Characterization of aggregates of hepatitis C virus glycoproteins

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Hepatitis C virus (HCV) encodes two glycoproteins, E1 and E2, which assemble in oligomeric structures. Studies of HCV glycoprotein assembly using heterologous expression systems have shown that these glycoproteins can follow two pathways: a productive pathway leading to the formation of a non-covalent heterodimer; and a non-productive pathway leading to the formation of large disulfide-linked aggregates. The non-covalent HCV glycoprotein complex is probably the functional complex which plays an active role in the entry process in host cells. The aggregates are believed to be waste products; however, one can imagine that, in infected cells, they could provide HCV glycoproteins with additional functions. To further understand the potential role played by HCV glycoprotein aggregates in HCV infection, a MAb (H14) was produced which specifically recognizes these aggregates but not the non-covalent E1E2 heterodimer. The H14 epitope was shown to be present on both HCV glycoproteins and was sensitive to deglycosylation. An additional characterization of HCV glycoprotein aggregates, with the help of MAb H14, indicates that they share an epitope with a cellular protein called Mac-2 binding protein. The presence of such an epitope on HCV glycoprotein aggregates could potentially lead to the production of autoantibodies recognizing Mac-2 binding protein in HCV-infected patients.

Introduction

Hepatitis C virus (HCV) encodes two glycoproteins, E1 and E2, which assemble in oligomeric structures (Dubuisson et al., 1994; Grakoui et al., 1993; Lanford et al., 1993; Ralston et al., 1993). Purified HCV glycoprotein complexes expressed by using vaccinia virus recombinants have been shown to be non-covalently associated (Ralston et al., 1993). In contrast, a fraction of E1 and E2 present in lysates of cells infected with vaccinia virus–HCV recombinants has been reported to be associated via disulfide linkages (Grakoui et al., 1993). Other studies on the formation of intracellular complexes have shown that, in the presence of non-ionic detergents, two forms of E1E2 complexes are detected: a heterodimer of E1 and E2 stabilized by non-covalent interactions, and disulfide-linked aggregates (Dubuisson et al., 1994; Dubuisson & Rice, 1996). Additional studies indicate that the non-covalent heterodimer is composed of native HCV glycoproteins whereas the disulfide-linked aggregates are formed by misfolded proteins (Deleersnyder et al., 1997). The formation of stable non-covalent E1E2 complexes is slow (t_{1/2} ≈ 2 h) due to the slow folding of these proteins. Indeed, formation of intramolecular disulfide bonds is slow for E1 (Dubuisson & Rice, 1996) and unidentified events following the acquisition of intramolecular disulfide bonds limit the folding of E2 (Deleersnyder et al., 1997; Dubuisson & Rice, 1996; Habersetzer et al., 1998; Michalak et al., 1997). In addition, E1 expressed in the absence of E2 does not fold properly, suggesting that E2 plays a chaperone-like role in the folding of E1 (Michalak et al., 1997). The non-covalent heterodimeric complex is believed to be the pre-budding form of the HCV glycoprotein oligomer and accumulates in the endoplasmic reticulum (ER) (Deleersnyder et al., 1997). The transmembrane domains of E1 and E2 have been shown to be major determinants for ER localization of these proteins (Cocquerel et al., 1999, 1998; Duvet et al., 1998).

There are several possible fates for newly synthesized proteins inside cells. The major distinction between these fates is whether a protein succeeds in folding correctly, or whether it aggregates. Aggregation has been commonly regarded as a nuisance which affects in vitro protein refolding studies. It is now apparent that aggregation is also a problem within cells.
The production of properly assembled E1E2 oligomers is inefficient (Deleersnyder et al., 1997). This does not seem to be due to mutations introduced during cDNA synthesis or PCR amplification of the original clone used in these studies. Indeed, similar results are obtained with a vaccinia virus recombinant expressing the sequence of the structural proteins of a recently characterized infectious cDNA clone (Kolykhlov et al., 1997; J. Dubuisson & C. M. Rice, unpublished data). Aggregates of HCV glycoproteins were first observed when expressed by viral vectors such as vaccinia virus and Sindbis virus which can induce a high level of protein synthesis (Dubuisson et al., 1994; Grakoui et al., 1993). However, such aggregates have also been reported when HCV proteins are expressed in a non-viral vector which drives a lower level of protein synthesis (Duvet et al., 1998). This tendency to aggregate is therefore probably an intrinsic property of HCV glycoproteins. Indeed, slow folding of HCV glycoproteins may increase the fraction of these proteins shunted into a competing non-productive pathway, such as aggregation (Fischer & Schmid, 1990). In this study, we produced a MAb which specifically reacts with HCV glycoprotein aggregates but not with non-covalently associated glycoproteins. Characterization of these aggregates indicates that they share a common epitope with a cellular protein.

Methods

Cell culture. HepG2 and CV-1 cell lines were obtained from the ATCC. Cell monolayers were grown in Dulbecco’s modified MEM supplemented with 10% foetal bovine serum (FBS).

Viruses. vTF7-3 (a vaccinia virus recombinant expressing the T7 DNA-dependent RNA polymerase) (Puerst et al., 1986), vaccinia virus–HCV recombinants vHCV1-1488 (expressing CE1E2p7NS2NS31207), vHCV170-809 (E1E2p7), vHCV371-609 (E2p7) and vHCV1-383 (CE1) (Fourmillier-Jacob et al., 1990; Grakoui et al., 1993; Michalak et al., 1997), and vaccinia virus recombinants expressing truncated forms of HCV glycoproteins [vHCV170–311 (E131), vHCV170–361 (E186), vHCV371–715 (E2129) and vHCV371–661 (E2483)] (Michalak et al., 1997) were used in this work. The Sindbis virus recombinant expressing a truncated form of HCV polyprotein (amino acid residues 1–1207: SNrep/HCV-H1-1207) has been described previously (Dubuisson et al., 1994).

Antibodies. Anti-HCV E1 (A4) and E2 (H2 and H47) MAbs have been described previously (Deleersnyder et al., 1997; Dubuisson et al., 1994; A. Pillez & J. Dubuisson, unpublished data) and were produced in vitro by using a MiniPerm apparatus (Heraeus) as recommended by the manufacturer. To produce other MAbs, HepG2 cells co-infected with vTF7-3 and vHCV1–1488 were lysed with 0.5% NP-40 in TBS (20 mM Tris–HCl, pH 7.4; 137 mM NaCl). HCV glycoproteins were purified by immunoaffinity on Protein A–Sepharose (Amersham Pharmacia Biotech) with anti-E1 MAb A4. HCV glycoprotein complexes bound to the immunoabsorbents were injected into BALB/c mice to produce HCV-specific, antibody-secreting hybridomas as described (Harlow & Lane, 1988). Screening was performed in 96-well plates containing HepG2 cells that had been co-infected with vTF7-3 and vHCV1–1488, and fixed with isopropanol as described (Dubuisson et al., 1994). Anti-Mac-2 binding protein (M2BP) antibody was kindly provided by R. Timpl (Max Planck Institute für Biochemie, Martinsried, Germany).

Metabolic labelling and immunoprecipitation. Subconfluent monolayers in 35 mm dishes were infected with the appropriate recombinant at an m.o.i. of 5 p.f.u. per cell. After 1 h, medium containing 5% FBS was added. Between 4 and 5 h post-infection, monolayers were washed once with pre-warmed medium lacking methionine and cysteine, and incubated in the same medium for an additional half hour. Infected cells were then pulse-labelled for 5 min with 100 Ci/ml 35S-Protein Labelling Mix (NEN Life Science Products). Cells were washed twice with pre-warmed medium containing 10-fold excess methionine and cysteine, followed by a chase for various times. Cells were then lysed with 0.5% TBS. Iodoacetamide (20 mM) was included in the lysis buffer for experiments in which disulfide bond formation was determined. Cell lysates were clarified by centrifugation in an Eppendorf centrifuge for 5 min at 4 °C. In steady-state labelling, cells were labelled at 4 h post-infection with 50 Ci/ml 35S-Protein Labelling Mix in medium lacking methionine and cysteine. Immunoprecipitations were carried out as described previously (Dubuisson & Rice, 1996). The precipitates were boiled for 5 min in SDS–PAGE sample buffer (under non-reducing conditions; β-mercaptoethanol was omitted) and run on a 10% polyacrylamide gel (Laemmli, 1970). After electrophoresis, gels were treated with sodium salicylate (Chamberlain, 1979), dried and exposed at −70 °C to pre-flashed Hyperfilm–MP (Amersham Pharmacia Biotech). 14C-Methylated protein molecular mass markers were purchased from Amersham Pharmacia Biotech.

Western blotting. For Western blotting studies, proteins were separated by SDS–PAGE, transferred to nitrocellulose membranes (Hybond–ECL; Amersham Pharmacia Biotech) by using Trans-Blot apparatus (Bio-Rad). After transfer, nitrocellulose membranes were incubated with specific antibodies (MAb or polyclonal antibody) followed by goat anti-mouse or swine anti-rabbit immunoglobulin conjugated to horseradish peroxidase or alkaline phosphatase (DAKO) as described previously (Harlow & Lane, 1988). The presence of proteins specifically recognized by the primary antibody was revealed by chemiluminescence (ECL; Amersham Pharmacia Biotech) as recommended by the manufacturer or with BCIP/NBT alkaline phosphatase substrate (Sigma).
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Bianco virus expressing HCV glycoproteins. At 8 h post-infection, cells were fixed for 10 min at 4°C with isopropanol. For immunofluorescence detection, cells were stained for 2 h at room temperature with MAb H14 (diluted 1/200), followed by incubation for 1 h at room temperature with donkey anti-mouse (Rhodamine-linked) immunoglobulins (diluted 1/100; Jackson Immunoresearch). For alkaline phosphatase detection, cells were stained for 2 h at room temperature with MAb H14 (diluted 1/200) or A4 (diluted 1/400), followed by incubation for 1 h at room temperature with rabbit anti-mouse (alkaline phosphatase-linked) immunoglobulins (diluted 1/200; DAKO) and revealed with BCIP/NBT alkaline phosphatase substrate.

■ Amino acid sequencing. Internal amino acid sequences were determined as described previously (Rosenfeld et al., 1992). Briefly, the cellular protein recognized by MAb H14 was purified by immunoprecipitation as described (Harlow & Lane, 1988) and separated by SDS–PAGE. After Coomassie blue staining, the band of interest was cut and treated with acetonitrile and ammonium carbonate to clear the sample of impurity. After a Speedvac dehydration, the protein was digested with endoproteinase Lys-C (Boehringer Mannheim) at 37°C for 18 h. Peptides of interest were purified by reverse-phase HPLC, eluted with acetonitrile in 0.1% trifluoroacetic acid, lyophilized and sequenced in an automated microsequencer.

Results

Identification of a MAb which specifically recognizes aggregates of HCV glycoproteins

HCV glycoproteins have a tendency to aggregate and recent data suggest that this tendency to form aggregates is probably an intrinsic property (Dubuisson, 1999; Duvet et al., 1998). To better characterize HCV aggregates, we produced a MAb which specifically recognizes disulfide-linked HCV glycoproteins but not non-covalently associated proteins. For this purpose, MAbHs were screened by immunoprecipitation followed by SDS–PAGE analysis under non-reducing conditions to select those which only precipitate large aggregates. One MAb (H14) showed the expected pattern (Fig. 1). When analysed under non-reducing conditions, proteins precipitated by H14 migrated as high molecular mass aggregates. Under reducing conditions, MAb H14 precipitated both E1 and E2 in a similar fashion to conformation-sensitive (H2) (Deleersnyder et al., 1997) or -insensitive (A4) (Dubuisson et al., 1994) MAb. For MAb H2, the pattern was similar under non-reducing and reducing conditions. For MAb A4, bands corresponding to E1 and E2 as well as heterogeneous aggregates were observed under non-reducing conditions. It is worth noting that MAb H2 precipitates the oxidized forms of E1 and E2 only (Deleersnyder et al., 1997) whereas MAb A4 precipitates both the reduced and oxidized forms of at least E1 (Dubuisson & Rice, 1996) (Fig. 1). Together these data indicate that MAb H14 is a new tool to characterize HCV glycoprotein aggregates.

Fig. 1. MAb H14 precipitates aggregates of HCV glycoproteins. HepG2 cells were co-infected with vTF7-3 and vHCV170–809. At 4–5 h post-infection, cells were labelled for 2 h with [35S]methionine and lysed with Triton X-100. Cell lysates were used for immunoprecipitation with MAb H14. Immunoprecipitates were analysed by SDS–PAGE (10% polyacrylamide) under reducing conditions. HCV-specific proteins are indicated on the left and the sizes (in kDa) of molecular mass markers are indicated on the right.

Fig. 2. MAb H14 precipitates a cellular protein (*). HepG2 cells were co-infected with vTF7-3 and vHCV170–809 (V), vTF7-3 alone (M) or left uninfected (U). At 4–5 h post-infection, cells were labelled for 2 h with [35S]methionine and lysed with Triton X-100. Cell lysates were used for immunoprecipitation with MAb H14. Immunoprecipitates were analysed by SDS–PAGE (12% polyacrylamide) under reducing conditions. HCV-specific proteins are indicated on the left and the sizes (in kDa) of molecular mass markers are indicated on the right.
Fig. 3. Western blotting analysis of the cellular protein (M2BP) recognized by MAb H14. (A) HepG2 cell lysates were immunoprecipitated with MAb H14 or an isotype-matched control MAb. Immunoprecipitates were separated by SDS–PAGE and revealed by Western blotting with a polyclonal antibody to M2BP. (B) HepG2 cell lysates were immunoprecipitated with an anti-M2BP polyclonal antibody or a species-matched control antibody. Immunoprecipitates were separated by SDS–PAGE and revealed by Western blotting with MAb H14. I.P., Immunoprecipitation; W. B. Western blotting.

Fig. 4. MAb H14 recognizes the intracellular form of M2BP but not its secreted form. (A) HepG2 cells were pulse-labelled for 5 min with \(^{[35}\text{S}]\)methionine and chased for the indicated times (min). Cell lysates and supernatants (lane S, 240 min chase only) were used for immunoprecipitation with MAb H14 or an anti-M2BP polyclonal antibody. Immunoprecipitates were analysed by SDS–PAGE (10% polyacrylamide) under reducing conditions. (B) Analysis of the endo-H sensitivity of the intra- and extra-cellular forms of M2BP. Lysates and supernatants of \(^{[35}\text{S}]\)methionine-labelled HepG2 cells were immunoprecipitated by an anti-M2BP polyclonal antibody and then treated with endo-H (+) or left untreated (−). Immunoprecipitates were analysed by SDS–PAGE (10% polyacrylamide) under reducing conditions. iM2BP, Intracellular form of M2BP; sM2BP, secreted form of M2BP.

Fig. 5. HCV glycoproteins but not their deglycosylated form are recognized by MAb H14 in Western blotting analysis. HepG2 cells were co-infected with vTF7-3 and vHCV170-809 (V) or vTF7-3 alone (M) and lysed with Triton X-100. Cell lysates were immunoprecipitated with MAb A4 and then treated with PNGase F (+) or left untreated (−). Immunoprecipitates were separated by SDS–PAGE (10% polyacrylamide) and revealed by Western blotting with MAb H14. HCV-specific proteins are indicated on the left and the sizes (in kDa) of molecular mass markers are indicated on the right.

MAb H14 recognizes a cellular protein as well as HCV glycoprotein aggregates

We have previously shown that ER chaperones BiP and calreticulin interact with aggregates of HCV glycoproteins (Choukhi et al., 1998). Based on these data, we wanted to know whether the epitope recognized by MAb H14 is present on HCV glycoprotein aggregates or whether it recognizes an ER chaperone interacting with them. To determine whether this MAb recognizes a cellular protein, uninfected HepG2 cells were radiolabelled and cell lysates were used for immunoprecipitation studies with MAb H14. As shown in Fig. 2, the major band recognized by MAb H14 was a cellular protein of approximately 70 kDa suggesting that HCV glycoprotein aggregates might interact with this protein. To identify the cellular protein recognized by MAb H14, internal amino acid sequences of this protein were determined. For this purpose, the 70 kDa protein was purified by immunoaffinity and digested with endoproteinase Lys-C to generate peptides. Two internal peptides were purified and sequenced (KSLGWLK and KAAIPSALDT) and they matched with the sequence of M2BP (Koths et al., 1993; Ulrich et al., 1994). Recognition of M2BP by MAb H14 was confirmed by immunoprecipitation of cell lysates with MAb H14, followed by Western blotting detection with a polyclonal antibody raised against M2BP (Fig. 3A). Similarly, M2BP precipitated by the polyclonal antibody was recognized by MAb H14 when analysed by Western blotting (Fig. 3B). Contrary to our expectations, the protein recognized by MAb H14 is not an ER chaperone. M2BP is a large oligomeric protein composed of 90 kDa glycosylated...
subunits which is secreted as a ring-like structure (Sasaki et al., 1998). When analysed in pulse–chase experiments with the polyclonal antibody, M2BP appeared as a 70 kDa band which increased in intensity during the first hour of chase (Fig. 4A). The intensity of this 70 kDa protein started to decrease after 2 h of chase and a band of approximately 90 kDa appeared, suggesting that some modifications had occurred. The intensity of the 90 kDa band was low and this is probably due to the secretion of M2BP since a similar band was precipitated from the supernatant after a 4 h chase (Fig. 4A). When analysed in pulse–chase experiments with MAb H14, the profile of detection of M2BP was very similar for the 70 kDa form (Fig. 4A). However, neither the intracellular 90 kDa protein nor its secreted form were precipitated by MAb H14, suggesting that the epitope recognized by this MAb is masked by some modification occurring in the secretory pathway. This modification probably occurs in the Golgi apparatus since the 70 kDa form of M2BP was endo-H-sensitive whereas the 90 kDa form was endo-H-resistant (Fig. 4B). Together, these data indicate that MAb H14 recognizes an immature form of M2BP.

After confirming that MAb H14 recognizes an epitope present on a cellular protein, we wanted to know whether the precipitation of HCV glycoprotein aggregates is due to their interaction with M2BP or whether they share an epitope with this cellular protein. Surprisingly, Western blotting analyses indicated that H14 recognizes both HCV glycoproteins E1 and E2 (Fig. 5); however, the intensity of the E1 band was lower. HCV glycoproteins were not recognized by the anti-M2BP polyclonal antibody (data not shown). It is worth noting that the deglycosylated forms of HCV glycoproteins were not recognized by H14 (Fig. 5), suggesting that H14 recog-
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HCV glycoproteins expressed in different cell lines (chick cells, insect cells, BHK-21, CV-1 and HepG2) were precipitated by MAb H14 suggesting that aggregates are formed in these different cell lines (data not shown). In addition, MAb H14 precipitated HCV glycoproteins expressed by viral (vaccinia and Sindbis viruses) and non-viral vectors (data not shown), indicating that the formation of HCV glycoprotein aggregates is not influenced by the expression system. Similar ratios of aggregates were observed even when the expression level was 10–20 times lower.

Since MAb H14 recognizes an epitope on both E1 and E2 (Fig. 5), we wanted to know whether the H14 epitope would be accessible on these proteins expressed alone, in the absence of any denaturation. For this purpose, HCV glycoproteins were analysed by immunoprecipitation under non-denaturing conditions. As shown in Fig. 6, MAb H14 precipitated E1 or E2 when expressed alone, suggesting that the H14 epitope is not buried in these glycoproteins. It is worth noting that these proteins were no longer recognized by MAb H14 when deleted at their C-terminus, suggesting that the H14 epitope might be present in close proximity to their transmembrane domain. However, the same truncated forms of E2 were recognized by MAb H14 in Western blotting experiments (data not shown), indicating that the H14 epitope is present on these truncated proteins but is not accessible unless the proteins are denatured. Since truncated forms of E2 also have a tendency to aggregate (Michalak et al., 1997), these data suggest that the H14 epitope is masked in these aggregates.

The kinetics of formation of HCV glycoprotein aggregates were analysed in pulse–chase experiments. As shown in Fig. 7, HCV glycoprotein aggregates recognized by MAb H14 were already formed during the pulse and the intensity of HCV glycoproteins precipitated by H14 remained rather stable during the chase. It is worth noting that MAb H14 can precipitate different glycoforms of E1 (Fig. 7). These data

Fig. 8. Immunodetection, by MAb H14, of cells infected by recombinant viruses expressing HCV glycoproteins. (A) CV-1 cells were infected with vTF7-3 and vHCV170-809 (E1E2p7) or vTF7-3 alone. At 8 h post-infection, cells were fixed with isopropanol and labelled with MAb H14 (secondary donkey anti-mouse IgG–Rhodamine). (B) CV-1 cells were infected with a Sindbis virus recombinant expressing a truncated form of HCV polyprotein (SINrep/HCV-H1-1207) at an m.o.i. of 0.5. At 8 h post-infection, cells were fixed with isopropanol and labelled with MAb H14 or A4 (secondary rabbit anti-mouse IgG–alkaline phosphatase).
suggest that HCV glycoprotein aggregates are formed very rapidly but are very slowly degraded.

To further characterize HCV glycoprotein aggregates recognized by MAb H14, we analysed their expression by immunofluorescence. As shown in Fig. 8(A), aggregates of HCV glycoproteins were localized in ER-like structures as usually observed for HCV glycoproteins (Dubuisson et al., 1994). However, when compared to the detection with other anti-E1 or anti-E2 MAbs, a smaller number of cells showed a strong ER-like signal with MAb H14 (Fig. 8B). We observed that only approximately 20% of the cells expressing HCV glycoproteins exhibited a strong ER-like signal with MAb H14, suggesting that the level of aggregation is higher in a small proportion of infected cells. Alternatively, the H14 epitope might be masked on some aggregates because aggregation is most likely heterogeneous. Similar results were observed in HepG2 cells infected by vaccinia virus–HCV recombinants (data not shown). It is worth noting that some fluorescence was also detected in control cells which did not express HCV glycoproteins (Fig. 8A). In these cells, the fluorescence accumulated in some spots which probably represent the subcellular localization of the immature form of M2BP recognized by MAb H14. However, we cannot exclude the recognition of some other cellular proteins by MAb H14. As observed in Fig. 2, high molecular mass bands were also observed after immunoprecipitation of cell lysate with MAB H14.

Discussion

HCV glycoproteins have a tendency to aggregate and recent data suggest that this tendency to form aggregates is probably an intrinsic property of these proteins (Dubuisson, 1999). To further understand the potential role played by HCV glycoprotein aggregates in HCV infection, we produced a MAb (H14) which specifically recognizes these aggregates but not the non-covalent E1E2 heterodimer. Characterization of HCV glycoprotein aggregates with the help of MAb H14, indicates that these aggregates share an epitope with a cellular protein (M2BP).

Aggregates of HCV glycoproteins share an epitope with M2BP. Viruses are major candidates for the induction of autoimmune diseases. Autoimmunity, in the form of autoantibodies, is common after many virus infections and may well result from the mimicking of host proteins by viral antigens. HCV induces a number of diseases of presumed autoimmune background, like mixed cryoglobulinaemia, glomerulonephritis, panarthritis, arthritis, thyroiditis and skin lesions (Houghton, 1996). On the other hand, a number of autoantibodies are observed during the course of HCV infection (Manns & Obermayer-Straub, 1997). Of particular interest are liver/kidney microsomal (LKM) antibodies. LKM antibodies in chronic hepatitis C recognize several autoepitopes that differ from those of autoimmune hepatitis. Hepatitis C-associated LKM antibodies are more heterogeneous. They recognize either conformational or several distinct linear epitopes on cytochrome P<sub>450</sub>2D6 (Manns & Obermayer-Straub, 1997). They may also react with other microsomal proteins. Apart from their molecular masses of 59 and 70 kDa, these microsomal antigens have not yet been identified (Durrazzo et al., 1995). The identification of a common epitope between HCV glycoprotein aggregates and M2BP suggests that M2BP might be a target for autoantibodies. This suggestion is reinforced by the fact that the microsomal form of M2BP is a protein of about 70 kDa and by the observation that among 14 HCV-positive sera tested, one reacted against M2BP (J. Dubuisson, unpublished data). Future studies should help us to evaluate the prevalence of such autoantibodies in HCV-infected patients.

HCV glycoprotein aggregates recognized by MAb H14 are expressed in a small proportion of the infected cells. Indeed, a large number of cells expressing HCV glycoproteins did not show a signal above background when analysed by immunofluorescence with MAB H14. These data suggest that, in some physiological conditions, aggregates of HCV glycoproteins are less likely to be formed. We can expect that an appropriate concentration of the chaperones and/or foldases involved in HCV glycoprotein folding could reduce the formation of aggregates. Recently, we have identified ER chaperones potentially involved in the folding and assembly of HCV glycoproteins (Choukhé et al., 1998). However, overexpression of these chaperones did not improve the folding of these glycoproteins. Since several chaperones can be involved in assisted folding of proteins in the ER, it is likely that a proper balance of chaperone activities is required for optimal folding. It is also likely that other chaperone(s) and/or foldase(s), which have not been identified yet, are necessary to assist in HCV glycoprotein folding. The use of MAB H14 should help us to determine the conditions in which cells produce these aggregates at a lower level.

Why do some proteins aggregate in vivo? Since protein folding in the cell is so complex, there is a significant likelihood of defects arising in the process. Potentially, thermodynamic destabilization of the native or an intermediate state, alteration of the folding kinetics, prolonged or inappropriate associations with chaperones or foldases, preferential formation of off-pathway or toxic conformations could all lead to loss of functional protein. Inability of an essential protein to form its native structure under physiological conditions may be the basis of a variety of human diseases (for review, see Thomas et al., 1995). In the case of HCV glycoproteins, it is hard to prove that in the course of an HCV infection, aggregates such as those characterized in this work can be formed. This is due to the absence of a tissue culture system which allows efficient replication of HCV. However, the use of different expression systems (viral or non-viral) to study the assembly of HCV glycoproteins allows us to conclude that aggregation of HCV glycoproteins is not an artifact linked to the expression system.
In the context of HCV infection, inefficient folding of the HCV glycoproteins might downregulate particle formation and virus replication to minimize exposure of viral antigens to the immune system and/or reduce pathogenicity. Alternatively, the production of HCV glycoprotein aggregates could provide these glycoproteins with additional functions in infected cells or on the particle. The non-covalent HCV glycoprotein complex previously characterized (Deleersnyder et al., 1997) is most likely the pre-budding form of the functional complex which will play an active role in the entry process in infected cells. However, it cannot be excluded that a portion of HCV glycoprotein aggregates makes up the envelope of the mature particle, providing the viral particle with additional functions. More likely, in infected cells, aggregates of HCV glycoproteins could interact with host proteins in the ER compartment whose transport or function could be altered as a consequence of these interactions. Further work will be necessary to evaluate the potential role of HCV glycoprotein aggregates in the physio-pathology of HCV infection.

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