Role of individual cysteine residues in the processing and antigenicity of the measles virus haemagglutinin protein

Aizhong Hu* and Erling Norrby

Microbiology and Tumorbiology Centre, Karolinska Institute, 171 77 Stockholm, Sweden

The haemagglutinin (H) protein is the dominant envelope glycoprotein of measles virus. The protein contains 13 cysteine residues among its 617 amino acids and all are located in its ectodomain. In previous studies, the capacity of a panel of monoclonal antibodies (MAbs) to react with continuous and discontinuous epitopes was defined. It was shown that the absence of disulphide bonds impaired the capacity of the protein to react with MAbs specific for the discontinuous epitopes. In the present study, our objective was to determine the contribution of individual cysteine residues to the folding of H protein into its native conformation. Site-directed oligonucleotide mutagenesis was used to create 13 mutants, each with a serine replacing a cysteine. The mutated genes were directly expressed in the BHK-21 cells by use of a vaccinia virus-driven T7 polymerase system. Investigations of the antigenic structure and intracellular processing properties of the mutant proteins reveal the following outcome. (i) Replacements of cysteine residues 139, 154, 188, 386, 570 or 606 had no detectable effect on the antigenic structure and intracellular processing of the H protein. However, a mutant with a replaced cysteine residue 154 displayed modified migration properties. (ii) Alterations of cysteine residues 381 or 494 displayed a moderate effect on H protein properties. The two mutants expressed discontinuous epitopes, indicating that they were partially folded, but they did not oligomerize, did not reach the medial Golgi complex and failed to be transported to the cell surface. (iii) Substitutions of cysteine residues 287, 300, 394, 579 or 583 resulted in a complete loss of binding of the MAbs that recognize the discontinuous epitopes, with no effect on the binding of a MAb reacting with a continuous epitope. No dimeric form of the proteins was observed and only high mannose oligosaccharides were demonstrated in these mutants, suggesting that the modified proteins did not oligomerize and were retained in the endoplasmic reticulum. In conclusion, cysteine residues 287, 300, 381, 394, 494, 579 and 583 appear to play a particularly critical role in the antigenic structure and processing of the H molecules and they probably participate in the inter- or intramolecular disulphide bonding.

Introduction

The haemagglutinin (H) protein of measles virus (MV) is a structural component of the virion envelope that stimulates high titres of neutralizing antibodies and plays an important role in the initial stage of viral infection. An acute MV infection is followed by life-long immunity in which the presence of efficient neutralizing antibodies against the H protein is critical (Norrby & Oxman, 1990). Furthermore, animals immunized with vaccinia virus expressing the H protein are protected from a lethal challenge with intracerebral inoculation of MV (Drillien et al., 1988). The nucleotide sequence of the H gene has been determined (Alkhatib & Briedis, 1986; Gerald et al., 1986). The predicted M, of the H protein is about 69K. The protein contains five predicted sites for N-linked glycosylation, four of which are utilized (Hu et al., 1994b). Thirteen cysteine residues exist in the H protein and most of them appear in clustered positions. These clusters include cysteines 1 to 3 (residues 139, 154 and 188), cysteines 4 and 5 (residues 287 and 300), cysteines 6 to 8 (residues 381, 386 and 394), cysteines 10 to 13 (residues 570, 579, 583 and 606). These 13 cysteines are strictly conserved both in number and position among the MV isolates. A complete conservation of the same cysteine residues of the H protein of rinderpest virus has also been observed (Tsukiyama et al., 1987). In the attachment proteins of canine distemper virus (Curran et al., 1991; Kövamees et al., 1991a) and phocid distemper virus (Curran et al., 1992; Kövamees et al., 1991b), which have only 30 to 40% amino acid identity with MV, all cysteines except the one corresponding to MV H residue 583 are conserved. This suggests that cysteine residues are of critical importance to the structure and function of the H protein.

Cysteine is highly conserved, indicating the critical
role played by this amino acid and the unique bond it can form. It is well established that disulphide bonds are essential for the stability of certain proteins and are critical for their various biological activities (Giese et al., 1987; Mark et al., 1984; Matsumura et al., 1989; Pace et al., 1988; Wang et al., 1984). The reactivities of a continuous and several discontinuous antigenic epitopes with monoclonal antibodies (MAbs) depend on specific amino acids at widely separated regions of the MV H protein (Sheshberadaran & Norrby, 1986; Hu et al., 1993). Folding and oligomerization are a prerequisite for the formation of conformational antigenic epitopes, and MAbs specific for the conformational epitopes reacted exclusively with the disulphide-linked dimeric form of the protein (Hu et al., 1994a). These observations prompted the study reported here.

H protein mutants were prepared by site-directed mutagenesis so that each of the 13 cysteines was replaced by a serine. The role of the individual cysteine residues in H protein oligomerization, intracellular processing, cell surface expression and immunoreactivity with different MAbs was examined after transient expression of the mutants in BHK-21 cells.

Methods

Cells and virus. BHK-21 cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% inactivated fetal calf serum (FCS). Propagation and purification of recombinant vaccinia virus expressing T7 RNA polymerase (vTF7-3) grown in BHK-21 cells (Fuerst et al., 1986) were carried out essentially as described previously (Mackett et al., 1985).

Oligonucleotide-directed mutagenesis. Plasmid pEH1 containing the full-length cDNA encoding the MV H protein has been described previously (Eschle, 1988; Hu et al., 1994b). Thirteen 25-mer oligonucleotides with the desired mismatch nucleotide in the middle of the sequences were synthesized. Single-stranded DNA was prepared as described by Sambrook et al. (1989). Oligonucleotide-directed site-specific mutagenesis was performed according to the instructions of the kit supplier (Boehringer). The mutagenesis was carried out so that each cysteine codon in the original sequence was individually substituted by a serine codon (Table 1). Confirmation of successful mutagenesis was obtained by dideoxynucleotide chain termination sequencing of alkali-denatured plasmid DNA according to the protocol of the reagent supplier (Pharmacia). A total of 13 cysteine mutants were generated. They were designated Cys1 to Cys13 from the amino terminus to the carboxy terminus, corresponding to the cysteine residues at H protein residues 139, 154, 188, 287, 300, 381, 386, 394, 494, 570, 579, 583 and 606, respectively (Alkhatib & Briedis, 1986).

Transfection. The technique has been described previously (Hu et al., 1994b). Briefly, BHK-21 cells were passaged the day before transfection and grown to about 70% confluence in MEM supplemented with 10% FCS in 25 cm² tissue culture plates. The medium was removed and the cells were washed with serum-free MEM three times. The cells were then infected with the recombinant vaccinia virus vTF7-3 at an m.o.i. of about 15 p.f.u./cell and incubated at 37°C for 45 min. The virus inoculum was then removed and replaced with 1 ml of fresh Opti-MEM (Gibco BRL). The cells were routinely transfected with 4 µg (exceptions are indicated) of plasmid using 15 µg lipofectin as instructed by the supplier (Gibco BRL).

Metabolic labelling and pulse-chase analysis. At 5 h post-transfection, the medium was replaced with medium containing 5% of the normal content of methionine and incubated at 37°C for 30 min. The cells were pulse-labelled with 100 µCi/ml of [35S]methionine for 30 min and the medium was then removed. The cells were washed with cold PBS three times to remove the unincorporated radio-labelled methionine and then overlaid with chase medium for 3 h. The labeled cells were harvested in 1 ml radioimmunoprecipitation assay (RIPA) buffer (2% Triton X-100, 0.15 M-NaCl, 0.6 M-KCl, 0.5 M-MgCl2, 5 mM-EDTA, 1 mM-phenylmethylsulphonyl fluoride, 1% aprotinin and 0.01 M-Tris-HCl pH 7.8). Nuclei and cell debris were removed by centrifugation at 15000 g in a microfuge and the supernatants were kept for immunoprecipitation.

Immunoprecipitation. Immunoprecipitation was carried out essentially as described by Sheshberadaran et al. (1983). Briefly, 100 µl of cell lysates was mixed with 2 µl MAb and the final reaction volume was adjusted to 500 µl using RIPA buffer. The mixture of antigen and antibody was incubated at 4°C overnight. Protein A-Sepharose CL-4B beads (Pharmacia) were subsequently added to the mixture, which was then incubated for 1 h at 4°C with frequent vortexing. Immunoprecipitates were washed three times with RIPA washing buffer (RIPA buffer without protease inhibitors) and once with 0.15 M-NaCl and 0.01 M-Tris-HCl pH 8.0. The purified immune complexes were dried, mixed with protein sample buffer [3% SDS, 3% 2-mercaptoethanol (2-ME), 10% glycerol and 0.1% EDTA], boiled for 3 min and finally fractionated by SDS-PAGE. For the analysis of non-reduced proteins, 2-ME was omitted from the sample buffer. The gels were analysed by autoradiography.

Drug treatment. The drug endoglycosidase H (Endo H; Boehringer Mannheim) was employed under conditions described previously (Hu et al., 1993).

Indirect immunofluorescence. The technique was modified from Norrby et al. (1982). BHK-21 cells were grown to 70% confluence on microscope coverslips before transfection. At 5 h post-transfection, the cells were washed with PBS and fixed with 3% paraformaldehyde in PBS for 30 min. After washing three times with PBS, the cells were permeabilized with 0.1% Triton X-100 in PBS for 30 min. Following three washes with PBS, cells were treated with primary antibody at room temperature for 30 rain. Cells were then rinsed with PBS and
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incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin (IgG) (Cappel Laboratories) for 30 min. Cells were washed again with PBS before mounting of the coverslips on glass slides for photography.

Results

Construction and expression of mutated H cDNA encoding proteins with cysteines substituted by serines

The 13 mutated forms of the MV H gene were designated Cys1 to Cys13 (Table 1). The proteins produced by these mutated genes were named HC1 to HC13, respectively. Recently we reported that two forms of H protein resulting from heterogeneous glycosylation at different glycosylation sites were expressed in BHK-21 cells (Hu et al., 1994b). Preliminary pulse-chase experiments of the cysteine mutants revealed that some were unstable and could not be detected by the end of the period of incubation with chase medium. In order to identify all the mutant proteins, the transfected cells were continuously labelled with [35S]methionine for 3 h. The cell lysates were prepared and immunoprecipitated with MAb I-29, which reacts with a continuous epitope on the H protein (Hu et al., 1994a). The polypeptides were separated by SDS-PAGE under reducing conditions. Two categories of expressed proteins were observed. The mutants HC1, HC2, HC3, HC7, HC10 and HC13 essentially displayed a phenotype similar to the wild-type H protein, in which two proteins of 77K and 79K were identified (Fig. 1a). Mutants HC4, HC5, HC6, HC8, HC9, HC11 and HC12 exhibited only the 77K protein. These mutant proteins were different from the wild-type H and had defects in oligomerization and intracellular processing (described below). The mutant Cys1 had low expression efficiency in three independent mutagenesis experiments and subsequent expression analyses. The protein expression level could be increased by increasing the amount of plasmid (6 µg instead of 4 µg) used for transfection as in the experiment shown in Fig. 1(a). This might be due to the instability of the mRNA and/or the protein as a consequence of the nucleotide alteration and amino acid change. A slight mobility difference was observed in mutant HC2 compared with the wild-type (Fig. 1a) and this effect was pronounced when the protein was analysed under non-reducing conditions (see below).

Effect of cysteine-to-serine substitutions on the oligomerization of the H protein

To analyse whether the substitution of cysteines in the H protein affects its folding and oligomerization, the polypeptides were analysed by SDS-PAGE under non-reducing conditions. The results obtained from the entire panel of mutants are shown in Fig. 1(b), and the corresponding reducing gel is shown in Fig. 1(a). Small amounts of the monomeric form of the protein and some aggregated materials on the top of the gel were observed with the mutants as well as the wild-type H protein. Mutants HC1, HC3, HC7, HC10 and HC13 displayed a diffuse band at a position corresponding to around 160K, which is characteristic of the dimeric form of the H protein. The mutants HC4, HC5, HC6, HC8, HC9, HC11 and HC12 exhibited no obvious dimeric form of the H protein. Mutants HC6, HC8 and HC9 showed two faint bands and they probably represented complexes between the misfolded H protein and cellular protein(s). Alter-
Fig. 2. Processing of the wild-type and mutant H proteins. BHK-21 cells infected with vaccinia virus vTF7-3 at 15 p.f.u./cell for 45 min were then transfected with plasmids using lipofectin (Gibco BRL). At 5 h post-transfection, the transfected cells were metabolically labelled with [35S]methionine for 30 min, followed by incubation in chase medium for 3 h. Cell lysates were prepared and immunoprecipitated with MAb I-29. The immune complexes were digested with Endo H and the polypeptides were analysed by SDS-PAGE under reducing conditions. No protein was observed when mutant Cys1 was used for transfection, owing to the low level of protein expression. The wild-type (HWT) and mutant H proteins used are indicated. He and Hs are used to indicate the Endo H-resistant and Endo H-sensitive forms of the H protein, respectively.

Alternatively, they could also represent the dimeric form of the H protein, but the quantity was significantly less than that of the wild-type protein. The mutant HC2 showed an altered migration pattern and this mobility shift was reproducible. The migration difference may be related to the structural changes associated with alterations in disulphide bonding or in other kind(s) of post-translational modification(s) due to the substitution of the cysteine residue. This analysis demonstrates that mutants HC1, HC2, HC3, HC7, HC10 and HC13 are folding- and oligomerization-competent, whereas the rest of the mutants are not.

Effect of cysteine-to-serine substitutions on the processing of the H protein in transiently transfected BHK-21 cells

We recently showed that BHK-21 cells transfected with the wild-type H gene expressed the H protein, which contained N-linked oligosaccharides primarily of the complex type (Hu et al., 1994b). This suggested that much of the protein had been processed in the medial Golgi complex, an indication that a glycoprotein is efficiently transported to the cell surface. To determine the effect of individual cysteine-to-serine substitutions on the processing of the H molecules, the transfected cells were labelled with [35S]methionine for 30 min, followed by incubation in chase medium for 3 h, to allow processing and maturation of the H molecules. The H proteins were immunoprecipitated with MAb I-29, digested with Endo H and analysed by SDS-PAGE. The results are shown in Fig. 2. Mutants HC2, HC3, HC7, HC10 and HC13 displayed Endo H-resistant protein bands, comparable (although weaker) to that of the wild-type H protein. This indicated that these mutations had only slight effects on oligosaccharide processing of the H molecule. The mutants HC4, HC5, HC6, HC8, HC9, HC11 and HC12 exhibited a protein band (clearly seen with longer exposure of the film) with slightly faster...
Table 2. MAb reactivity, oligomerization, Endo H sensitivity and cell surface expression of wild-type and mutant H proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>16-DE6</th>
<th>I-44</th>
<th>I-41</th>
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<th>Endo H sensitivity§</th>
<th>Cell surface expression¶</th>
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* Data presented in this table are from three independent experiments.
† For MAb reactivity, the transfected cells were labelled with [35S]methionine for 3 h. Cell lysates were prepared, immunoprecipitated with different MAbs and polypeptides were analysed by SDS-PAGE under reducing conditions. The data are presented as the amount of the protein immunoprecipitated by MAbs relative to that precipitated by MAb 1-29: +, more than 80%; −, less than 10%.
‡ For oligomerization, the transfected cells were labelled with [35S]methionine for 3 h. Cell lysates were prepared and immunoprecipitated with MAb 1-29. The polypeptides were analysed by SDS-PAGE under non-reducing conditions. Whether or not the dimeric form of the H protein could be visualized is indicated.
§ To determine the sensitivity of mutant H protein oligosaccharide chains to digestion with Endo H, the transfected cells were metabolically labelled with [35S]methionine for 30 min and then incubated in chase medium for 3 h. Cell lysates were prepared and immunoprecipitated with MAb 1-29. The immunoprecipitated H proteins were digested with Endo H and polypeptides were analysed by SDS-PAGE under reducing conditions. S, Oligosaccharide chains were sensitive to Endo H digestion; R, oligosaccharides were resistant to Endo H digestion; ND, not determined owing to the low quantity of protein.
¶ Cell surface expression was analysed by indirect immunofluorescence with MAb 1-29.

mobility than the Endo H-resistant protein band of the wild-type. This protein might be a cellular protein, since it was observed when the H mutant without any glycosylation (Hu et al., 1994b) was analysed comparatively (data not shown). These results suggest that the alterations of cysteine residues in mutants HC4, HC5, HC6, HC8, HC9, HC11 and HC12 caused a pronounced change in the extent of carbohydrate processing. Alternatively, the absence of the processed form of the glycoprotein could be due to enhanced degradation. The reactivity of the mutant H1 to Endo H could not be determined owing to the failure of pulse-chase analysis on the protein.

Subcellular localization of the cysteine mutant H proteins

The subcellular localization of the cysteine mutant proteins was examined by indirect immunofluorescence. Immunostaining was carried out on fixed intact or fixed and permeabilized, transfected BHK-21 cells by use of MAb 1-29 and of FITC-conjugated goat anti-mouse IgG. The staining pattern can be basically classified into two categories. One group, comprising the wild-type H protein, HC2, HC3, HC7, HC10 and HC13, displayed internal staining throughout the cytoplasmic reticulum as well as in the juxtanuclear region (Fig. 3a), and cell surface staining was detectable (Fig. 3b). The other group exhibited no surface staining (Fig. 3d) and the internal staining pattern was limited to a reticular perinuclear structure (Fig. 3c), indicating the accumulation of the protein in the endoplasmic reticulum (ER). The mutants HC1, HC6 and HC9 displayed stronger staining than the rest of the mutants in the group, possibly due to their partial folding capacity in the ER. The results of the indirect immunofluorescence analysis for the entire panel of mutants are summarized in Table 2.

Effect of cysteine-to-serine substitutions on the discontinuous epitopes of the H protein

To examine the antigenic profile of each mutant, cell lysates were prepared, immunoprecipitated with MAbs
Fig. 4. Immunoreactivity of the single cysteine-to-serine mutant proteins with a panel of MAbs. BHK-21 cells were infected with vaccinia virus vTF7-3 at 15 p.f.u./cell for 45 min and then transfected with plasmids using lipofectin (Gibco BRL). The transfected cells were metabolically labelled, at 5 h post-transfection, with [35S]methionine for 3 h. Equal amounts of cell lysates of labelled proteins were immunoprecipitated with different MAbs as illustrated and the polypeptides were analysed by SDS-PAGE under reducing conditions. The mutant proteins and their Mr values are indicated.

recognizing one continuous and three discontinuous epitopes (Hu et al., 1994a) and the polypeptides were separated by SDS-PAGE under reducing conditions. Three categories of immunoreactivity between mutants and MAbs reacting with the discontinuous epitopes were identified (Fig. 4). The first was the mutants that retained full reactivity with the MAbs, including HC1, HC2, HC3, HC7, HC10 and HC13. These mutants showed equal reactivity with a MAb recognizing a continuous epitope and MAbs reacting with the discontinuous epitopes, indicating that they were conformationally competent. The second category comprised the mutants HC6 and HC9, which retained complete reactivity with different MAbs, but only the 77K protein was detected. These two mutants seemed to be partially folded and were capable of expressing the discontinuous epitopes. However, they were defective in oligomerization and intracellular processing (Fig. 1b and Fig. 2). The third category included the mutants that lacked immunoreactivity with the MAbs recognizing discontinuous epitopes, such as HC4, HC5, HC8, HC11 and HC12. These mutants were found to react only with a MAb recognizing a continuous epitope. Some, for example HC7 and HC10, when immunoprecipitated by MAb I-29 occasionally displayed a very strong signal for an unknown reason. However, the 77K and 79K forms of the H protein were clearly observed with a shorter exposure of the film (data not shown). These results indicate that mutations causing replacement of cysteine residues at positions 287, 300, 394, 579 or 583 have dramatic effects on the discontinuous epitopes of the H protein.

Discussion

This paper presents the results of mutational analysis of the 13 cysteines in the H protein of MV. To study their contribution to the antigenicity and processing of the MV H protein, each cysteine was replaced by a serine. This kind of mutation results in minimal secondary effects on protein structure, caused by the substitution of an oxygen atom (serine) for a sulphur atom (cysteine) (Wang et al., 1984; Giese et al., 1987). Our results showed that substitutions of a serine for any one of cysteine residues 287, 300, 394, 579 or 583 in the H protein resulted in dramatic changes in the properties of the expressed protein. First, no obvious dimeric form of these proteins was observed when proteins were analysed under non-reducing conditions and only nascent unprocessed proteins were detected when gels were run under reducing conditions. Secondly, these mutant proteins contained only high mannose oligosaccharides and failed to be transported to the cell surface. Thirdly, they also exhibited a drastic decrease in reactivity with
the MAbs specific for discontinuous epitopes. Although the mutants with replaced cysteine residues 381 or 494 displayed the capacity to react with the MAbs specific for discontinuous epitopes, these two proteins did not oligomerize, were retained in the ER and failed to be transported to the cell surface. We suggest that the general loss of conformation of these molecules was due to the absence of a critical cysteine residue. We speculate that the absence of cysteine residues 287, 300, 381, 394, 494, 579 or 583 leads to incomplete intra- or intermolecular disulphide bonding. In contrast, alterations of the cysteine residues 139, 154, 188, 386, 570 or 606 displayed no obvious effects on these properties, suggesting that these cysteines either do not participate in disulphide bonding or form bonds that do not contribute significantly to the overall tertiary structure of the protein.

Cysteines 287, 300, 381, 394, 494, 579 and 583 appear to be necessary for full activity of the discontinuous epitopes and most of these residues are also critical for transport of the H molecules. On the basis of the results presented in this report, we propose that these cysteines form intra- or intermolecular disulphide bridges, and cysteines 139, 154, 188, 386, 570 and 606 are normally unpaired. Nevertheless, we cannot draw a conclusion concerning disulphide bond patterns among these cysteine residues. One might expect that if a particular disulphide bond was essential for the expression of a particular epitope, loss of either cysteine in that pair would lead to the total loss of reactivity for that epitope. However, if the disulphide bond was not important to the epitope, the loss of a cysteine would not affect the expressed phenotype. We found that the H proteins expressed by two mutants (Cys6 and Cys9) were in the former category, whereas the glycoproteins expressed by five other mutants (Cys4, Cys5, Cys8, Cys11 and Cys12) displayed unaltered phenotypes. These results make it impossible to deduce pairing of cysteines based on antigenic activity and suggest the need for chemical analysis to determine the disulphide bonding pattern of the H protein.

The maintenance of the antigenicity and processing of the H protein was dependent on at least one interchain disulphide bridge. Cysteine residues 381 and 494 were likely to be intramolecularly disulphide-bonded. One, three or all five of the other critical cysteine residues (287, 300, 394, 579 and 583) could be used for intermolecular connection(s). Thus, two, or less likely one, intramolecular disulphide bonds would be expected to be formed between the five cysteines. The predicted disulphide linking pattern of the MV H protein may be unique in that not every highly conserved cysteine was indicated as being involved in disulphide bonding and that cysteine(s) may be involved in intermolecular disulphide pairing and may also be subject to other kinds of modification. Cysteine residues in the ectodomain of influenza virus H protein (Gething et al., 1980), human immunodeficiency virus envelope glycoprotein gp120 (Leonard et al., 1990), herpesvirus glycoproteins (Long et al., 1992) and West Nile flavivirus membrane proteins (Nowak & Wengler, 1987) are, without exception, used to form intramolecular disulphide bonds. The assignment of these cysteines is consistent with the observations that the oligomers of some of these viral glycoproteins contain no intermolecular disulphide links and are dissociated by SDS (Doms & Helenius, 1986; Earl et al., 1990; Pinter et al., 1989; Segal et al., 1992). However, cysteine residues of human platelet-derived growth factor (Giese et al., 1987; Haniu et al., 1993) and human macrophage colony-stimulating factor (Glocker et al., 1993) participate in either intra- or intermolecular disulphide bonding.

Assuming that the cysteine residues 139, 154, 188, 386, 570 and 606, which were not essential for the antigenicity and processing properties of the protein, were not used for disulphide bonding, structural assignments for these residues are more difficult. At least two of them, cysteine residues 139 and 154, may relate to the structure of the H protein, since the mutant with a replaced cysteine residue 139 exhibited instability and the mutant with a substituted cysteine residue 154 displayed altered migration properties in reducing and non-reducing gels compared with the wild-type protein. Further studies will be required to determine free thiols and thioester-linked cysteine residues and their possible physiological role.

Our results showed that replacements of cysteine residues 287, 300, 381, 394, 494, 579 and 583 altered the extent of processing of oligosaccharides in H protein from the high mannose to the complex form. Conformation has been shown to be important for the processing and transport of viral glycoproteins (Garoff, 1985; Pfeffer & Rothman, 1987). There appeared to be a good correlation between correct conformation, as measured by the reactivity with MAbs specific for the discontinuous epitope, extent of oligosaccharide processing, as measured by sensitivity to Endo H, and transport efficiency, as measured by cell surface expression. The mutants HC2, HC3, HC7, HC10 and HC13 closely resembled wild-type H protein in that they were detectable by the MAbs reacting with discontinuous epitopes, had complex-type oligosaccharides and could be detected on the cell surface. The mutants HC4, HC5, HC8, HC9, HC11 and HC12, which were not detectable by MAbs specific for conformational epitopes and which did not have complex-type oligosaccharides, could not be detected on the cell surface. The mutants HC1, HC6 and HC9, which had molecules recognized by the MAbs detecting conformational epitopes and which did not
acquire the Endo H-resistant form of carbohydrates, did not express a detectable level of H protein on the cell surface. It is possible that transport is more sensitive to conformational alteration than antibody binding. Alternatively, these three mutants could be sensitive to degradation. Therefore, the capacity of the H protein to be transported through the exocytic pathway is dependent upon the presence of certain cysteines that aid in the folding of the protein to obtain a native structure.

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