[SIII-4]

The Mechanism of Membrane Fusion During the Infection of HIV

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Abstract

The fusion between viral envelope and target cell membrane is a central step of viral infection, and the fusion proteins located at viral envelope mediate such process. Gp41 of HIV is one of the fusion proteins whose structure and mechanism of membrane fusion had been extensively studied. Functionally important motives of gp41 are the N-terminus fusion peptide, the coiled-coil and the membrane proximal C-peptide regions. The role of these regions during the fusion process had been thoroughly examined. Specially, insertion of the fusion peptide into membrane and conformational change of the coiled-coil and C-peptide regions are assumed to be critical for the fusion mechanism. In addition, the coiled-coil region has been shown to interact with membrane, and the C-peptide region regulates the interaction in a dose dependent manner. Furthermore, fusion defective mutations of the coiled-coil region dramatically changed its binding affinity to membrane. These results suggested that the membrane binding property of the coiled-coil region is important for the fusion activity of gp41, and such property could be modulated by the interaction with the C-peptide region.

Introduction

The envelope protein of human immunodeficiency virus type I (HIV-I) consists of a receptor binding peripheral protein (gp120) and a transmembrane protein (gp41) which forms an oligomeric complex. Gp120 binds to CD4 (1) and chemokine receptors (2) on the target cell membrane and gp41 mediates the fusion of viral and target cell membranes. Upon binding to the receptor(s), the gp120/gp41 complex undergoes structural changes. Particularly, gp41 transforms from a native or non-fusogenic conformation to a fusion competent or fusogenic conformation (3). This change results in an exposure of the N-terminal hydrophobic region of the gp41, so called fusion peptide (4), which is assumed to interact directly with lipid membrane (5) during the fusion process.

Following the fusion peptide region, there are coiled-coil region and membrane proximal C-peptide region (Fig. 1). The coiled-coil region is assumed to be important for oligomerization of gp41 and the C-peptide region interacted with the coiled-coil region (6). Crystal structure of the core domain of gp41 consisted of the coiled-coil and C-peptide regions revealed a helix bundle structure in which the coiled-coil forms trimer and three C-peptide regions buttress the central coiled-coil trimer resulting a 6-membered helical bundle structure (7). The trimeric coiled-coil structure is commonly found in the ectodomain of various viral fusion proteins, and the similarity of the structure suggested that the viral fusion protein might have a common mechanism of membrane fusion. It was assumed that the energy generated from the formation of the stable conformation was used as a driving force for membrane fusion.

Recently, there were reports that the coiled-coil region or ectodomain of gp41 without the

fusion peptide region could directly interact with membrane. A peptide representing the coiled-coil region could bind to the synthetic lipid bilayer (8), or it induced hemolysis of erythrocytes and promoted cell aggregation (9). The ectodomain of gp41 that lack the fusion peptide was also showed binding affinity to lipid bilayer and the susceptibility to protease was increased by the binding to membrane suggesting conformational change after binding to membrane (10), although there was a controversial report that a shorter version of the extracellular domain maintains 6-membered helical bundle structure with or without lipid vesicle or detergents.

Results

The relationship between the membrane-binding activity of the coiled-coil region and its role in the fusion process has been investigated with wild-type coiled-coil sequence and mutants that harboring fusion defective mutations. The ability to interact with lipid bilayer has been examined by measuring the amount of fluorescence active calcein leaked from liposome. The peptide (N-38) or chimeric protein that harboring the coiled-coil region of gp41 (Trx-N) effectively disrupted liposome (Fig. 2). The membrane disrupting activity of these peptides or protein at the concentration of 2 μM was about 90% compare to the detergent-induced lysis. In contrast, the core region of gp41 representing amino acid 534-668 (gp41-ec) did not induce disruption of membrane. They showed marginal effect on the integrity of membrane at the concentration higher than 10 μM.

The effect of fusion defective mutation on the membrane disrupting activity of the coiled-coil region was further examined. The mutations used in this study were localized at the center of the coiled-coil region, and the variants of Trx-N that harboring the mutated coiled-coil regions were used for further study. The mutations were hydrophilic amino acid substitutions located at the hydrophobic face of coiled-coil (V570D and Y586E) at the outside of the hydrophobic face (V556D). In addition, two possible conformational mutants with proline substitution (I573P and L587P) were examined their interaction with liposome. When these mutant Trx-N proteins tested, most of the mutants showed significantly reduced activity of membrane disruption. Specially, Y586E and I573P mutations almost completely abolished the membrane disrupting activity of the coiled-coil region results indicating that the hydrophobic face of the coiled-coil region was important for the interaction with membrane. Other mutations also reduced such activity significantly; the membrane disrupting activity of V570D, V556D, and L587P mutants were only 40, 34, and 25%, respectively, compare to the lysis by 0.1% Triton X-100 (Fig. 3). The decreased membrane disrupting activity of these mutants suggested that the hydrophobic residues of the coiled-coil region as well as the helical conformation were necessary for the membrane interaction. Previously, it was shown that these mutations were not affect the expression level and processing of gp41 or its complex formation with gp120. Hence these mutations might be directly involved in the fusogenic function of gp41.

Discussion

The coiled-coil regions of gp41 have been characterized in this study regarding to their membrane interaction. The entire region spanning the coiled-coil composed of 51 amino acids was also shown to possess membrane-binding property as the shorter peptides. This property was also observed in the coiled-coil region of viral fusion protein, such as hemagglutinine of influenza virus. The significance of the strong affinity of the coiled-coil region of gp41 against membrane has been

implicated to direct interaction during membrane fusion. However, the relationship between such affinity and fusion activity of gp41 have not been extensively examined. Interestingly, all of the tested fusion defective mutants showed significant reduction in their membrane binding affinity suggesting that the membrane binding property of the coiled-coil region is closely related or necessary for the fusion activity of gp41.

A model of the gp41-mediated membrane fusion had been proposed from the structure and membrane binding property of the extracellular regions of gp41 (11). The stable 6-membered helical bundle structure and the fusion inhibitory activity of the peptides such as C34 or DP178 that were derived from the C-peptide region proposed a conformational change derived fusion model as described in Fig.4A. In this model, the fusion peptide and the coiled-coil region of gp41 exposed after the attachment of virus to the target cell, and the fusion peptide inserts into the target membrane. Peptides from C-peptide region may tightly bind to the exposed coiled-coil region so that prevent further conformational change. Then, the coiled-coil and C-peptide region of gp41 further form a stable helix bundle structure. The energy liberated form this conformational change is suggested to be used to juxtapose of the two membranes and induce lipid mixing.

Another model, which our observation supports, is a transient membrane disruption model by the coiled-coil region of gp41. The spontaneous binding of the coiled-coil region of gp41 implied that the exposed region after the attachment of virus to target cell could interact with membrane unless the interaction between gp120 and C-peptide region prevent to form the helix bundle structure. It may be detached from the membrane to form the helix bundle structure when the C-peptide region of gp41 is available. The change of membrane on and off state of the coiled-coil region may significantly perturb the lipid bilayer structure, or reduce the hydration barrier of the two membranes (Fig. 4B). In this case, any mutation at the coiled-coil that reduced the binding to the membrane may result in defects in the fusion activity of gp41. Further study on the conformational change and membrane interaction of the core region of gp41 would elucidate detailed mechanism of membrane fusion.

Acknowledgements

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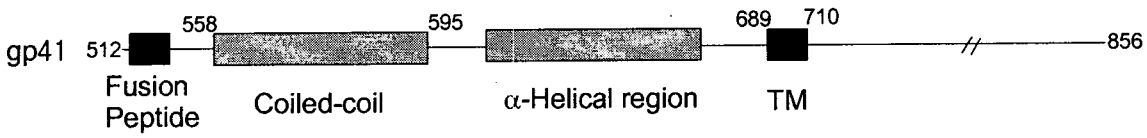
gp41
512
558
595
689
710
856
Fusion Peptide
Coiled-coil
α-Helical region
TM

Fig. 1

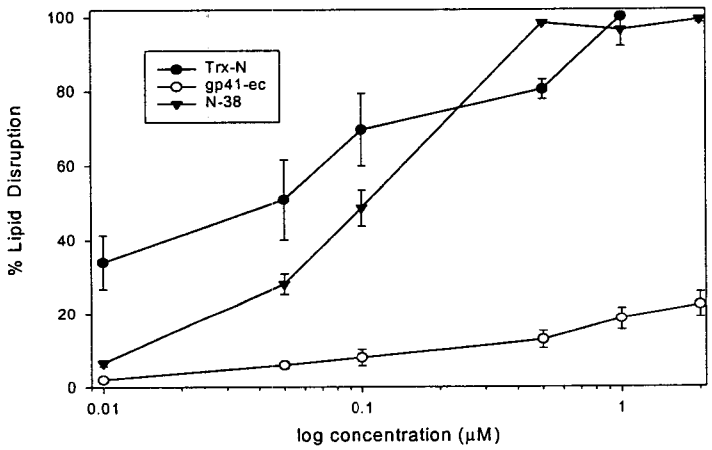
% Lipid Disruption
log concentration (μM)
Trx-N
gp41-ec
N-38
0
20
40
60
80
100
0.01
0.1
1

Fig. 2

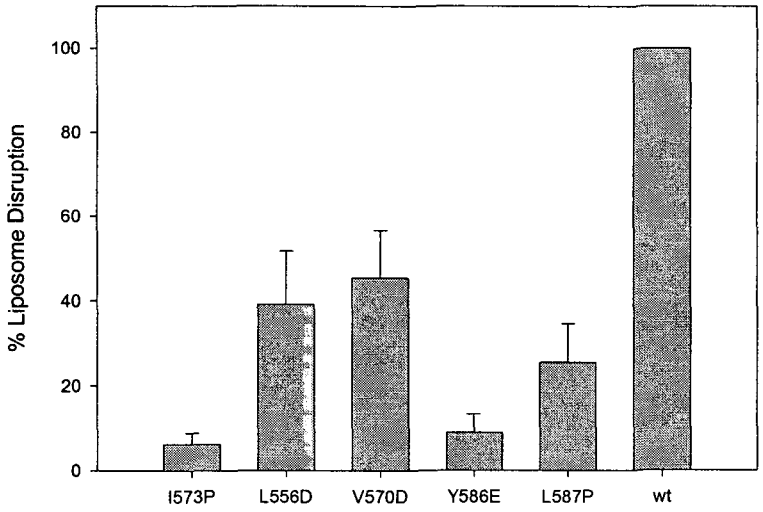
% Liposome Disruption
100
80
60
40
20
0
I573P
L556D
V570D
Y586E
L587P
wt

Fig.3

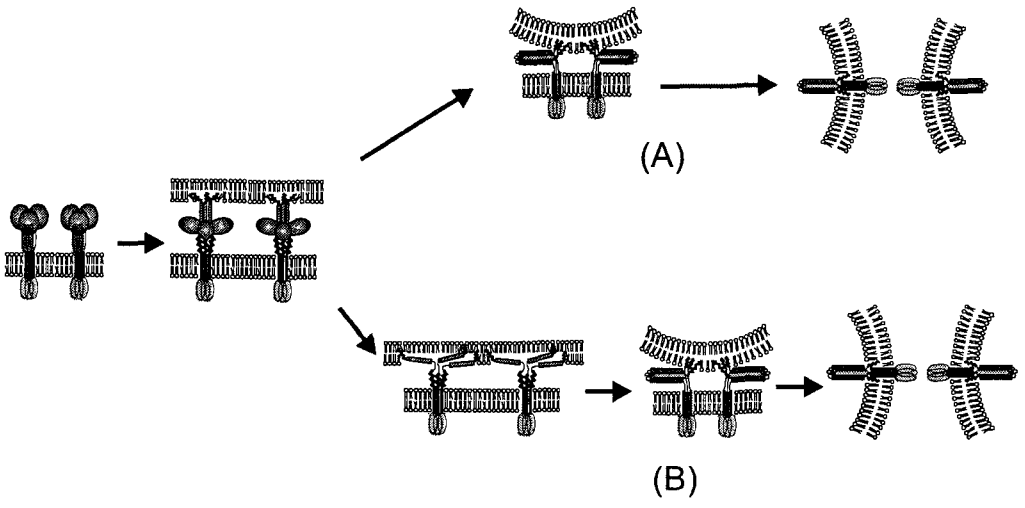
(A)
(B)

Fig. 4