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Conservation of inner domain modules in the surface envelope glycoproteins of an ancient rabbit lentivirus and extant lentiviruses and betaretroviruses

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Abstract

The consensus sequence of endogenous lentiviral elements in the genome of European rabbits (RELIK) was used to extend a model of conserved lentiviral and betaretroviral surface envelope glycoprotein (SU) inner domain structures. Here it is shown that nearly all the inner domain elements of human and simian immunodeficiency virus gp120 mediating conformational changes upon CD4 binding were conserved in the SU of RELIK. Many of these inner domain elements and a carboxy-terminal region outside the gp120 core are also conserved in the SU of other lentiviruses and betaretroviruses, suggesting conserved mechanisms of SU conformational changes induced by receptor binding.

Keywords: Lentivirus; Betaretrovirus; Envelope; gp120; gp90; EIAV; FIV; HIV; SIV; MMTV

Introduction

The lentiviruses are a diverse group of retroviruses that induce immunosupression or chronic connective tissue inflammation in infected animals. There are five known groups of lentiviruses: the primate lentiviruses (PLV), including the human immunodeficiency virus type 1 (HIV-1) and related simian immunodeficiency viruses (SIV); the bovine immunodeficiency (BIV) and Jembrana disease (JDV) lentiviruses; the small ruminant lentiviruses (SRLV), including the caprine arthritis-encephalitis (CAEV) and visna viruses; the feline immunodeficiency viruses (FIV); and the equine infectious anemia virus (EIAV). Recently, a family of replication-defective endogenous lentiviral elements was identified in the genome of the European rabbit named rabbit endogenous lentivirus type K (RELIK), constituting a sixth lentiviral group (Katzourakis et al., 2007). The exogenous replication-competent lentivirus that originated the endogenous RELIK elements (also referred to as RELIK here as opposed to RELIK elements to refer to the endogenous forms) is estimated to have infected the germ line of rabbits about 7 million years ago, probably a few million years before the emergence of the primate lentiviruses (Katzourakis et al., 2007).

Sequence similarity between lentiviruses is generally evident in most genes and gene products within lentiviral groups. However, sequence similarity between lentiviruses of different groups is weak and mostly limited to *pol* gene-encoded proteins and the transmembrane subunit of the envelope glycoprotein. In particular, the rapid evolution of the receptor-binding surface unit glycoprotein (SU) of lentiviruses hindered the establishment of clear sequence and structural similarities of this protein between different lentiviral groups until the structure of HIV-1 gp120 was determined.

The CD4-bound HIV-1 gp120 is composed of the inner and outer domains and a bridging sheet minidomain formed by four antiparallel β-strands, with two strands emanating from the inner and outer domains, respectively (Kwong et al., 1998) (Fig. 1A). Strands β2 and β3 of the bridging sheet emanate from the inner domain and form the stem of the V1/V2 stem–loop structure. In the CD4-bound structure of HIV-1 gp120, the inner domain is formed also by a five-strand β-sandwich and a two-strand, two-helix bundle. The outer domain is inserted between strand β8 and helix α5 of the two-strand, two-helix bundle of the inner domain. The inner domain is also connected to the amino and carboxy-terminal regions before and after strands β0 and β25,

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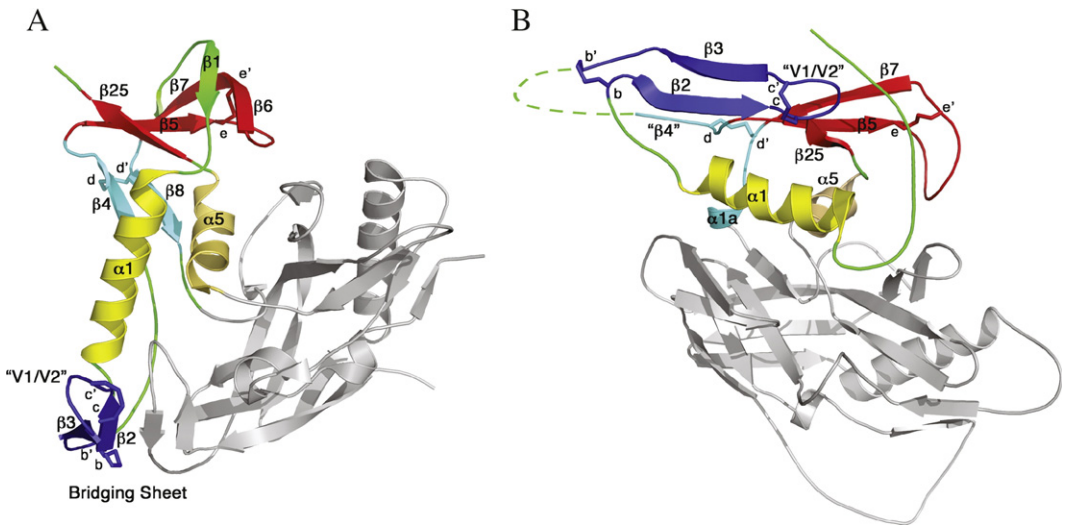

Fig. 1. Ribbon diagram of the structures of HIV-1 gp120 bound to CD4 (A) and SIV gp120 (B). CD4 and the Fab fragment of antibody 17b are not shown in (A). The structures are oriented by fixing strands β5, β25 and β7 of the inner domain β-sandwich in similar positions in both structures. The outer domain is colored gray. The inner domain structural elements are color coded and labeled. The part of the inner domain β-sandwich conserved in PLV, RELIK and SRLV is shown in red. Inner-domain disulfide bonds are shown as sticks and the corresponding cysteine residues labeled according to the text and Figs. 2, 3 and 4. The base of the V1/V2 loop (deleted from the structures) and the bridging sheet are indicated. This figure was prepared with the MacPyMOL software version 0.99 (Delano Scientific, Palo Alto, CA).

respectively. CD4 binds at the interface of the inner and outer domains and the bridging sheet, inducing conformational changes in gp120 that stabilize the latter structure which forms part of the coreceptor binding site (Kwong et al., 1998).

Structural similarities between gp120 and the SU of other lentiviruses were identified using the structure of gp120 to interpret short interspersed conserved motifs (Hötzel and Cheevers, 2000, 2001). Two motifs that include part of the inner domain β-sandwich are highly conserved in most lentiviruses. The C2 or β4/β5 motif, located centrally in the SU of most lentiviruses, overlaps strands β4 and β5 of gp120 and includes a strictly conserved cysteine residue in β4. The C5 or β25 motif is located in the carboxy-terminal region of the gp120 core and the SU of all lentiviruses. The β4/β5 motif, but not the β25 motif, was also found in the carboxy-terminal region of the SU of betaretroviruses and related endogenous elements, indicating that the vertex of the gp120 inner domain formed by the β-sandwich is highly conserved in both the lentiviruses and betaretroviruses and may represent a common structure for interaction with the transmembrane protein (Hötzel and Cheevers, 2001, 2003; Yang et al., 2003). The sequence similarity between gp120 and SRLV gp135 also extends to almost the entire inner domain β-sandwich, including strands β5, β6, β7 and β25 as well as strands β4 and β8 (Hötzel and Cheevers, 2000). In addition, EIAV gp90 has a very similar spacing of cysteine residues as gp120 in the region that includes the whole V1/V2 stem–loop structure up to the conserved cysteine of the β4/β5 motif, suggesting that EIAV gp90 includes at least the inner domain part of the bridging sheet as well as a structure similar to the V1/V2 loop (Hötzel, 2003).

The description of the free SIV gp120 structure allowed the inference of large conformational changes that occur in the inner domain upon binding of gp120 to CD4 (Chen et al., 2005). These conformational changes involve different modules that move independently relative to each other (Figs. 1A and B). One of these modules is the β-sandwich that is held together by two disulfide bonds (Fig. 1B). The other modules are the amphipathic helix α1, the V1/V2 stem–loop structure, helix α5 and strands β4 and β8, which in the unliganded SIV gp120 adopt a partially disordered and helical conformation (helix α1a), respectively. Whether these modules and receptor-induced inner domain conformational changes are unique to the primate lentiviruses or occur more broadly in the lentiviruses is not known due the lack of structural information and the limited SU sequence conservation. The identification of RELIK provides an opportunity to expand the model of inner domain conservation to address this question. Here, the consensus sequence of RELIK SU was analyzed to identify sequences and structural elements conserved in the SU of lentiviruses to extend the model of inner domain structural conservation, define a previously unrecognized conserved carboxy-terminal region in the SU of lentiviruses and betaretroviruses and determine conserved mechanisms of conformational changes induced by receptor binding in the SU of lentiviruses.

Results

RELIK SU includes an inner domain β-sandwich structure

Examination of the consensus RELIK SU sequence revealed the presence of the β4/β5 motif conserved in most lentiviruses (Hötzel and Cheevers, 2001). BLAST alignments of RELIK SU with HIV-1 gp120 or SRLV gp135 also revealed the presence of sequences similar to those of gp120 strands β6 to β8 and the corresponding sequences of SRLV gp135 (Fig. 2A), including the four cysteine residues forming two disulfide bonds in the gp120 β-sandwich that are conserved in the SU of PLV and SRLV (Hötzel and Cheevers, 2000). In fact, the closest