges had a relative affinity of 33 nM (Fig. 3). A peptide corresponding to the central conserved region of BRSV-G, in which the native folding of the synthetic peptide guided the disulphide bridge formation by slow dialysis (see Methods) had an affinity similar to the peptide with the [1-4, 2-3] disulphide bridges.

Fig. 3. Binding of MAb 20 to the peptide with [1-4, 2-3] disulphide bridges (■), the peptide with [1-3, 2-4] disulphide bridges (○) and the peptide with blocked cysteine residues (★) (see Methods).

in Fig 2(a) because the oxidized fragments were still bound by disulphide bridges. The digestion was successful because the fragments corresponding to the N-terminal part and the C-terminal part were observed only after reduction of the digested peptides (Fig. 2(b)). These fragments were similar to the fragments that resulted from digestion of the reduced peptide (data not shown). Therefore, only two disulphide connections remain possible: the [1-3, 2-4] and the [1-4, 2-3] disulphide connections. Further disulphide bridge assignment is not possible with proteolytic digestion.

Fig. 4. Pepscan results obtained with overlapping peptides of the ectodomain of BRSV-G, strain 391-2 (Lerch). (a) MAb 20 (dilution 1:100000). (b) Field sera of naturally infected cows. Cow 1, dilution 1:200; cow 2, 1:150; cow 3, 1:550; cow 4, 1:250. Numbers on the horizontal axes correspond to the N-terminal amino acid of the 12-mer peptide. Absorbance values at 405 nm obtained with each peptide in an ELISA are plotted vertically.
Analysis of structural details of the protein surface by pepscan analysis

Because fine details of the antigenic site can be used to check the validity of a structural model, the reactivities of an anti-BRSV-G MAb (MAb 20) and representative polyclonal sera were tested against all overlapping dodecapeptides of the ectodomain of BRSV-G. The results (Fig. 4a) showed that MAb 20 bound three successive overlapping peptides that shared the amino acid sequence CEGNLACLSL. The highest reactivity was directed against the peptide with amino acid sequence STCEGNLACLSL. Furthermore, all polyclonal bovine sera also bound exclusively to the same peptides within the central conserved region [amino acid sequence (ST)CEGNLACL(SL)] (Fig. 4b). This region corresponds to the C-terminal half of the central conserved region.

The contribution of each amino acid side-chain of the epitope to antibody binding was determined by testing the reactivity of MAb 20 to peptide analogues in which each amino acid position of the epitope was replaced by all 19 naturally occurring amino acids (Fig. 5). If substitution of an amino acid by most other amino acids results in loss of antibody binding, that specific amino acid is important for antibody binding. The results showed that seven out of twelve amino acids are important for binding and are most likely to have high surface accessibility: Cys\textsuperscript{176}–Cys\textsuperscript{182} and to a lesser extent Ser\textsuperscript{184}. Remarkably, unusual substitution of methionine for Thr\textsuperscript{175}, Glu\textsuperscript{177}, Gly\textsuperscript{178}, Ala\textsuperscript{181} and Leu\textsuperscript{183} was favoured. All these substitutions cluster directly C- and N-terminally of Cys\textsuperscript{176} and Cys\textsuperscript{182}. Therefore, it is likely that MAb 20 interacts directly with sulphur atom(s) of a disulphide bridge between Cys\textsuperscript{176} and Cys\textsuperscript{182}.

To check whether a disulphide bridge between Cys\textsuperscript{176} and Cys\textsuperscript{182} is important for antibody binding, pepscan analysis was performed with the reduced peptides under reducing conditions. The results showed that a disulphide bridge was not important for antibody binding because reactivity with the oxidized and reduced peptides was similar (data not shown). However, a peptide with all four –SH groups blocked with 2-iodoacetamide had a 23-fold lower affinity with MAb 20 than the native oxidized peptide (Fig. 3).

Homology search

Disulphide bridges between Cys\textsuperscript{173}–Cys\textsuperscript{188} and Cys\textsuperscript{176}–Cys\textsuperscript{182} ([1–4, 2–3]) may induce a disulphide-constrained surface-exposed loop (Fig. 6). These loops, called cystine
nooses, have recently been identified as a new structural motif (Lapthorn et al., 1995). A search in the Brookhaven structural database (release 71) yielded seven unrelated proteins that contained cystine nooses with five residues between cysteines (Table 1). There is no obvious sequence similarity between the sequences in Table 1 and any of the RSV sequences in the proposed cystine noose. Several of the seven independent loop structures in Table 1 show the same fold despite the lack of sequence similarity. Therefore, despite the lack of sequence similarity between the sequences in Table 1 and any of the RSV sequences, structural homology with one of the listed structures may be possible.

Discussion

In this study, we put forward a 3D model for RSV-G, based on a dissection of the protein into several modules. We propose that the ectodomain of protein G contains a hydrophobic globular region bounded by two hydrophilic, polymeric mucin-like regions. Because the mucin-like regions may form linear stalks (Jentoft, 1990), the structural integrity of the whole region may remain unaltered despite its high genetic variability, as long as the Ser, Thr and Pro content remains high enough to support the rigidity of the stalk. The structural function of the mucin-like regions may be to elevate the central conserved region to a particular height, and the physical nature of the mucin-like regions may facilitate transport of the virus through the mucous barrier in the airways. The nature of the mucin-like region implies that further structural analysis is not possible with current technologies. Therefore, the stalk model may prove helpful.

Because of the dense carbohydrate coat, the mucin-like regions are probably not very antigenic and not involved in high-affinity protein-protein interactions. However, the hydrophobic protein module located between two mucin-like regions may be very antigenic. This is supported by the results of the pepscan analysis. The important antigenic site of BRSV-G that we mapped with pepscan analysis (residues 174–185) corresponds to the C-terminal half of the central conserved region. Furthermore, this region is also an important antigenic site for HRSV-G, subtype A (residues 174–188) (Norrby et al., 1987; Àkerlund-Stopner et al., 1990). In pepscan, epitopes could have been missed, especially in the mucin-like regions, because synthetic peptides do not contain the carbohydrate side-chains that may be essential for epitope integrity.

The global structure of RSV-G suggests that further structural analysis of RSV-G can be limited to the

Table 1. Cystine nooses with five residues between cysteines

<table>
<thead>
<tr>
<th>Protein</th>
<th>Residue no.</th>
<th>Sequence*</th>
<th>PDB-code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine RSV G-protein</td>
<td>176–182</td>
<td>CEGNLAC</td>
<td></td>
</tr>
<tr>
<td>Bovine RSV G-protein</td>
<td>176–182</td>
<td>CEGNPAC</td>
<td></td>
</tr>
<tr>
<td>Oxive RSV G-protein</td>
<td>176–182</td>
<td>CEGDSAC</td>
<td></td>
</tr>
<tr>
<td>Human RSV-A G-protein</td>
<td>176–182</td>
<td>CGNNQLC</td>
<td></td>
</tr>
<tr>
<td>Human RSV-B G-protein</td>
<td>176–182</td>
<td>CSWNGTC</td>
<td></td>
</tr>
<tr>
<td>Bovine neurophysin li complex</td>
<td>28–34</td>
<td>CGDEEGLGC</td>
<td>1BN21†</td>
</tr>
<tr>
<td>Lipase</td>
<td>4–10</td>
<td>CYERLGC</td>
<td>1HPLA†</td>
</tr>
<tr>
<td>Human neutrophil elastase</td>
<td>152–158</td>
<td>CRPOVNC</td>
<td>1HNNE†</td>
</tr>
<tr>
<td>Thaumatin I</td>
<td>71–77</td>
<td>CGGQLRC</td>
<td>1THI;</td>
</tr>
<tr>
<td>Fasciculin</td>
<td>53–59</td>
<td>CTSPDKC</td>
<td>1FAS;</td>
</tr>
<tr>
<td>GCSF (Rbg-Csf)</td>
<td>37–43</td>
<td>CAAHLC</td>
<td>1BGCE</td>
</tr>
<tr>
<td>GCSF (Rcg-Cshii)</td>
<td>37–43</td>
<td>CASHLQC</td>
<td>1BGFE</td>
</tr>
<tr>
<td>Aspartic proteinase</td>
<td>51–57</td>
<td>CTKSEGC</td>
<td>1ASI</td>
</tr>
</tbody>
</table>

* Amino acids in bold type are identical with an amino acid in RSV-G.
† These loops fold in a similar hairpin loop structure (0.73–0.95 Å RMSD between Cα).
‡ These loops fold in a similar omega loop structure (1.44 Å RMSD between Cα).
central conserved region, which is probably the only part with a relatively fixed and compact structure.

Analysis of the proteolytic digestion products of the 32-residue synthetic peptide corresponding to the central conserved region of BRSV-G eliminated one of the three theoretically possible disulphide connections ([1-2, 3-4]). Evidence for the [1-4, 2-3] disulphide connection was obtained from affinity measurements with peptide variants with different disulphide bridge connections. The 32-residue peptide in which the [1-4, 2-3] disulphide bridges were formed and the synthetic peptide in which the disulphide bridges were allowed to form spontaneously had a much higher affinity with MAb 20 than the 32-residue peptide in which the [1-3, 2-4] disulphide bridges were formed. Therefore, it is likely that the central conserved region in the native protein adopts [1-4, 2-3] disulphide bridges corresponding to a cystine noose.

The epitope of MAb 20 is contained exactly within the inner disulphide bridge, which agrees with a disulphide-constrained loop. Strikingly, the immunodominant site of human immunodeficiency virus type 1 (HIV-1) has also been mapped to seven amino acids contained in a cystine noose (including both cysteines) in the envelope glycoprotein gp41 (Gnann et al., 1987). The number of amino acid side-chains in the epitope of MAb 20 that are important for binding is very high compared with other epitopes elucidated by the same technique (Meloen et al., 1995; Langedijk et al., 1991). By definition, the disulphide bridge in a cystine noose motif connects the strands of a loop inducing high surface accessibility for the residues contained in the loop (Lapthorn et al., 1995), which agrees with the surface accessibility of amino acids 176-182 according to pepscan analysis. Cys176 is more essential for antibody binding than any other residue in the epitope. Additionally, methionine is a preferable substitution C- and N-terminally of Cys 176 and Cys182, suggesting that the antibody may bind sulphur atoms in a disulphide bridge between Cys176 and Cys182. Many of the cystine nooses listed in Table 1 have exposed disulphide bridges.

Because reactivity of the antibody with the oxidized and reduced dodecapeptides was similar, a covalent bond between Cys176 and Cys182 was obviously not essential for antibody binding to the dodecapeptide in pepscan. However, when the –SH groups of the reduced peptide were blocked with 2-iodoacetamide, the reactivity of the antibody was significantly lowered. This is probably due to steric hindrance caused by the acetamide groups. Additionally, pepscan analysis with the systematically substituted peptides also showed that a covalent bond between both Cys residues was not essential. According to pepscan data (Fig. 5), the antibody could bind peptides in which Cys182 was substituted by Asp or Glu. Probably, binding of MAb 20 is based on an induced-fit of residues in the epitope. The high turn propensity of some residues between Cys176 and Cys182 would enable such an induced fit.

Table 1 shows that it is difficult to predict structural similarity between the RSV-G cystine noose and another noose of known 3D structure based on sequence identity. However, despite sequence differences, a limited set of canonical structures may be possible in five residue cystine nooses. Sequence identity would suggest that the cystine noose in thaumatin is the best model structure, although, the side-chain exposure in the cystine noose of bovine neurophysin corresponds better with the side-chain exposure according to pepscan analysis. Detailed structure determination is necessary to see whether the structure of BRSV-G matches one of the model structures.

The structural consequence of the hairpin structure of the cystine noose may be the anti-parallel alignment of both polymeric mucin-like regions. From the transmembrane region, the polypeptide chain corresponding to the first mucin-like region may form a stalk that lifts the cystine noose high above the membrane, and from the noose the second mucin-like region may run antiparallel to the first stalk, back to the viral membrane (Fig. 6). The anti-parallel alignment agrees with the possible compensatory amino acid mutations found in the first and second mucin-like regions (Cane & Pringle, 1995). Consequently, the second mucin-like region is structurally less important, and if the first mucin-like region is at the centre of the oligomeric G complex (Fig. 6), the second, exposed mucin-like region may be an antigenic site. This agrees with the antigenic importance of the second mucin-like region (Rueda et al., 1991; Garcia-Barreno et al., 1990). This global model may explain the dramatic genetic changes in the second mucin-like region of RSV-G in antibody escape mutants. In vitro studies showed that viruses could be selected in which G was truncated by between 1 and 42 amino acids at the C-terminal end (Rueda et al., 1991), or in which the last 81 amino acids were changed due to frameshift mutations (Garcia-Barreno et al., 1990). Such mutations would have little structural effect and the elevation of the cystine noose would be unaffected. The highly schematic structure of RSV-G should be regarded as approximate. In particular, the mucin-like regions are oversimplified. Ideal mucin chains have an average length of 2.5 Å per residue. Therefore, the theoretical length of the first mucin-like region with the central conserved region would far exceed the length of distal tips of other viral spikes (reviewed in Lamb, 1993). Therefore, some coils may be expected in the mucin-like region.

In conclusion, the global 3D model of RSV-G emphasizes the prominence of the central conserved region. This region has an important role in the infection
mechanism (Trudel et al., 1992). The C-terminal part of the central conserved region forms a cystine noose. Because the residues between the disulphide bridge in a cystine noose display high surface accessibility, and the domain may be the distal tip of the protein, these residues may define the binding specificity of RSV-G to a receptor. An immunodominant cystine noose has also been suggested to play an important role in the activity of the HIV-1 envelope glycoprotein gp41 (Oldstone et al., 1991).

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References


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