Ubiquitination of the human immunodeficiency virus type 1 Env glycoprotein

Citation for published version:

Digital Object Identifier (DOI):

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published in:
Journal of Virology

Publisher Rights Statement:
Copyright © 2000, American Society for Microbiology. All Rights Reserved.

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Ubiquitination of the Human Immunodeficiency Virus Type 1 Env Glycoprotein

ANDREAS BÜLTMANN, JOSEF EBERLE, AND JÜRGEN HAAS*
Max-von-Pettenkofer Institut, Genzentrum, LMU München, 81377 Munich, Germany

Received 12 October 1999/Accepted 7 March 2000

Expression of the human immunodeficiency virus type 1 (HIV-1) Env glycoprotein is stringently regulated in infected cells. The majority of the glycoprotein does not reach the cell surface but rather is retained in the endoplasmic reticulum or a cis-Golgi compartment and subsequently degraded. We here report that Env of various HIV-1 isolates is ubiquitinated at the extracellular domain of gp41 and that Env expression could be increased by lactacystin, a specific proteasome inhibitor, suggesting that the ubiquitin/proteasome system is involved in control of expression and degradation.

The human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein gp160 is produced as a precursor polyprotein, which is cleaved in a Golgi or post-Golgi compartment by a cellular furin-type protease into gp120 and gp41 subunits (13, 19, 22, 25, 26). Although Env cleavage efficacy during its transport through the secretory pathway depends on both cell type and virus isolate, it is very inefficient; the majority of the Env glycoproteins remain uncleaved and retained in the endoplasmic reticulum (ER) or a cis-Golgi compartment (8). Accordingly, the majority of the gp160 glycoproteins remain endoglycosidase H sensitive, which is indicative of proteins that do not reach the medial Golgi (14, 27). Retained and probably misfolded Env is subsequently degraded, which was reported to occur in either lysosomes (31) or a Golgi-associated compartment (14). However, the current view of protein degradation holds that lysosomes are reached through either endosomes or late Golgi compartments.

The ER is the entry site of proteins into the secretory pathway. It is responsible for proper folding of proteins before transport to the cis-Golgi compartment and acts to ensure that misfolded and nonassembled proteins are eliminated (reviewed in references 10 and 15). Recently, the ubiquitin/proteasome system located in the cytoplasm was identified as the major site of degradation for ER-resident proteins as well as proteolytic substrates of the secretory pathway destined for the proteasome system located in the cytoplasm (12, 18, 23). In HIV-infected cells, GRB78 BIP was shown to bind to Env (9) together with other ER-based chaperones including calnexin (16, 21) and calreticulin (21). As the majority of Env is retained in the ER and subsequently degraded without reaching late Golgi compartments, we asked whether the ubiquitin/proteasome system is involved in HIV-1 Env degradation and whether ubiquitinated Env can be detected in infected cells.

First, we tested whether Env glycoprotein of the MN isolate can be reproducibly detected using antiubiquitin and anti-Env antibodies (Fig. 1a). Upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) Env glycoprotein was detected in infected cells. The majority of the glycoprotein does not reach the cell surface but rather is retained in the endoplasmic reticulum or a cis-Golgi compartment and subsequently degraded. We here report that Env of various HIV-1 isolates is ubiquitinated at the extracellular domain of gp41 and that Env expression could be increased by lactacystin, a specific proteasome inhibitor, suggesting that the ubiquitin/proteasome system is involved in control of expression and degradation.

*Corresponding author. Mailing address: Max-von-Pettenkofer Institut, Genzentrum, LMU München, Feodor-Lynen-Str. 25, 81377 Munich, Germany. Phone: 49 89 2180 6882. Fax: 49 89 2180 6889. E-mail: haas@lmb.uni-muenchen.de.
Cells were disrupted and separated into membrane and cytosolic fractions by centrifugation at 20,000 × g. Cells were pretreated with 5 μM lactacystin (+) or culture medium alone (−) for 1 h. Lactacystin was also present during the pulse and chase periods. Cells were metabolically labeled for 1 h, and membrane (m) and cytosolic (c) fractions were isolated by centrifugation at 20,000 × g for 15 min. The pellet was resuspended and washed once in hypotonic buffer. The lysates were split, and each fraction was preprecipitated with human antisera 95-2 against HIV-1 (a), first precipitated with a polyclonal serum against ubiquitin (U5379), and reprecipitated with antisera 95-2 (b), or precipitated with a monoclonal antibody against MHC class I (W6/32) (c). Immunoprecipitates were subjected to electrophoresis on either an 8% (gp160) or 12% (MHC class I) gel. Transiently transfected 293T cells expressing Env MN were pulsed for 1 h with [35S]Cys-Met and subsequently chased for 0 and 5 h. Lactacystin was also present during the pulse and chase periods. Cells were disrupted and separated into membrane and cytosolic fractions by centrifugation. After both 0 and 5 h of chase, ubiquitinated Env was found only in the membrane (pellet), not in the cytosolic fraction (supernatant). Due to the low Env cleavage efficiency in 293T cells, almost no gp120 could be detected even after 5 h of chase. To control for proper fractionation, major histo compatibility complex (MHC) class I molecules were precipitated from both fractions. As previously reported, MHC class I molecules were precipitated from the membrane but not in the cytosolic fraction under the conditions used. Therefore, we have no evidence for a contamination of the two fractions and conclude that the main portion of ubiquitinated Env is still membrane bound and not located within the cytosol in soluble form. Addition of lactacystin, a specific proteasome inhibitor, increased the amount of both Env and ubiquitinated Env after 0 and 5 h of chase (Fig. 2). Thus, these data suggest that ubiquitinated Env is at least partly degraded by proteasomes and that Env is ubiquitinated prior to translocation into the cytosol.

Ubiquitin is usually attached to lysine residues in long polymeric chains (7, 11) but can also be detected in monoubiquitinated (20, 29) and in lysineless proteins (5, 6). To determine the site of ubiquitination, C-terminal Env deletion mutants were tested by reprecipitation with antiubiquitin and anti-Env antibodies (Fig. 3a and b). Ubiquitinated Env MN was detected in transfected 293T cells after a 1-h pulse if gp160, gp140ac, or gp130ac was expressed, but not for gp120, suggesting that ubiquitin is bound to the extracellular part of gp41. Further evidence for this binding site was received by proteinase K digest of isolated microsomes from 293T cells expressing Env (Fig. 3c). Ubiquitinated Env was detected by reprecipitation in undigested as well as in proteinase K-digested microsomes, indicating that proteolytic deletion of the cytoplasmic Env domain does not disrupt ubiquitination. Since we were not able to detect the proteinase K-digested Env protein by Western blot analysis with monoclonal antibody 1577 directed against residues 735 to 752 of gp41, the cytoplasmic gp41 domain had been completely degraded (Fig. 3d). Sequence comparison between the four ubiquitinated Env glycoproteins shown in Fig. 1 revealed three conserved lysine residues as potential sites of ubiquitination. Our results indicating that ubiquitinated Env is still membrane associated and that ubiquitin is bound to the extracellular, luminal part of gp41 suggest that ubiquitination, translocation, and ER degradation of Env constitute an integrated process, as recently discussed for other luminal and integral membrane proteins (4, 24). To test whether ubiquitinated Env can also be detected in HIV-1-infected cells, we metabolically labeled C8166 cells infected with either HIV-1 MN or HIV-1 MvP 899 and reprecipitated cell lysates with antiubiquitin and anti-Env antisera (Fig. 4). In accordance with the experiments shown in Fig. 3, mapping the ubiquitination site, we detected ubiquitinated gp160 but not gp120 in cells infected with both HIV-1 isolates.

In this study, we showed that the extracellular domain of HIV-1 gp41 is ubiquitinated. Since the proteasome inhibitor lactacystin increased the amount of Env, we assume that ubiquitination occurs prior to degradation in the proteasome. This is consistent with and might explain observations by us and other groups that the majority of Env molecules are retained in dislocated to the cytosolic proteolytic system by retrograde transport, in which at least for some proteins components of the Sec61 translocon and the proteasome appear to be involved (17, 30). We thus tested by particulate fraction experiments whether ubiquitinated Env is membrane associated or can be found in the cytosol (Fig. 2). Transfected 293T cells were metabolically labeled for 1 h and chased for 0 and 5 h. Cells were disrupted and separated into membrane and cytosolic fractions by centrifugation. After both 0 and 5 h of chase, ubiquitinated Env was found only in the membrane (pellet), not in the cytosolic fraction (supernatant). Due to the low Env cleavage efficiency in 293T cells, almost no gp120 could be detected even after 5 h of chase. To control for proper fractionation, major histocompatibility complex (MHC) class I molecules were precipitated from both fractions. As previously reported, MHC class I molecules were precipitated from the membrane but not in the cytosolic fraction under the conditions used. Therefore, we have no evidence for a contamination of the two fractions and conclude that the main portion of ubiquitinated Env is still membrane bound and not located within the cytosol in soluble form. Addition of lactacystin, a specific proteasome inhibitor, increased the amount of both Env and ubiquitinated Env after 0 and 5 h of chase (Fig. 2). Thus, these data suggest that ubiquitinated Env is at least partly degraded by proteasomes and that Env is ubiquitinated prior to translocation into the cytosol.
the ER and stay endoglycosidase H sensitive (14, 27). Intrigu-
ingly, recent reports suggested alternative explanations for ubi-
quitylated viral proteins. It was shown that p6^gag^ of HIV-1 and
simian immunodeficiency virus, as well as p12^gag^ of murine
leukemia virus and US9 of herpes simplex virus type, 1 are
ubiquitinated and can be found within virus particles, in addi-
tion to free ubiquitin (2, 5, 20). The significance of ubiquiti-

REFERENCES

Increased immune response elicited by DNA vaccination with a synthetic


immunodeficiency viruses: implications for pathogenesis and vaccines. Sci-

the Sec61p complex, an integral component of the ER membrane, by the

tination and degradation at the ER surface. Science 278:1806–1809.


