Supplementary data

Disruption of the SU-TM disulphide-bond by SDS-PAGE

Results

The *env*^{ampho} gene was expressed in BHK-21 cells using SFV vectors. The cells were labeled with [^{35}S]Cys for 6 h and then lysed in the presence of NEM. Env was isolated with pAb and separated by non-reducing SDS-PAGE (8 %). The SU-TM complexes and their precursor gp85 were eluted and analyzed by non-reducing or reducing SDS-PAGE (12 %) together with a sample of the original immunoisolated Env preparation (Fig. I). Analysis of the latter sample revealed gp85 as the major band with SU-TM complexes running behind and apparently some non-covalently linked SU in front (lane1). Analysis of the eluted SU-TM complexes showed that ~10 % underwent bond-disruption during elution/re-running and appeared as non-linked subunits (lanes 2, 4 and 5). One might argue that the non-linked SU represents contamination of the SU that seems to migrate at the front of gp85 in the immuno-isolated sample (lane 1). However this is unlikely as there was no evident gp85 contamination.

Fig.I

Disruption of SU-TM disulphide-bond by SDS-PAGE. Analyses by non-reducing SDS-PAGE (12 %) of immunoisolated Env (lane 1), gel-isolated SU-TM complexes (lanes 2, 4 and 5) and gel-isolated gp85 (lane 3). Analyses by reducing SDS-PAGE of gel-isolated SU-TM complexes (lane 6). Note the migration difference of oxidized (lanes 2,4 and 5) and reduced (lane 6) SU.

Thiol-mapping of disulphide linked trypsin fragments from intact or isomerized SU-TM complexes

Results

[^{35}S]Cys labeled Env^{ampho} from VLPs was treated with MB or NEM in its non-isomerized or isomerized form, deglycosylated with N-glycanase F, digested with trypsin and the peptides analyzed by SDS-tricin-gel electrophoresis under non-reducing conditions (Schagger and von Jagow, 1987). From the protein sequence and the disulphide status, as adopted from that of SU^{Friend} and TM^{Mo}, it can be predicted that trypsin digestion of non-isomerized Env generates one heavily labeled 14,7 kD complex of disulphide-linked fragments and several much smaller ones under non-reducing conditions (Fig. IIA) (Fass and Kim, 1995; Linder et al., 1992). The 14.7 kD complex contains one fragment (4.9 kD), with the CXXC-motif, together with two additional ones (1.8 and 6.0 kD) from the C-terminal region of the SU subunit, and two fragments (0.9 and 1.6 kD) from TM. Analyses of the trypsin digest by SDS-PAGE showed an intensively labeled band-doublet in the 16.5-15 kD range and several faster migrating bands (Fig. IIB, lanes 1-3). The band doublet demonstrated a clear migration shift when comparing the NEM (MW 125 kD) and the MB (MW 523 kD) treated samples, suggesting that they contain the modified thiol. As the slower migrating band could be converted into the faster one by increasing trypsin concentration in the Env digestion the latter probably represents a complex of complete digestion products corresponding to the expected 14.7 kD complex (data not shown). This was supported by the selective capture of the 15 kD material by streptavidine-agarose under non-reducing conditions and analysis of its composition under reducing conditions (data not shown).

Trypsin digestion of isomerized Env^{ampho} is predicted to yield a major disulphide-linked complex of SU fragments of 12.2 kD that is not modified by MB nor NEM (Fig. IIA). This corresponds to the 14,7 kD complex, but with a disulphide-bonded Cys-pair in the CXXC-motif and without the two peptides (9.0 and 1.6 kD) derived from TM (see above). Analysis of disulphide-linked tryptic SU fragments of MB- and NEM-treated isomerized Env by non-reducing SDS-PAGE indeed revealed a major peptide-complex of 12 kD that did not display the NEM/MB specific size-shift characteristic for the 15 kD peptide-complex derived from non-isomerized Env (Fig. IIC, lanes 2-4, SU^{isom}). The lack of modification was confirmed by streptavidin-capture analysis (data not shown). In Fig. IIC we have included analysis of the tryptic peptide-complex of NEM-alkylated intact Env (lane 1, SU-TM) and trypsin digested recombinant RBD^{ampho} as controls (lane 5, RBD). The major disulphide-linked tryptic peptide-complex derived from RBD^{ampho} migrates in the 3-4 kD size-range further supporting the identities of the 12 and 15 kD peptide-complexes. Thiol-mapping of disulphide linked tryptic Env-peptides was also done using non-isomerized and isomerized Env^{Friend}. As predicted (Linder et al., 1992) digestion of non-isomerized Env^{Friend} yielded a major, thiol-labeled complex of disulphide linked fragments of ~12 kD, whereas digestion of isomerized Env resulted in a non-modified, major 9 kD complex (data not shown). Altogether we conclude that the isomerization active thiol-group of Env is localized to a C-terminal 12 kD fragment of SU.

Methods

Trypsin-digestion was done by incubating MB-labeled and deglycosylated proteins with 10-100 μg/ml of TPCK-treated trypsin (SIGMA-Aldrich Chemie) for 15 h at 25°C

in lysis-buffer. Trypsin was inactivated by adding four times molar excess of trypsin inhibitor. Recombinant RBD^{ampho} comprised the 234 first residues of mature Env^{ampho} and was provided by J. Casasnovas, Karolinska Institutet.

References

Fass, D., and Kim, P. S. (1995). Dissection of a retrovirus envelope protein reveals structural similarity to influenza hemagglutinin. Curr Biol *5*, 1377-1383.

Linder, M., Linder, D., Hahnen, J., Schott, H. H., and Stirm, S. (1992). Localization of the intrachain disulfide bonds of the envelope glycoprotein 71 from Friend murine leukemia virus. Eur J Biochem *203*, 65-73.

Schagger, H., and von Jagow, G. (1987). Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem *166*, 368-379.

Fig. II. Thiol-mapping of trypsin fragments

A. Predicted disulphide-bond status of the tryptic fragments of MLV Env^{ampho} when non-isomerized and isomerized. Shown are the amino acid sequences of all Cys containing tryptic fragments of the Env ecto-domain. The order is from N- to C-terminus of the Env polypeptide. The MWs of the tryptic peptides are indicated below the amino acid sequences. MWs of disulphide-linked tryptic peptide complexes are indicated in square brackets. **B.** Thiol-mapping of disulphide linked trypsin fragments from non-isomerized Env^{ampho}. Non-reducing SDS-PAGE of disulphide-linked, [^{35}S]Cys labeled tryptic peptide-complexes of NEM (lanes 1 and 3) or MB alkylated (lane 2) non-isomerized Env. **C.** Thiol-mapping of disulphide linked trypsin fragments from SU of isomerized Env^{ampho}. Non-reducing SDS-PAGE of disulphide linked SU-peptide-complexes of NEM (lanes 2 and 4) or MB treated (lane 3) isomerized Env.

Shown are also analyses of disulphide linked tryptic peptide-complexes of non-isomerized Env that had been alkylated with NEM (lane 1) and tryptic peptide-complexes of RBD^{ampho} (lane 5) for comparisons.

Figure I

A

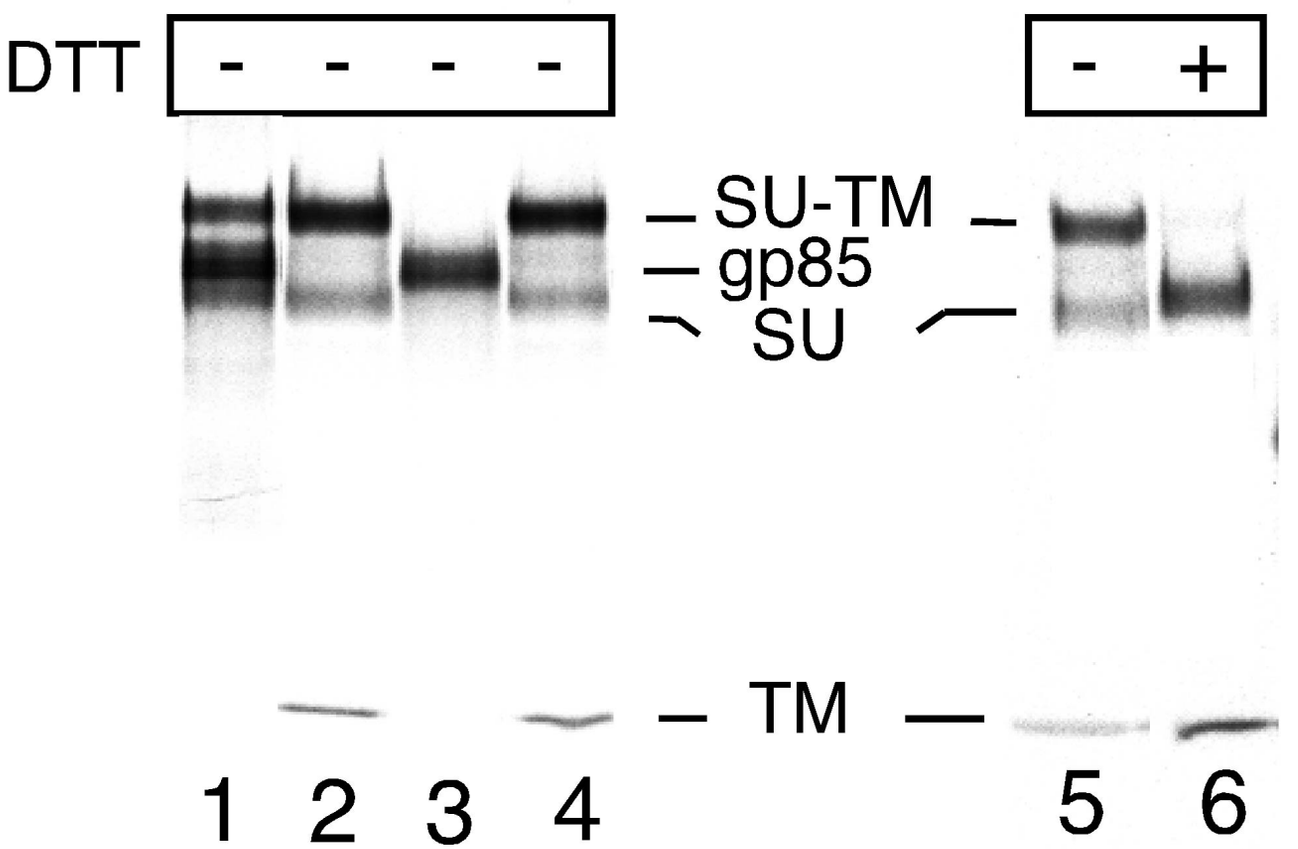
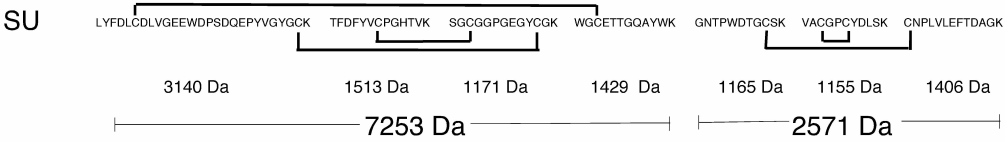


Figure II

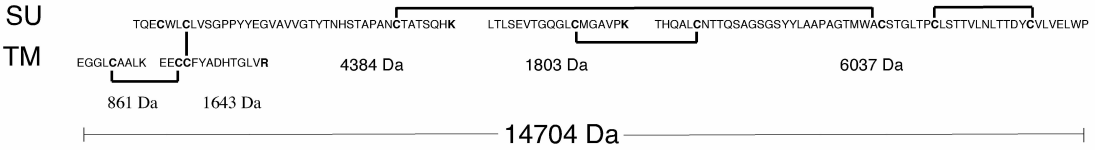
A

Disulphide bonded peptides in N-terminal region (RBD) of SU.

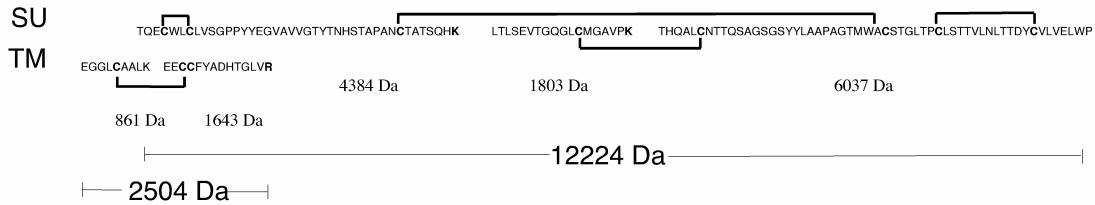


Disulphide bonded peptides in C-terminal region of SU and N-terminal region of TM.

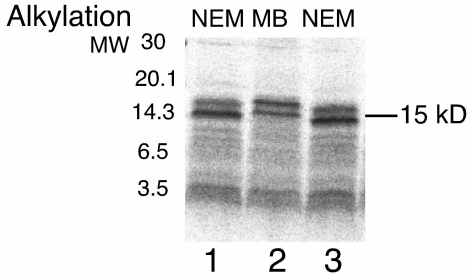
Non-isomerized



Isomerized



B



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