

Cell surface expression of functional hepatitis C virus E1 and E2 glycoproteins

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Received 7 April 2003; revised 13 May 2003; accepted 21 May 2003

First published online 12 June 2003

Edited by Hans-Dieter Klenk

Abstract Hepatitis C virus (HCV) glycoproteins E1 and E2 are believed to be retained in the endoplasmic reticulum (ER) or *cis*-Golgi compartment via retention signals located in their transmembrane domains. Here we describe the detection of E1 and E2 at the surface of transiently transfected HEK 293T and Huh7 cells. Surface-localized E1E2 heterodimers presented exclusively as non-covalently associated complexes. Surface-expressed E2 contained *trans*-Golgi modified complex/hybrid type carbohydrate and migrated diffusely between 70 and 90 kDa while intracellular E1 and E2 existed as high mannose 35 kDa and 70 kDa precursors, respectively. In addition, surface-localized E1E2 heterodimers were incorporated into E1E2-pseudotyped HIV-1 particles that were competent for entry into Huh7 cells. These studies suggest that functional HCV glycoproteins are not retained exclusively in the ER and transit through the secretory pathway.

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Key words: Hepatitis C virus; Glycoprotein; E1E2-pseudotyped particle; Surface expression

1. Introduction

Hepatitis C virus (HCV) is a positive sense RNA virus and comprises the hepacivirus genus in the family *Flaviviridae*. Approximately 200 million people are chronically infected with HCV world-wide with infection rates as high as 20% in some countries. The genome of HCV encodes an approximately 3300 amino acid polyprotein that is cleaved co- and post-translationally into structural (core, E1, E2) and non-structural (NS2, 3, 4, 5a and 5b) proteins. Two small proteins of unclear function (p7 and F) are also generated from the polyprotein by distinct mechanisms. The virus cannot be readily propagated in cell culture so studies of the structure and function of the viral glycoproteins have been limited to heterologous systems that utilize transient transfection or infection with recombinant viruses that express the viral glycoproteins [1,2].

The HCV glycoproteins, E1 (polyprotein residues 191–383)

and E2 (384–746), are type 1 transmembrane proteins each comprising an N-terminal glycosylated ectodomain and C-terminal transmembrane domain (TMD). When expressed in cell culture systems, E1 and E2 form heterodimers in a TMD domain-dependent manner [3]. Two forms of E1E2 complexes can be detected with the use of monoclonal antibodies (MAbs). Conformation-dependent MAbs immunoprecipitate E1E2 complexes stabilized by non-covalent interactions whereas MAbs to linear epitopes also recognize heterogeneous disulfide-linked aggregates that are believed to represent misfolded complexes destined for degradation [4].

Previous studies investigating the localization of E1 and E2 have indicated that they are retained in the endoplasmic reticulum (ER) due to the presence of static retention signals in their TMDs and in the juxtamembrane region of E1 [5–8]. Expression of the entire structural region in mammalian and insect cells results in the formation of HCV-like particles that remain associated with budding membrane invaginations of the rough ER and membranous vacuoles [9–11]. Insect cell-derived HCV-like particles are capable of binding target cells, however, their ability to mediate membrane fusion, an obligatory step preceding viral entry, has not been demonstrated [12].

Thus evidence that hepatitis C virions mature through the *trans*-Golgi network is lacking despite an observation pointing to *trans*-Golgi network modifications of HCV sugar moieties [13]. Whereas our understanding of HCV maturation is incomplete, the maturation pathway of flaviviruses, which are phylogenetically related to HCV, involves the *trans*-Golgi network [14].

Here we describe the detection of native E1 and E2 expressed at the cell surface. Cell surface-localized E1E2 exist as non-covalently associated heterodimers, with surface-localized E2 exhibiting *trans*-Golgi modified complex/hybrid type carbohydrates. Plasma membrane-localized E1E2 heterodimers can be incorporated into retroviral pseudoparticles and represent a functional form of the glycoproteins mediating viral entry. These studies suggest that functional HCV glycoproteins are not retained exclusively in the ER and transit through the secretory pathway.

2. Materials and methods

2.1. Cell lines and antibodies

HEK 293T cells and Huh7 cells were maintained in Dulbecco's minimal essential medium containing 10% fetal calf serum and 2 mM L-glutamine (DMF10). MAbs A4, A11 and H53 were a kind gift from Dr. Jean Dubuisson. Immunoglobulin G was purified from plasma obtained from an HCV-infected individual using protein

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Abbreviations: HCV, hepatitis C virus; TMD, transmembrane domain; MAb, monoclonal antibody; ER, endoplasmic reticulum; hIgG, human immunoglobulin G; C, core; ECL, enhanced chemiluminescence

G Sepharose (hIgG; Amersham Pharmacia Biotech). MAb 9E10, directed to the myc epitope tag, was purified from the cell line MYC 1-9E10.2 using protein G Sepharose (Amersham Pharmacia Biotech). Mouse MAb specific to calnexin (BD Transduction Laboratories) and rabbit anti-giantin antibody (Covance Research Products) were used at 1/500 and 1/2000 dilutions in Western blotting, respectively.

2.2. Vectors

pBRTM/HCV 1-3011con was a generous gift from Dr. C. Rice, Laboratory of Virology and Infectious Disease, Center for the Study of Hepatitis C, The Rockefeller University. The full-length infectious clone pCV-H77c was a generous gift from Dr. J. Bukh and Dr. R. Purcell, Hepatitis Viruses Section, NIAID, National Institutes of Health [15]. The HIV-1 luciferase reporter vector NL4-3.LUC.R-E was obtained from Dr. N. Landau through the NIH AIDS Research and Reference Reagent program [16]. pCDNA4HisMax HCV protein expression vectors were prepared as follows. DNA fragments encoding core (C), E1, E2 and p7 were amplified using PCR with the primers Fcore: ggtGGAATTctgatgagcagaatcctaacc and p7r: ccTCTAG-Actcgtcgatgcccgtgagg (polyprotein numbering 1–813; pCE1E2p7); CE1E2 with Fcore and E2r: ccTCTAGActcgcgcctccgttgggatag (1–746; pCE1E2), E1E2p7 with FE1: ggtGGAATTctggcaacagggaaccttctgg and p7r (165–813; pE1E2p7), E1E2 with FE1 and E2r (165–746; pE1E2) from HCV-Hcon. A fragment encoding E1E2 was also amplified from pCV-H77c DNA using E1f and E2r to generate the vector pE1E2H77c. A fragment encoding E2 (363–746) was amplified

with E2f (ccgGGTACcctcatggtggggaactgg) and E2r to generate pE2. PCR products were digested with *Eco*RI and *Xba*I or *Kpn*I and *Xba*I for pE2 and cloned into similarly digested pcDNA4HisMax vector (Invitrogen). Inserts were fully sequenced on an ABI automated sequencer. Vectors were transfected into HEK 293T and Huh7 cells using Fugene 6 (Roche) according to the manufacturer's recommendations.

2.3. Immunofluorescence

Huh7 cells, grown on coverslips, were transfected with plasmid DNA and Fugene 6 and 24 h later, placed on ice for 30 min. Cells were either left untreated (unfixed) or fixed in 4% paraformaldehyde for 20 min and permeabilized in 0.5% Triton X-100 for 1 min. Antibody was diluted in FACS buffer (phosphate-buffered saline (PBS) containing 5% bovine serum albumin (BSA), 1 mM EDTA, 0.02% sodium azide) and added to unfixed cells or fixed and permeabilized cells, and incubated on ice for 30 min. After three washes, Alexa 488-conjugated goat anti-mouse antibody diluted in FACS buffer was added to cells and incubated for a further 30 min on ice. After three further washes, unfixed cells were fixed and coverslips were mounted in DAKO fluorescence mounting medium and visualized on an Axi-overt 25 microscope.

2.4. Radioimmunoprecipitation and biotinylation

HEK 293T cells seeded in six-well culture plates at 5×10^5 cells/well were transfected 24 h later with plasmid DNA using Fugene 6

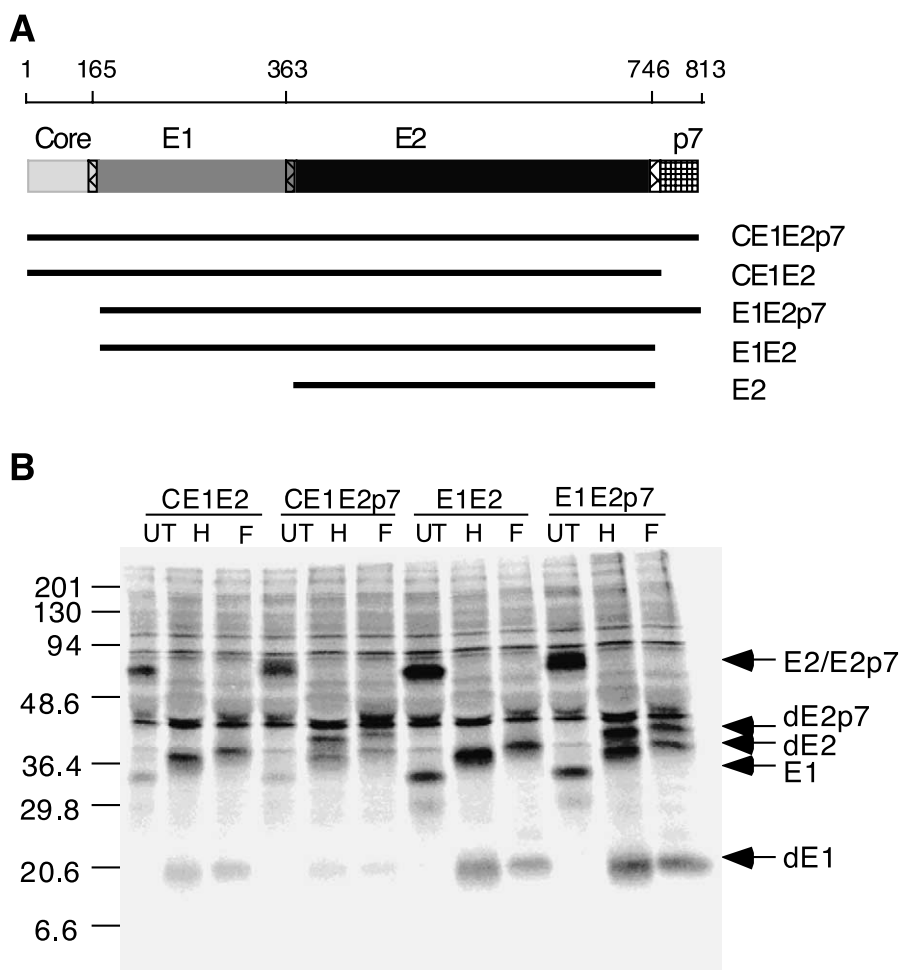


Fig. 1. Expression of HCV glycoproteins E1 and E2 in HEK 293T cells transfected with pCE1E2, pCE1E2p7, pE1E2 and pE1E2p7. A: Schematic representation of HCV regions investigated in this study. The numbering refers to the first or last residue included in each construct. Cross hatches represent hydrophobic domains incorporating signal peptide and TMDs. B: E1 and E2 glycoproteins were immunoprecipitated with IgG obtained from an HCV-infected individual (hIgG). Glycoproteins were not treated (UT) or deglycosylated with endoglycosidase H (H) or PNGase F (F) and followed by reducing SDS-PAGE and phosphorimager analysis. The locations of E1, E2, E2/p7 or deglycosylated forms (d) are indicated by arrows.

(Roche). At 24 h post-transfection, the cells were labelled with 150 μ Ci Tran-³⁵S-label (ICN, Costa Mesa, CA, USA) in methionine/cysteine deficient medium for 15 min and then chased in complete medium for a further 4 h. Cell lysates were prepared in lysis buffer (1% Triton X-100, 0.05 M Tris-HCl pH 7.4, 0.6 M KCl) and clarified at 10 000 $\times g$ for 10 min. In some experiments, cells were treated with membrane impermeant sulfo-NHS-long chain biotin (Pierce) prior to lysis. Sulfo-NHS-long chain biotin (1.25 mM in PBS) was added to cells and incubated on ice for 30 min, washed and then quenched with 50 mM glycine in PBS for 30 min. Proteins were precleared using Sepharose coupled to BSA for a minimum of 1 h followed by immunoprecipitation with protein G Sepharose and antibody. Proteins were electrophoresed under reducing or non-reducing conditions in SDS-PAGE followed by electrophoretic transfer to nitrocellulose. Surface-biotinylated proteins were detected with streptavidin coupled to horseradish peroxidase (HRP) and enhanced chemiluminescence (ECL; Roche). Radiolabelled proteins were visualized by exposing the nitrocellulose membrane to a phosphorimager screen after the ECL procedure.

2.5. Subcellular fractionation

pE1E2-transfected HEK 293T cells were homogenized (1 ml, 250 mM sucrose, 1 mM EDTA, 10 mM Tris-HCl pH 7.4) by passing through a 25G needle fitted to a 1 ml syringe 10 times. The homogenate was centrifuged (500 $\times g$, 10 min) and the post-nuclear supernatant applied to a 10–24% continuous iodixanol (Accudenz) gradient (3 mM KCl, 5 mM Tris-HCl pH 7.5, 0.3 mM EDTA, 0.75% NaCl) and centrifuged for 90 min (SW 41, 37 000 rpm). Gradients were fractionated from the bottom of the tube and proteins precipitated using methanol/chloroform before analysis on SDS-PAGE and Western blotting.

2.6. E1E2-HIV-1 pseudotype particle entry assay

293T cells seeded at 350 000 cells/well in six-well culture dishes were transfected with 1 μ g each of NL4-3.LUC.R-E- and either pE1E2H77c, pCDNA4HisMax or pE2. Three days later, the tissue culture fluid containing pseudotyped HIV-1 particles was collected, filtered (0.45 μ m) and added to Huh7 cells (30 000/well) in 48-well culture plates. Following 7 h incubation at 37°C, the inoculum was removed and the cells cultured for a further 3 days. Luciferase activity was measured in a Microumat LB (Berthold) luminometer using the Promega luciferase reagent system.

3. Results

3.1. Expression of HCV glycoproteins

Previous studies examining the subcellular localization of E1 and E2 used cells infected with recombinant vaccinia viruses expressing E1 and E2 [17]. These studies concluded that the HCV structural proteins are retained in the ER or a *cis*-Golgi compartment based on the lack of immunofluorescent detection of E1 or E2 at the cell surface, endoglycosidase H sensitivity of E1 and E2 carbohydrate groups and the colocalization of E1 and E2 with ER-resident proteins in subcellular fractionation/immunofluorescence experiments. In order to examine E1E2 localization in an expression system that did not require vaccinia virus infection, we cloned the structural region of HCV-Hcon (genotype 1a) into the vector pCDNA4-HisMax (Invitrogen). This cytomegalovirus promoter-driven expression vector also encodes a translational enhancer for increased levels of protein expression [18]. HEK 293T cells were transfected with vectors pCE1E2, pCE1E2p7, pE1E2 or pE1E2p7 (Fig. 1A) and proteins were biosynthetically labelled with [³⁵S]Met/Cys. After cell lysis, E1 and E2 were immunoprecipitated with IgG purified from the plasma of an HCV-infected individual (hIgG) and subjected to SDS-PAGE. Both E1 and E2 were detected for each expression vector (Fig. 1B, UT). In addition to E2, E2p7 was detected in vectors encoding CE1E2p7 or E1E2p7 suggesting that co-

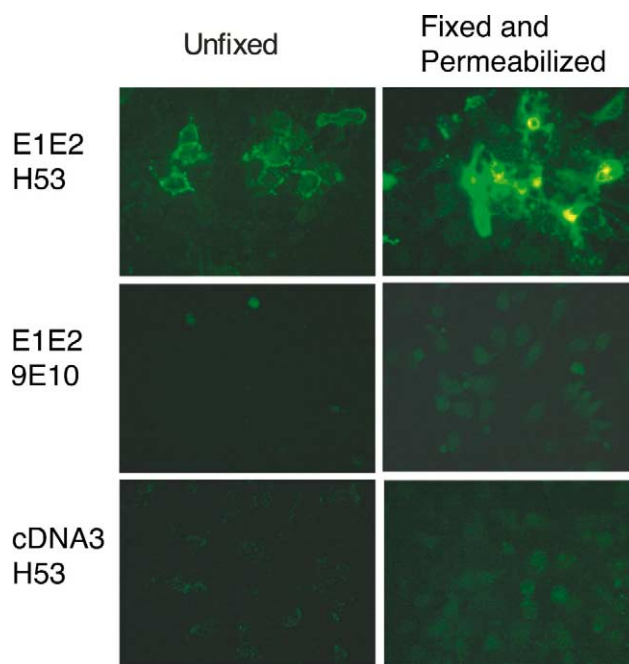


Fig. 2. Immunofluorescence of Huh7 cells transfected with pE1E2 or a control vector, cDNA3. Twenty-four hours after transfection, cells were placed on ice and stained directly with conformation-dependent E2 specific MAb H53 or irrelevant MAb 9E10 (unfixed). Alternatively, cells were fixed in 4% paraformaldehyde, permeabilized in 0.1% Triton X-100 before the addition of H53. Bound antibody was detected with Alexa 488 goat anti-mouse antibody. Fluorescence was examined using an Axiovert microscope.

or post-translational cleavage at the E2/p7 junction was not complete. The carbohydrate content of biosynthetically labelled E1 and E2 was examined by deglycosylation with endoglycosidase H or PNGase F and reducing SDS-PAGE. After digestion with either enzyme both E1 and E2 were reduced to their theoretical backbone molecular weights of 21 kDa and 40 kDa, respectively (Fig. 1B). These results indicate that E1 and E2 contain high mannose carbohydrate suggestive of retention in the ER or in a *cis*-Golgi compartment.

In order to determine if the HCV glycoproteins expressed from these vectors also exhibit an intracellular localization, we examined transfected Huh7 cells by immunofluorescence using the conformational anti-E2 MAb H53. Unexpectedly, we observed strong cell surface staining for unfixed pE1E2-transfected Huh7 cells (Fig. 2) under conditions that preclude staining of the ER-resident chaperone, calnexin (data not shown). By contrast, fixation and permeabilization prior to staining gave rise to intense perinuclear fluorescence (Fig. 2), in agreement with previous studies [7]. Immunofluorescence was not observed for pE1E2-transfected cells stained with the control MAb 9E10 nor for control pCDNA4-transfected cells stained with MAb H53 confirming that the fluorescence observed is due to a specific interaction between E2 and MAb H53 (Fig. 2). Similar data were obtained for Huh7 cells transfected with pCE1E2, pCE1E2p7 and pE1E2p7 (data not shown). These results indicate that a significant proportion of E2 is surface-expressed.

3.2. Subcellular fractionation of E1E2-transfected cells

We next employed iodixanol density gradient centrifugation in order to determine if E1 and E2 could be detected in non-

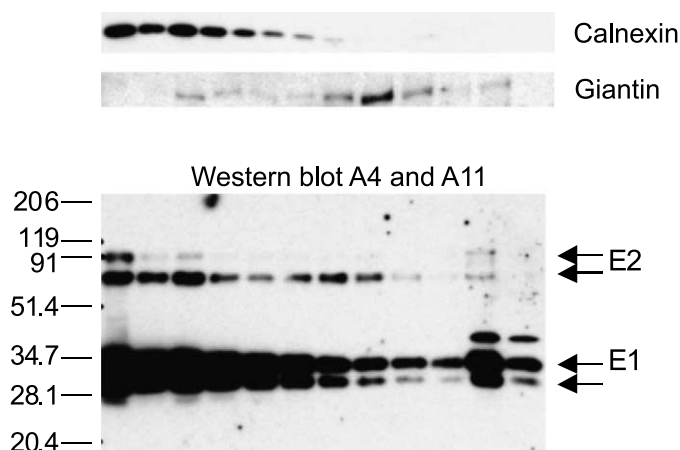


Fig. 3. Subcellular fractionation of HEK 293T cells transfected with pE1E2. Twenty-four hours after transfection, cells were homogenized and a post-nuclear supernatant applied to a 10–24% iodixanol gradient and ultracentrifuged for 90 min. Fractions were collected from the bottom of the tube (left) and analyzed in reducing SDS–PAGE and Western blot assay for the presence of calnexin, giantin or E1 (A4) and E2 (A11). The direction of sedimentation is right to left.

ER compartments. Unlike sucrose, iodixanol allows the separation of intracellular organelles under isotonic conditions. pE1E2-transfected HEK 293T cells were homogenized and the post-nuclear supernatant layered onto 10–24% continuous iodixanol gradients. Fractions were analyzed for the presence of the ER-resident protein, calnexin, *cis-medial* Golgi-resident protein giantin, E1 (MAb A4) and E2 (MAb A11) using Western blotting. Fig. 3 shows that the majority of E1 and E2 sedimented in fractions corresponding to ER. However, we also observed a small amount of E1 and E2 in low-density fractions, with a small proportion of E2 migrating in SDS–PAGE at a higher molecular weight than was observed for the ER-localized material. This higher molecular weight is suggestive of *trans*-Golgi oligosaccharide modifications consistent with a small proportion of E1E2 entering the secretory pathway.

3.3. Biochemical analysis of HCV proteins expressed at the cell surface

The results of immunofluorescence (Fig. 2) and iodixanol density gradient experiments (Fig. 3) indicate that a small proportion of total E1E2 escapes the ER and enters the secretory pathway. We therefore employed the sensitive technique of surface biotinylation in order to ask if we could directly detect E1 and E2 at the cell surface. To biochemically compare the total E1 and E2 with the surface-localized component, pE1E2H77c-transfected HEK 293T cells were pulse-chase biosynthetically labelled and then biotinylated with the membrane impermeant agent sulfo-NHS-long chain biotin. E1E2 complexes were immunoprecipitated with MAb H53 or hIgG and subjected to SDS–PAGE and Western blotting using streptavidin–HRP for detection of biotinylated proteins. Total HCV glycoproteins were visualized by scanning nitro-

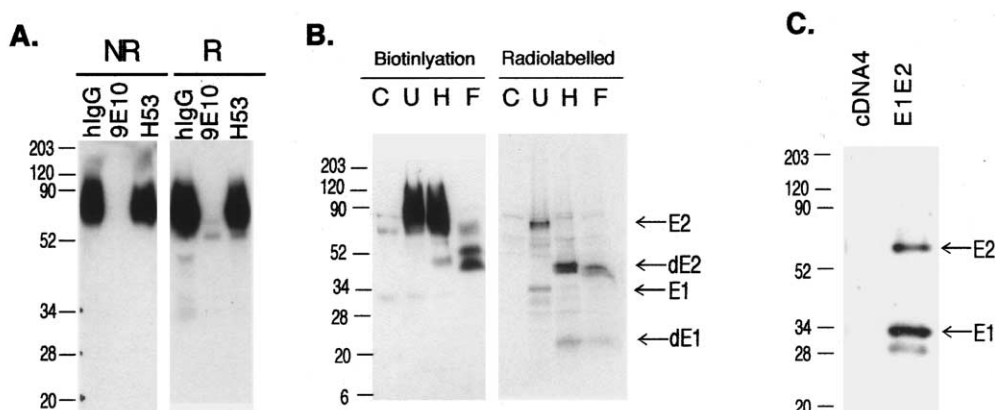


Fig. 4. Cell surface biotinylation of HEK 293T cells transfected with pE1E2. A: Following surface biotinylation, E2 was immunoprecipitated with MAb H53, hIgG or irrelevant MAb 9E10. Samples were analyzed on non-reducing (NR) and reducing (R) SDS–PAGE followed by Western blotting. Biotinylated proteins were visualized with avidin–HRP and ECL detection. B: pE1E2- (lanes U, H, F) or cDNA4- (lane C) transfected cells were pulse-chase-labelled with [35 S]Met/Cys followed by surface biotinylation. E1 and E2 were immunoprecipitated with hIgG and deglycosylated with endoglycosidase H (H), PNGase F (F) or untreated (U and C). Samples were analyzed in reducing SDS–PAGE followed by Western blotting. Following visualization of biotinylated proteins with avidin–HRP and ECL, blots were phosphorimaged to examine total immunoprecipitated protein. C: Surface-expressed E2 is present in a complex with E1. HEK 293T cells were transfected with pE1E2H77c and 24 h later surface-expressed proteins biotinylated. Cells were lysed and applied to avidin-agarose, then subjected to reducing SDS–PAGE and Western blotting. E1 and E2 were detected by ECL using MAb A4 and A11, and rabbit anti-mouse HRP.

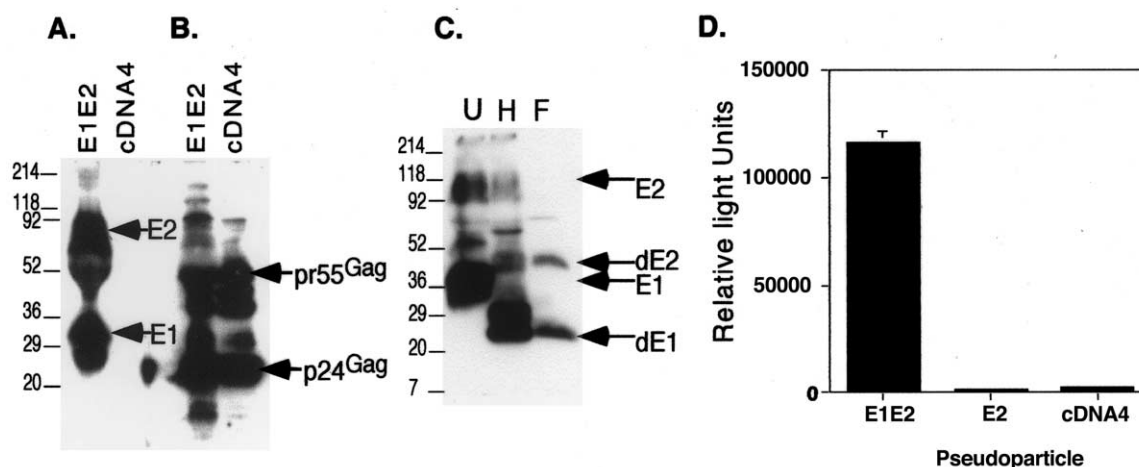


Fig. 5. Surface-localized E1E2 heterodimers are incorporated into retroviral pseudoparticles and mediate receptor binding and entry into Huh7 cells. 293T cells were co-transfected with pE1E2H77c and NL4-3.LUC.R-E-. Supernatants containing E1E2-pseudotyped retroviral particles were partially purified on 20% sucrose cushions and protein content analyzed in reducing SDS-PAGE and Western blotting with (A) MAbs A4 and A11 or, (B) HIV-1 p24 specific MAb 183. C: Carbohydrate content of HCV-pseudotyped retroviral particles. Partially purified particles were either untreated (U) or deglycosylated with endoglycosidase H (H) or PNGase F (F). E1 and E2 were detected in Western blotting with MAbs A4 and A11. D: Supernatants containing HCV-pseudotyped retroviral particles were applied to Huh7 cells and 3 days later luciferase activity quantitated in a luminometer. Data shown are the mean \pm S.D. from triplicate transfections and are representative of two independent assays.

cellulose membranes in a phosphorimager following the ECL procedure. Both H53 and hIgG immunoprecipitated biotinylated E2 (Fig. 4A). This E2 band migrated close to its expected monomer position in non-reducing SDS-PAGE, consistent with the absence of covalently linked E2 aggregates (Fig. 4A). Biotin-E2 was found to be endoglycosidase H resistant suggesting that it transits through the *trans*-Golgi network; PNGase F reduced the majority of biotin-E2 to its backbone molecular weight of 40 kDa (Fig. 4B). In contrast, metabolically labelled E1 and E2 remained sensitive to endoglycosidase H suggesting that the majority of E1 and E2 is retained in the ER (Fig. 4B). Direct biotinylation of E1 at the cell surface was difficult to achieve, the anti-E1 MAb A4 co-precipitating substantial amounts of biotin-E2 and a very faint band corresponding to E1 (data not shown). However, evidence that E1 was complexed with E2 at the cell surface was obtained by using avidin-agarose to pull down biotinylated E2 before detection of E1 with MAb A4 in immunoblot (Fig. 4C). This result suggests that E2 may obscure E1 from biotinylation within the cell surface-localized complex. E1E2 surface expression was also observed for pCE1E2, pCE1E2p7 and pE1E2p7 vectors indicating that the presence of the other structural proteins did not affect E1E2 translocation (data not shown).

3.4. HIV-1 particles pseudotyped with surface-localized E1E2 are entry-competent

In order to investigate if surface-localized E1E2 could mediate viral entry, we produced E1E2-pseudotyped HIV-1 luciferase reporter virus and tested its ability to establish a single cycle of replication in Huh7 cells. HEK 293T cells were co-transfected with pE1E2H77c and the HIV-1 luciferase reporter vector, NL4-3.LUC.R-E-. NL4-3.LUC.R-E- is derived from the pNL4-3 proviral clone and contains the firefly luciferase open reading frame in place of *nef* but lacks functional *vpr* and *env* [16]. We first confirmed the production of particulate E1E2 (Fig. 5A) and HIV-1 structural proteins, Pr55^{Gag}

and p24^{Gag} (Fig. 5B) by sedimenting pseudotyped particles through a sucrose cushion prior to SDS-PAGE and Western blotting with MAb A4/A11 mixture or anti-p24 MAb 183. The particulate E2 was partially resistant to endoglycosidase H (Fig. 5C) consistent with the incorporation of cell surface-localized E1E2 complex into HIV-1, which assembles and buds at the plasma membrane. However, E1 appeared to remain sensitive to deglycosylation with endoglycosidase H (Fig. 5C). We next tested if E1E2-HIV-1 pseudotypes were entry-competent. Filtered supernatants from co-transfected HEK 293T cells were applied to Huh7 cells and luciferase reporter expression assayed 72 h later. Fig. 5D shows that E1E2-HIV-1 pseudotypes gave rise to substantial luciferase activity when compared to the controls, HIV-1 pseudotyped with E2 alone or lacking envelope glycoprotein. These results indicate that E1E2-HIV-1 pseudotypes can mediate viral entry resulting in reporter gene expression. These data also indicate that surface-expressed E1E2 heterodimers are indeed functional.

4. Discussion

Previous studies of HCV glycoproteins have focussed on intracellular E1E2 forms due to observations indicating that they are retained in an ER- or *cis*-Golgi-derived compartment [5–8]. Intracellular forms of E1E2 are heterogeneous comprising non-covalently associated E1E2 heterodimers together with covalently associated aggregates [4,17,19]. In addition, a significant proportion of non-covalent complexes are associated with ER chaperones BiP and calnexin [4]. These characteristics have hampered rigorous investigations of E1E2 structure and function. In this study, we found that the majority of E1 and E2 expressed in transiently transfected cells are indeed ER-associated. However, a significant proportion of E1 and E2 were also detected in non-ER compartments and at the cell surface indicating that the ER retention is not absolute. The detection of surface-expressed E2 may

have been facilitated by the use of sensitive detection methods including surface biotinylation and fluorescence microscopy employing a high-intensity fluorophore (Alexa 488). Furthermore, the use of iodixanol instead of sucrose in fractionation experiments revealed the presence of E1E2 in non-ER or non-*cis*-Golgi compartments. Unlike sucrose, iodixanol allows subcellular fractionation under isotonic conditions thereby limiting disruption to organelles. Such techniques have not been used previously to study E1E2 localization.

Whereas surface-localized E2 was readily modified by the membrane-impermeable biotinylation reagent, surface-biotinylated E1 was not easily detected even after immunoprecipitation with the high affinity anti-E1 MAb A4. However, significant amounts of E1 were co-precipitated by avidin-agarose in a complex with biotinylated E2. These data suggest that in surface-localized complexes, E1 is shielded by E2, precluding E1 biotinylation. This relationship between E1 and E2 is reminiscent of the organization of flavivirus glycoproteins where the large envelope protein E shields the small envelope protein M [20,21].

We and others have produced entry-competent retrovirus particles pseudotyped with E1E2 heterodimer [22]. As retroviruses assemble and bud at the plasma membrane, these findings are entirely consistent with our observation that E1E2 complexes are surface-expressed. Moreover, the results presented here and elsewhere [22] indicate that surface-localized E1E2 is functional because it has the capacity to mediate receptor-binding and membrane fusion when incorporated into pseudovirions. The absence of complex carbohydrate on E1, despite maturation through the secretory pathway, may also be due to E2 shielding E1 from carbohydrate modification enzymes in a correctly folded heterodimeric complex. We found that surface-localized E1E2 was present exclusively as non-covalently associated heterodimers, in contrast to the heterogeneous nature of intracellular forms. The HCV glycoproteins may therefore enter distinct folding pathways whereby ER retention signals are negated in a small proportion of non-covalently associated E1E2 complexes that proceed via the secretory pathway to the cell surface. The HCV maturation pathway may therefore share some characteristics with that of flaviviruses where the majority of viral proteins remain associated with the ER and relatively few assembled particles enter the secretory pathway prior to budding [23]. It would now be of interest to examine the subcellular localization and cell surface distribution of E1 and E2 in systems that may more closely resemble HCV infection such as full-length HCV replicons.

Acknowledgements: We would like to thank Dr. Jean Dubuisson, Institut de Biologie de Lille, Institut Pasteur de Lille, for the provision of MAbs A4, A11 and H53. We thank Chris Vassos for excellent technical support. This work was supported by NHMRC project Grant 156714.

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