Post-translational folding of the influenza C virus glycoprotein HEF: defective processing in cells expressing the cloned gene

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The post-translational processing of the influenza C virus glycoprotein HEF was analysed. In cells infected with influenza C virus, HEF protein is synthesized as a glycosylated 80K polypeptide. A post-translational conformational rearrangement involving the formation of intramolecular disulphide bonds results in a decrease in its electrophoretic mobility. Therefore, SDS-PAGE under non-reducing conditions suggests an $M_r$ of about 100K, whereas under reducing conditions an 80K protein is observed which is in accordance with the sequence data. The 100K form was detected 10 min after synthesis of HEF, and transport to the cell surface took about 60 min. This result indicates that the conformational change presumably occurs in the endoplasmic reticulum. A difference in post-translational processing was observed when the HEF gene was expressed in the absence of other influenza C virus genes. In cells infected with recombinant simian virus 40, the 80K precursor was synthesized, but this protein was neither converted to the 100K form nor transported to the cell surface. Deletion of the short cytoplasmic tail of HEF (Arg–Thr–Lys) or replacement of the two basic amino acids by hydrophobic (Ile) or acidic residues (Glu) resulted in HEF protein which was partially converted to the 100K form. Influenza C virus glycoprotein obtained after transient expression of the HEF gene using the vaccinia virus system was completely converted to the 100K form. However, in neither expression system was HEF transported to the cell surface. The possibility is discussed that the interaction of HEF with another viral protein is required for the post-translational folding and transport of this glycoprotein. The M protein of influenza C virus is suggested as a candidate for the chaperone which might interact with the cytoplasmic tail of HEF.

Introduction

In contrast to influenza A and B viruses, which contain two different spike proteins, the haemagglutinin and the neuraminidase, the surface projections of influenza C virus consist of a single type of protein. As indicated by the three-letter designation HEF, the surface glycoprotein of influenza C virus has three activities. It is a haemagglutinin (receptor-binding protein), an esterase (receptor-inactivating enzyme) and a fusion factor (reviewed by Herrler & Klenk, 1991). The receptor-binding activity mediates the attachment of the virus to N-acetyl-9-O-acetylneuraminic acid (Neu5,9Ac₂) on glycoproteins or glycolipids of the cell surface (Rogers et al., 1986; Herrler & Klenk, 1987). The receptor-inactivating enzyme is an acetylesterase which releases the 9-O-acetyl group from Neu5,9Ac₂ (Herrler et al., 1985). The fusion activity depends on the post-translational proteolytic cleavage of the glycoprotein into the subunits HEF₁ and HEF₂ as well as on the exposure to an acidic environment (Ohuchi et al., 1982; Kitame et al., 1982). In low pH conditions, a conformational change of the glycoprotein has been observed (Formanowski et al., 1990). By analogy with influenza A viruses it has been suggested that in the course of the structural rearrangement a hydrophobic sequence present at the N terminus of HEF₂ becomes exposed and induces the fusion of the viral envelope with the membrane of the target cell.

Apart from the biological activities, there are also structural peculiarities that distinguish the influenza C virus glycoprotein from the glycoproteins of influenza A and B viruses. The cytoplasmic portion of HEF comprises only three amino acids (Arg–Thr–Lys) in contrast to 10 amino acids in the case of the haemagglutinins of influenza A and B viruses (Pfeifer & Compans, 1984; Nakada et al., 1984). One post-translational modification of HEF is acylation with fatty acids. Surprisingly, stearic acid was detected as the prevailing fatty acid attached to HEF, whereas palmitic acid is the predominant fatty acid found in all other membrane proteins yet analysed (Veit et al., 1990). An
unusual migration of HEF is observed upon SDS–PAGE under non-reducing conditions suggesting an 
Mr of about 100K (Herrler et al., 1979). A reduction of the apparent 
Mr to about 80K is observed after proteolytic cleavage of the glycoprotein into the subunits HEF 1 and
HEF 2, which are held together by disulphide bonds. An 
80K protein is also obtained when the uncleaved glycoprotein is analysed under reducing conditions. 
In neither case is there evidence for the loss of a peptide 
accounting for the difference of 20K (Meier-Ewert et al.,
1980, 1981a, b). The sequence information is also 
incompatible with a protein of 100K, even if oligo-
saccharides were attached to all of the eight potential 
glycosylation sites. Therefore, it is assumed that the high 
Mr form of HEF is due to a peculiar conformation that 
allows the binding of only a reduced amount of SDS and,
therefore, is responsible for the decrease of the electro-
phoretic mobility. These conformational constraints of 
HEF can obviously be abolished by proteolytic cleavage 
or by breaking the disulphide bonds.

Here we report that the formation of the 100K form of 
HEF is a post-translational event occurring soon after 
synthesis of the glycoprotein. The conformational change 
was also observed with HEF protein transiently 
expressed in cells using the vaccinia virus expression 
system, but not in cells infected with recombinant simian 
virus 40 (SV40). In both cases the glycoprotein was not 
transported to the cell surface. We discuss the possibility 
that a helper protein is required for maturation and 
transport of HEF.

Methods

*Cells.* MDCK cells were grown as previously described (Szepanski et al., 1992). CV-1p cells were kindly provided by Dr E. Fanning-
Honegger, Munich, Germany, and grown in Dulbecco's modified 
Eagle's medium (Gibco) containing 5% fetal calf serum.

*Viruses.* Influenza C virus, strain Johannesburg/1/66 (C/JHB/1/66), 
was grown in MDCK I cells as previously described (Herrler & Klenk, 
1987). As CV-1p cells lack receptors for influenza C virus, they were 
treated with bovine brain gangliosides (Sigma) prior to infection 
(Herrler & Klenk, 1987). Recombinant vaccinia virus, vTF7-3, was 
grown and plaque-purified in CV-lp cells (Fuerst et al., 1986).

*Cloning and site-specific mutagenesis of the HEF gene.* A copy of 
the HEF gene cloned into pBR322 was obtained by Dr J. Pfeifer. 
Subcloning in Bluescript M13 + KS and removal of the GC tails has 
been described previously (Pleschka, 1989). For site-specific muta-
genesis, the HEF gene was excised with KpnI and BamHI and cloned into 
M13mpl8 replicative form DNA. Oligonucleotide-directed muta-
genesis was performed using a commercial kit based on the phosphoro-
thioate method (Taylor et al., 1985). M13–HEF phages were screened by 
dideoxyribonucleotide sequencing (Sanger et al., 1977) for mutations at 
the positions of interest. The sequence of the cloned gene was found to 
conform with the sequence of the HEF gene of C/JHB/1/66 determined by 
sequencing of the viral RNA (Szepanski et al., 1992) except for a silent mutation at nucleotide position 321 (A to G), which was introduced to delete the internal BamHI restriction site.

*Recombinant SV40–HEF.* Wild-type and mutated HEF genes were 
cloned into the KpnI/BamHI site of the SV40 expression plasmid 
pA11SV13. The DNA sequence was verified by dideoxyribonucleotide 
sequencing. Cotransfection of CV-1p cells with pA11SV13 and the 
SV40-helper plasmid dl1055, as well as generation of a recombinant 
SV40 stock have been described recently (Veit et al., 1991). For co-
fection experiments, cells were infected at 80% confluence with 
SV40–HEF. At 24 h post-infection (p.i.), the confluent cells were 
treated with bovine brain gangliosides and infected with influenza C 
virus as described above.

*Transient expression.* For transient expression of HEF, the vaccinia 
virus expression plasmid pTM1 (Moss et al., 1990) was used. The 
purified plasmid DNA was digested with SacI and BamHI and treated with 
calf intestinal phosphatase (Boehringer Mannheim) for 2 h at 45 
°C followed by phenol extraction and sodium acetate (3 M, pH 6.0) 
precipitation. The HEF gene was excised from M13mpl8 as described 
above and digested with SacI and BamHI. Linearized vector (pTM1) and 
HEF DNA were ligated and amplified according to standard 
procedures (Sambrook et al., 1989). CV-1 cells were infected with 
vTF7-3 vaccinia virus at an m.o.i. of 30 p.f.u./cell. At 2 h p.i., the virus 
inoculum was replaced by the transfection inoculum containing 20 µg 
ptM1–HEF DNA and 25 µl lipofectin (BRL). At 18 to 20 h after 
transfection, the cells were harvested and analysed for the synthesis of 
HEF protein. For co-infection experiments, CV-1p cells were grown to 
confluence and infected with influenza C virus. At 4 h p.i., the cells were 
injected with vTF7-3. Two hours later, cells were transfected with 
pTM1–HEF. The synthesis of HEF was analysed 18 to 20 h after 
infected by vaccinia virus.

*Radioactive labelling.* Prior to labelling proteins, cells were starved 
for 1 h at 37 °C with methionine-free Dulbecco's modified Eagle's 
medium. At the times indicated, cells were incubated either with 50 µCi 
[35S]methionine for labelling periods of 1 to 4 h or with 100 µCi 
[35S]methionine for pulse–chase experiments. During the chase periods, 
the labelling medium was replaced with medium containing unlabelled 
methionine at a concentration of 300 µg/ml for the times indicated.

*Cell surface trypsinization.* MDCK I cells infected with influenza C 
virus were labelled at 24 h p.i. with 100 µCi [35S]methionine/ml for 
15 min and chased for the times indicated. TPCK/trypsin (Sigma) was 
added to the chase medium at a concentration of 50 µg/ml 15 min 
before lysis of the cells with RIPA buffer as described below. The lysis 
buffer was supplemented with the trypsin inhibitor aprotinin (50 µg/ 
ml), HEF protein was immunoprecipitated (see below) and analysed for 
proteolytic cleavage by SDS–PAGE under reducing conditions.

*Immunoprecipitation.* Labelled cells were washed twice with ice-cold 
PBS and lysed in RIPA buffer (1% Triton X-100, 0.1% SDS, 5 U/ml 
aprotinin, 0.15 M-NaCl, 20 mM-Tris–HCl, 10 mM-EDTA, 1 mM-PMSE, 
10 mM-iodoacetamide). Lysates were sonicated for 3 min and cellular 
debris was pelleted by centrifugation at 15000 g for 30 min. The supernatant was removed and 5 µl of polyclonal rabbit antisemur 
directed against C/JHB/1/66 (final dilution 1:1000, preincubated with 
unlabelled CV-1 lysate) was added. For surface immunoprecipita-
tion, intact cells were treated with the antisemur (1:100) for 30 min at 4 °C. 
After three washes with ice-cold PBS, they were lysed in RIPA buffer. 
The immunocomplexes were adsorbed to Protein A–Sepharose (20 µl) 
for 30 min and washed three times with RIPA buffer. The precipitated 
proteins were solubilized by heating in electrophoresis sample buffer for 
5 min at 96 °C. Aliquots of the samples were separated by 
SDS–PAGE in 10% polyacrylamide gels (Laemmli, 1970). For analysis of 
the proteins under reducing conditions, 2-mercaptoethanol was 
added to the sample buffer to give a final concentration of 1%.

*Esterase and haemadsorption assays.* For haemadsorption, infected 
or transfected cells were incubated with 1 ml of a 1% suspension of 
chicken erythrocytes for 20 min on ice. The supernatant was discarded
and unbound erythrocytes were removed by extensive washing with PBS. Esterase activity was measured using bovine submaxillary mucin as a substrate (20 mg/ml, Boehringer Mannheim). Cells were incubated at 37 °C for 3 h with gentle shaking in PBS containing the substrate. The amount of acetate released was determined with a commercial kit as described previously (Herrler et al., 1985).

Results

Processing and transport of HEF in influenza C virus-infected cells

In order to obtain information about the time course of the synthesis of the 100K form of HEF, pulse-chase experiments were performed. Infected cells were labelled for 5 min with [35S]methionine and, after different chase periods, analysed by SDS-PAGE under non-reducing conditions. As shown in Fig. 1, in influenza C virus-infected MDCK cells an 80K protein is detected after a labelling period of 5 min. After a chase of 5 min, the 80K and 100K forms of HEF are present in about equal amounts. At later chase times the 100K form becomes more prominent, whereas the 80K band almost disappears after a chase of 30 min. The protein pattern from Fig. 1 suggests that the 80K protein is a precursor form and the 100K protein is the mature form of HEF. The total amount of glycoprotein appears to increase with the chase times. This is not due to inappropriate chase conditions, because the increase is not observed with the viral nucleoprotein (NP) and matrix (M) protein. Because the antiserum is directed against the virus, it probably recognizes the mature glycoprotein more efficiently than the precursor. The same result was obtained when the processing of HEF was analysed in CV-1 cells infected with influenza C virus (not shown).

To relate the kinetics of the appearance of the 100K protein to the post-translational transport of HEF, we determined the time that the glycoprotein requires to reach the plasma membrane. The appearance of HEF on the cell surface was analysed by surface trypsinization. In cultured cells infected with influenza C virus, the viral glycoprotein is usually present in the uncleaved form (Herrler et al., 1979; Sugawara et al., 1981) designated HEF<sub>0</sub> (Herrler et al., 1988). As HEF can be cleaved by trypsin into HEF<sub>1</sub> and HEF<sub>2</sub> (Herrler et al., 1979; Sugawara et al., 1981), addition of the protease to intact cells allows differentiation of intracellular glycoprotein and glycoprotein on the cell surface. Only the latter is accessible to trypsin treatment. Therefore, the detection of cleavage products is indicative of the presence of HEF on the cell surface. As shown in Fig. 2, cells labelled with [35S]methionine for 15 min and chased for various times contain mainly the uncleaved form of the glycoprotein. Only after a chase time of 60 min (lane 5) is the characteristic doublet of the HEF<sub>1</sub> polypeptide (Herrler et al., 1979) detected. The same kinetics were observed when the transport of HEF to the cell surface in influenza C virus-infected CV-1 cells was analysed by surface immunoprecipitation. This result indicates that the glycoprotein takes about 60 min to reach the surface of these cells.

Expression of the HEF gene in cells infected with recombinant SV40

The synthesis and transport of the influenza C virus glycoprotein was also analysed with recombinant SV40 containing the cloned HEF gene. CV-1 cells were infected with SV40–HEF and labelled with [35S]methionine. After immunoprecipitation by antiserum against influenza C
virus, the proteins were analysed by SDS–PAGE. As shown in Fig. 3(a), under non-reducing conditions a protein with an estimated size of about 80K was detected (lane 3). This is different from the glycoprotein pattern obtained after infection of cells with influenza C virus, where HEF was present primarily in the 100K form and only a minor portion was found in the 80K form (lanes 1 and 2). This pattern was observed not only with MDCK cells (lane 1) but also with CV-1 cells (lane 2) infected with influenza C virus. As the latter cells were also used for infection with the recombinant virus, the difference in the glycoprotein pattern cannot be explained as an effect of the host cell. The 80K protein found in purified virus is known to represent the proteolytically cleaved form of HEF with the cleavage products HEF₁ and HEF₂ held together by disulphide bonds (Herrler et al., 1979). The 80K protein detected in SV40–HEF-infected cells is not cleaved into subunits, because the electrophoretic migration is not changed under reducing conditions (lanes 3 of Fig. 3a and b). This result suggests that the 80K protein observed in SV40–HEF-infected cells is the precursor form of HEF and that the glycoprotein is unable to adopt the 100K conformation.

HEF protein synthesized in cells infected with recombinant SV40 was analysed for transport to the cell surface by surface immunoprecipitation. As shown in Fig. 4(a), when cells infected with influenza C virus were labelled for 30 min, HEF protein was detected on the cell surface after a chase of 60 min (lane 3). In contrast, no glycoprotein was detected on the surface of cells infected with SV40–HEF even after a chase of 2 h (Fig. 4b) though, in parallel samples, it was readily detected intracellularly. Thus, HEF protein synthesized in cells after infection with recombinant SV40 is neither folded nor transported correctly. The defective processing of the glycoprotein in this system was also evident from the lack of HEF-specific biological activities on the cell surface. Cells infected with SV40–HEF were negative in both haemadsorption (not shown) and esterase assays (see Table 1).

Mutations in the short cytoplasmic domain affect the processing of HEF

A characteristic feature of the influenza C virus glycoprotein is a short cytoplasmic tail comprising only three amino acids, two of them positively charged
Fig. 5. Amino acid sequence of the cytoplasmic tail of the HEF protein of influenza C virus as well as of three mutant glycoproteins derived by site-directed mutagenesis. Point mutations are indicated by bold-printed letters.

<table>
<thead>
<tr>
<th>Transmembrane domain</th>
<th>Cytoplasmic tail</th>
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<tbody>
<tr>
<td>Gly–Ile–Ala–Ile–Cys</td>
<td>Wild-type</td>
</tr>
<tr>
<td>Gly–Ile–Ala–Ile–Cys</td>
<td>Mutant 1</td>
</tr>
<tr>
<td>Gly–Ile–Ala–Ile–Cys</td>
<td>Mutant 2</td>
</tr>
<tr>
<td>Gly–Ile–Ala–Ile–Cys</td>
<td>Mutant 3</td>
</tr>
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Fig. 6. Synthesis of HEF protein in CV-1 cells infected by SV40 (lane 1) or recombinant SV40 containing either wild-type (lane 2) or mutated HEF genes (lanes 3 to 5). The mutations affected the cytoplasmic tail as shown in Fig. 5: lane 3 corresponds to mutant 1, lane 4 to mutant 2 and lane 5 to mutant 3. HEF protein was labelled with $[^{35}\text{S}]$methionine, immunoprecipitated and analysed by SDS–PAGE under non-reducing conditions. The 100K and 80K forms of HEF are indicated by arrows.

(Arg–Thr–Lys). In order to analyse the importance of the basic amino acids, mutant glycoproteins were generated with a change in the cytoplasmic tail (Fig. 5). In two of the mutants, the arginine and lysine residues have been changed by site-directed mutagenesis into either hydrophobic amino acids (Ile) or acidic amino acids (Glu). In a third mutant, the three amino acids of the cytoplasmic tail were deleted. CV-1 cells were infected with recombinant SV40 containing the mutated glycoprotein genes. Proteins were labelled with $[^{35}\text{S}]$methionine, immunoprecipitated with antiserum against influenza C virus, and analysed by SDS–PAGE under non-reducing conditions. After labelling for 4 h, wild-type HEF is present only in the 80K form (Fig. 6, lane 2). In contrast, cells expressing any one of the three mutated glycoprotein genes contained, in addition to the 80K protein, a distinct band of the 100K form of HEF (lanes 3 to 5). This result indicates that the positively charged amino acids of the cytoplasmic tail affect the processing of the glycoprotein. However, despite the presence of the 100K form in cells expressing any one of the mutated HEF genes, no glycoprotein was transported to the cell surface as indicated by surface immunofluorescence and haemadsorption assays (not shown).

**Transient expression of HEF using vaccinia virus**

As HEF protein expressed with recombinant SV40 was not processed correctly, we analysed whether the defect can be overcome by using a different expression system. We chose transient expression with the vaccinia virus/T7 RNA polymerase system. The HEF gene was placed under the control of the bacteriophage T7 promoter and transfected into CV-1 cells which had previously been infected with the recombinant vaccinia virus vTF7-3. The synthesis of HEF was monitored by metabolic labelling, immunoprecipitation and SDS–PAGE. As shown in Fig. 7(a), HEF protein obtained with the vaccinia virus/T7 system (lane 2) comigrated with the HEF protein synthesized in MDCK cells after infection with influenza C virus (lane 1). In contrast to the results obtained with recombinant SV40, the 100K form of HEF was the predominant band observed by SDS–PAGE under non-reducing conditions with the transient expression system. Thus, in the vaccinia virus system, the HEF protein synthesized undergoes a post-translational change of conformation similar to that observed during an influenza C virus infection. In order to analyse whether the protein is transported to the cell surface, surface immunoprecipitation experiments were performed. As shown in Fig. 7(b), transiently expressed HEF protein was not detected on the surface of CV-1 cells (lane 2). In contrast, when the same type of cells was infected by influenza C virus, the viral glycoprotein was readily detectable on the cell surface (lane 1). The transport of HEF to the plasma membrane of influenza C virus-infected cells was not affected when the cells were co-infected with recombinant vaccinia virus (lanes 3 and 4). Therefore, the inability of transiently expressed HEF to be transported to the cell surface appears to be an intrinsic property of this viral glycoprotein and not an effect of the expression system. The lack of surface transport was confirmed by surface immunofluorescence and haemadsorption assays (not shown). The esterase activity of HEF could not be used to study the localization of the glycoprotein because, in contrast to the case with SV40, vaccinia virus appears to inhibit the viral enzyme. The inhibitory effect was observed not only with the transiently expressed protein, but also with cells co-infected with influenza C virus and vaccinia virus (Table 1).
Fig. 7. Synthesis of HEF protein after transient expression with the vaccinia virus expression system. (a) HEF protein synthesized in CV-1 cells after infection with influenza C virus (lanes 1 and 3) or transient expression of the gene with the vaccinia virus expression system (lanes 2 and 4). Influenza C proteins labelled with [35S]methionine were immunoprecipitated and analysed by SDS–PAGE under non-reducing (lanes 1 and 2) and reducing conditions (lanes 3 and 4). The arrows indicate the 100K and the 80K forms of HEF. (b) CV-1 cells were infected with influenza C virus (lane 1), infected with recombinant vaccinia virus vTF7-3 and transfected with expression plasmid pTM1-HEF (lane 2), co-infected with influenza C virus and vTF7-3 (lane 3), or co-infected with influenza C virus and vTF7-3 and transfected with pTM1-HEF (lane 4). HEF protein was labelled with [35S]methionine and analysed for transport to the cell surface by surface immunoprecipitation followed by SDS–PAGE under non-reducing conditions.

Discussion

The results presented above indicate that, in influenza C virus-infected cells, the glycoprotein HEF is synthesized as an 80K precursor that is post-translationally converted into a protein with slower electrophoretic migration. The conversion to the 100K form is only detected after SDS–PAGE under non-reducing conditions indicating that disulphide bonds are involved in this process. The formation of disulphide bonds is obviously accompanied by a conformational rearrangement, because the precursor and the mature form of HEF were found to differ from each other in antigenicity. Rabbit antiserum raised against purified virions appeared to recognize the 100K form more efficiently than the 80K precursor. In addition, some monoclonal antibodies directed against HEF did not recognize the 80K form at all (results not shown). The post-translational folding of a protein from an immature to a mature conformation is a common process. What is unusual in the case of the influenza C virus glycoprotein is the concomitant decrease in electrophoretic mobility of the glycoprotein under non-reducing conditions. This may be related to the fact that this protein has to adopt and maintain a conformation forming not only several antigenic sites (Sugawara et al., 1986, 1988), but also three functional regions: the receptor-binding site, the active site of the acetyl esterase, and the fusogenic site. The transition from the 80K to the 100K form is a relatively fast process occurring within 5 to 10 min after synthesis. By comparison, the transport to the cell surface takes about 60 min. This difference indicates that the conformational rearrangement of HEF is an early event and probably takes place in the endoplasmic reticulum.

The influenza C virus glycoprotein of strain Johannesburg/1/66 expressed with the SV40 system was neither folded correctly nor transported to the cell surface. This defect was not due to the expression system, because in cells co-infected with influenza C virus and SV40 the post-translational processing of HEF was not affected. In a previous study, the SV40 system has been used to express the HEF gene of strain C/Cal/78 (Vlasak et al., 1987). The authors reported that the glycoprotein was transported to the cell surface as judged by haemadsorption and esterase tests. This is in contrast to our results obtained with the glycoprotein of strain C/JHB/1/66. It may appear surprising that expression of the cloned gene of one strain results in a functional protein, whereas the glycoprotein of a related strain is defective in post-translational processing. However, the vector-expressed C/Cal/78 glycoprotein has not been analysed under non-reducing conditions. Therefore, it is not possible to estimate in that case the efficiency of the maturation process, i.e. the conversion of the precursor into the mature form. In addition, the time course of the transport to the cell surface has not been analysed. Though some functional protein has been detected when the cloned HEF gene of C/Cal/78 was expressed in CV-1 cells, it is possible that the maturation and surface transport are less efficient than that occurring in influenza C virus-infected cells. The HEF protein of C/Cal/78 differs from that of C/JHB/1/66 at 25 amino acid positions (including the deletion of an amino acid), which may be responsible for the different processing of the two vector-expressed proteins. A mutation in the course of the cloning procedure has been excluded by comparing the nucleotide sequence of the cloned gene with that of the genomic RNA of C/JHB/1/66. Future studies may show the extent of strain-dependent variation in the requirements of the HEF protein for the correct post-translational processing.
As an explanation of our results with the SV40 expression system we propose that the influenza C virus glycoprotein requires the cooperative effect of another viral protein for proper folding and transport to the cell surface. As HEF is the only glycoprotein known for influenza C virus, the non-glycosylated viral proteins are candidates for such a helper function. Owing to their location in the cytoplasm, an interaction of HEF with one of these proteins is expected to involve its cytoplasmic tail which is composed of only three amino acids (Arg-Thr-Lys). Because two of them are positively charged, an ionic interaction between HEF and a potential cytoplasmic chaperone might result in a cooperative effect on the folding and transport of the glycoprotein. In the absence of a chaperone, the basic amino acids of the cytoplasmic tail may even be detrimental for the post-translational processing of HEF. If they were deleted or replaced by either hydrophobic or acidic amino acids, a proportion of the glycoprotein molecules would have folded such that it appeared as a 100K protein by SDS-PAGE. In the absence of a chaperone, a cellular protein may interact with the positively charged amino acids and prevent the correct folding of HEF. The inappropriate interaction with a cellular protein may be abolished by the vaccinia virus infection, because the 100K form was observed when HEF was expressed with the vaccinia virus system. However, similar to the mutated glycoproteins in the SV40 system, the 100K protein was not transported to the cell surface. Therefore, the function of a chaperone is not restricted to neutralizing the positive charge of the basic amino acids. The helper protein would have to make the glycoprotein transport-competent. The extent to which the maturation of HEF depends on a chaperone may vary depending on the strain. Although the glycoprotein of strain C/Cal/78 has the same cytoplasmic tail as the HEF protein of C/JHB/1/66, functional glycoprotein was detected after expression of the corresponding gene of the former strain (Vlasak et al., 1987). However, this does not exclude the possibility that, even in this case, the maturation is more efficient in the presence of a helper protein.

Among the proteins of influenza C virus, the M protein is a prime candidate for serving as a helper of HEF. This protein is believed to function in virus maturation by interacting with both the ribonucleoprotein complexes and the cytoplasmic tail of the viral glycoprotein. The M protein may play a role not only at the plasma membrane for the budding process, but also at the endoplasmic reticulum for the maturation and the transport of the glycoprotein. An interaction between M protein and viral glycoproteins at intracellular membranes has been reported recently for paramyxoviruses (Sanderson et al., 1993). An example for a chaperone function required for the intracellular transport of a glycoprotein has been reported for herpes simplex virus and human cytomegalovirus. Glycoprotein H of these viruses is transported from the endoplasmic reticulum to the cell surface only after formation of a stable complex with another viral glycoprotein, gL (Hutchinson et al., 1992; Kaye et al., 1992; Spaete et al., 1993). In the case of influenza C virus, coexpression of the M and the HEF gene should allow us to draw conclusions about the cooperative effect of M protein on the post-translational processing of the HEF protein.

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References


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