Interaction of HIV-1 fusion peptide and its mutant with lipid membrane

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Abstract HIV_{WT} and HIV_{V2E} represent the 23 amino acids fusion peptide of HIV-1 gp41 N terminus and its position 2 mutant (Val→Glu). We have studied the structure-function relationship of HIV_{WT} and HIV_{V2E} when they interact with acidic and neutral lipid membranes. The results show that HIV_{WT} and HIV_{V2E} have the same conformational characteristics and tendencies of conformational transition but definitely different functions: HIV_{WT} destabilizes membrane and induces fusion by adopting predominant α-helix conformation when interacting with acidic POPG membrane, its phenylalanine residues can penetrate into the hydrophobic core of POPG bilayer; HIV_{V2E} also adopts predominant α-helix when interacting with POPG membrane, but it cannot destabilize POPG membrane and induce fusion, the phenylalanine residues of it are located near the surface of POPG bilayer. HIV_{WT} and HIV_{V2E} both adopt predominant β-sheet conformation to interact with neutral POPC membrane, and cannot destabilize POPC membrane and induce fusion, the position of phenylalanine residues of both HIV_{WT} and HIV_{V2E} are close to the surface of POPC bilayer. These results demonstrate that the N terminal hydrophobicity of fusion peptide and the secondary structure when interacting with lipid membrane play important roles for fusion peptide exerting its function.

Keywords: human immunodeficiency virus type 1, fusion peptide, lipid membrane.

The therapy for AIDS patients is a difficult problem of medicine because $CD4^+$ lymphocytes (helper/inducer T) are infected by human immunodeficiency virus type 1 (HIV-1). When HIV-1 attacks target cells, the virus envelope membrane fuses with the target cell membrane, resulting in two probable consequences. One is injection of viral nucleoprotein and genetic materials into the target cells. These materials replicate in host cells and pass to filial generation cells with the fission of host cells, leading to the death of host cells and the infection of healthy cells. The other is destabilization target cell membrane and imbalance of the inner and outer environments of cells, finally resulting in the death of target cells. So membrane fusion is a principal and key process for HIV-1 infection target cells. After HIV-1 gets into human body, its envelope glycoprotein gp160 is proteolytically cleaved into a surface glycoprotein gp120 and a transmembrane glycoprotein gp41; gp120/gp41 are activated and the N terminal fusion peptide is exposed by the action of CD4 receptor and cofactors. Freed et al. confirmed in 1990 that HIV-1 fusion peptide functions as a "bridge" which connects the viral envelope membrane and the target cell membrane, disrupts the stability of target cell membrane, triggers the fusion process and causes cell death[1,2].

It has been proven by the molecular biological point-mutation method that HIV-1 fusion peptide consists of about 25 amino acid residues and the mutants formed by the substitution of hydrophilic amino acids for the N terminal hydrophobic amino acids lose the fusion function[1,3,4]. This result suggests that the hydrophobicity and structure of fusion peptide play critical roles in the process of membrane fusion. Membrane fusion is a complicated course involving various physical and chemical interactions. In order to study the membrane fusion mechanism led by HIV-1 fusion peptide and establish the relationship between structure and function of HIV-1 fusion peptide, using fluorescence energy transfer, fluorescence quenching and light scattering techniques, we compared the difference of membrane fusion and insertion abilities between two synthetic peptides corresponding to HIV-1 gp41 N terminal fusion peptide (HIV_{WT}) and its position 2 mutant (HIV_{V2E}) when they interact with acidic and neutral lipid membranes. Besides, we studied the conformational changes before and after the two peptides interaction with lipid membrane by circular dichroism (CD) and Fourier transformation infrared spectroscopy (FTIR).

1 Materials and methods

(ⅰ) Materials. The synthetic peptides HIV_{WT} (AVGIGALFLGFLGAAGSTMGARS-NH_2) and HIV_{V2E}(AEGIGALFLGFLGAAGSTMGARS-NH_2) were purchased from Advanced ChemTech (Louisville, KY, USA), the purities are greater than 91% and 88% respectively. Their molecular weights tested by mass spectrum are consistent with calculated values. 10 mg/mL peptide stock solutions were prepared in DMSO and stocked at -20℃. POPG and POPC were from Sigma. Fluorescence-labeled phospholipids NBD-PE, Rh-PE and spin-labeled phospholipids 5-doxyl-PC, 12-doxyl-PC were obtained from Avanti Polar Lipids (Alabaster, AL, USA). Fluorescent probes ANTS and DPX were from Molecular Probes (Junction City, OR, USA). All other reagents were of analytical grade. HEPES buffer (5 mmol/L HEPES, 100 mmol/L NaCl, pH 7.4) was prepared.

(ⅱ) Methods

(1) Fluorescence energy transfer. 2 mg phospholipids were dissolved in 200 μL $CHCl_3/CH_3OH$ (2∶1, *v*/*v*) solution, dried on an rotary evaporator. The residue of solvent was further dried under high vacuum for at least 3 h, then the lipid film was dispersed in 500 μL HEPES buffer for 10 min and subjected to 6 freeze-thaw cycles prior to extrusion 10 times through two stacked 0.2 μm pore size polycarbonate membranes; large unilamellar vesicles (LUVs) were produced with diameters from 100 to 200 nm. The lipid concentration was determined according to Ames phosphate analysis [5]. POPG or POPC LUVs containing 0.6% (mol/mol) of NBD-PE and Rh-PE were mixed with unlabeled POPG or POPC LUVs at 1∶4 ratio (mol/mol), the phospholipid concentration was 100 μmol/L. The NBD fluorescence intensity was monitored at 530 nm with the excitation wavelength set at 467 nm. The initial fluorescence intensity was recorded as F_0 (reflecting the energy transfer situation between NBD and Rh), then peptide dissolved in DMSO was added to vesicle suspension at different ratios of peptide to lipid, and the NBD emission was recorded as F after the suspension was incubated at 37℃ for 10 min. The NBD emission of liposomes containing 0.12% (mol/mol) NBD-PE and Rh-PE was recorded as F_1, representing the fluorescence energy transfer when liposomes fused completely. The fluorescence increasing percentage of NBD (i.e., the degree of energy transfer decrease) represents the lipid fusion percentage, which is calculated according to the following formula: $(F-F_0)/(F_1-F_0) \times 100\%$. The measurement proceeded at 37℃ by using a Hitachi 850 spectrofluorometer.

(2) Leakage of liposome contents. Using the buffer (pH 7.4) containing 12.5 mmol/L ANTS, 45 mmol/L DPX, 20 mmol/L NaCl, 5 mmol/L HEPES, LUVs were prepared by the method described in (ⅱ)(1). The liposomes filtered through two stacked 0.2 μm pore size polycarbonate membranes were separated from unencapsulated ANTS and DPX on a sephadex G-75 column eluted with HEPES buffer. The concentration of purified liposome solution was determined by Ames phosphate analysis[5]. 1 mL liposomes (100 μmol/L) was put into a cuvette, the excitation and emission wavelengths were set at 355 and 520 nm, respectively. The fluorescence intensity of samples with or without peptide was measured after incubating at 37℃ for 10 min. Triton X-100 (0.5%, *v*/*v*) was added to liposomes to determine complete release. The percentage of leakage was calculated by the formula: $(I-I_0)/(I_1-I_0) \times 100\%$, where I_0 is the initial fluorescence intensity of LUVs containing ANTS and DPX, I_1 is the fluorescence intensity observed after addition of Triton X-100, and I is the fluorescence intensity after addition of peptide. The experimental temperature and instrument were the same as those described in (ⅱ) (1).

(3) Light scattering. 0.2 mg phospholipid was dissolved in 20 μL $CHCl_3/CH_3OH$ (2∶1, *v*/*v*) and dried under N_2 gas. The residue of solvent was removed under high vacuum for 2 h, then dispersed in 2 mL HEPES buffer and supersonicated for 20 min at 20—25℃ in water bath to make the solution clear. Small unilamellar vesicles (SUVs) were produced. Put 1 mL liposomes into a cuvette, immediately record the 90° light scattering values which change with time at 420 nm (slit width: 3 nm) after addition of peptide dissolved in DMSO to make the ratio of peptide to lipid 1∶25 (mol/mol). Blank control was the peptide solution with the same concentration. Measuremental temperature and instrument were the same as those mentioned in (ⅱ)(1).

(4) Electron microscopy. POPG and POPC LUVs were prepared according to the method

described in (ⅱ)(1). A fixed amount of peptide was added to 100 μmol/L liposomes to make the mole ratio of peptide/lipid 1∶25. After incubation at 37℃ for 10 min, a drop containing LUVs alone or a mixture of LUVs and peptide was deposited onto a carbon-coated grid and negatively stained with 1.5% phosphatotungstic acid. The grids were observed using an H-500 transmission electron microscope.

(5) Measurement of the membrane insertion depth of phenylalanine residues of peptide. Spin-labeled phospholipid *n*-doxyl-PC was used as fluorescence quencher to measure the depth of phenylalanine residues of peptide inserted into POPG and POPC membrane. 4 μmol/L (final concentration) peptide was added to 100 μmol/L SUVs, the suspension was incubated for 10 min at 37℃. Set the excitation wavelength at 265 nm and record the fluorescence emission spectra of peptide from 290—400 nm and the maximum fluorescence intensity at 305 nm. The data were handled according to the method of Chattopadhay and London[6]. Z_{cf}, the average distance between phenylalanine residues and the center of bilayer, was calculated by the following equation:

$$Z_{cf} = (-1/2L_{m-n}) \times [(1/pc) \times \ln(F_m / F_n) + L_{m-n}^2] + L_{c-m}, \quad (1)$$

where c is the two-dimensional concentration of the quenchers in the membrane (0.00002 molecules/nm^2, assuming that the cross section of a lipid molecule is 0.7 nm^2); F_m and F_n are the fluorescence intensities in the presence of *m*- and *n*-doxyl-PC, respectively; L_{c-m} is the vertical length between the bilayer center and the doxyl group of *m*-doxyl-PC (1.215 nm for m=5) and L_{m-n} is the vertical distance between *m*-doxyl and *n*-doxyl (0.63 nm for m=5, n=12).

(6) CD spectra. 25 μg peptide was dissolved in 30 μL HEPES buffer and 30 μL 1% SDS solution, respectively. The sample was put into a 0.1 mm pathlength cell after incubation for 10 min at 37℃. Measurements were carried out on a Jobin Yvon-Spex CD6 spectrometer at 37℃. Each of the CD data was obtained from the average of four scans with a step resolution of 0.5 nm and a band width of 2 nm. The spectra were recorded from 186 to 260 nm with a time constant of 1s.

(7) FTIR spectra. 200 μg peptide was dissolved in 50 μL D_2O buffer (5 mmol/L HEPES, 100 mmol/L NaCl, pH7.4), POPG SUVs, and POPC SUVs, respectively. POPG SUVs and POPC SUVs were produced in D_2O buffer. The mole ratio of peptide to lipid was 1∶50. After incubation for 10 min at 37℃, 25 μL sample solution was placed between two CaF_2 windows separated by a 50-μm Teflon spacer. FTIR spectra were recorded at 37℃ on a Bio-Rad FTS-165 spectrometer. Spectra were averaged from 200 scans at a spectral resolution of 4 cm^{-1}.

2 Results

(ⅰ) Fluorescence energy transfer. Fig. 1 shows the curves of HIV_{WT} and HIV_{V2E} inducing POPG and POPC membrane fusion. With peptide/lipid ratio increase, the efficiency of NBD fluorescence energy transfer in POPG liposomes decreases (the NBD fluorescence intensity increases), indicating that the percentage of POPG membrane fusion increases. The fusion percentage reaches 47.5% when peptide/lipid ratio is 1∶12.5. But HIV_{WT} almost loses the ability to induce neutral POPC membrane fusion. HIV_{V2E} cannot induce both POPG and POPC membrane fusion.

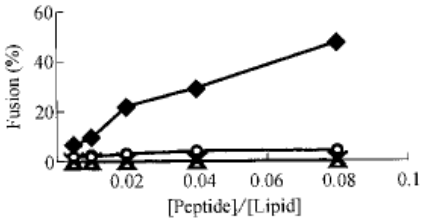


Fig.1. Lipid mixing of POPG or POPC LUVs induced by HIV_{WT} and HIV_{V2E}. The lipid concentration was 100 μmol/L, the peptide dissolved in DMSO was added, and the decrease in fluorescence energy transfer following liposome-liposome fusion was monitored at 530 nm, pH 7.4 and 37℃. ◆, HIV_{WT}/POPG; ○, HIV_{WT}/POPC; △, HIV_{V2E}/POPG; ×, HIV_{V2E}/POPC.

(ⅱ) Leakage of fluorescence contents. Fig. 2 demonstrates the leakage of the contents of POPG and POPC LUVs induced by HIV_{WT} and HIV_{V2E}. The percentage of leakage of POPG vesicles induced by HIV_{WT} increases with the peptide/lipid ratio increase, which is indicated by the increase of fluorescence intensity of ANTS. The percentage reaches 77.8% when peptide/lipid ratio is 1∶12.5, whereas HIV_{WT}

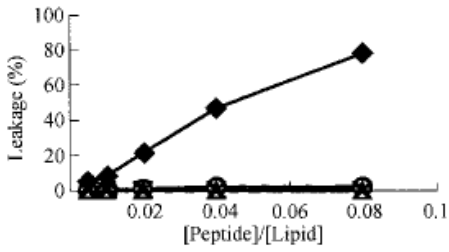


Fig. 2. Leakage of POPG or POPC LUVs induced by HIV_{WT} and HIV_{V2E}. Lipid concentration was 100 μmol/L, the peptide dissolved in DMSO was added, and the increase in fluorescence dequenching after membrane disturbing was monitored at λ_{ex}=355 nm, λ_{em}=520 nm, pH 7.4 and 37℃. ◆, HIV_{WT}/POPG; ○, HIV_{WT}/POPC; △,HIV_{V2E}/POPG; ×, HIV_{V2E}/POPC.

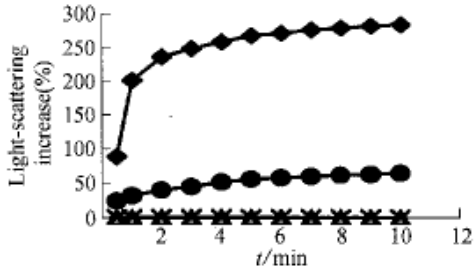


Fig. 3. Light-scattering increase of POPG or POPC SUVs induced by HIV_{WT} and HIV_{V2E}. Lipid concentration was 100 μmol/L, at time zero, the peptide dissolved in DMSO was added, the final concentration of peptide was 4 μmol/L. 90° light-scattering was monitored at 420 nm (slit width: 3nm), pH 7.4 and 37℃. ◆, HIV_{WT}/POPG; ●, HIV_{WT}/POPC; △, HIV_{V2E}/POPG; ×, HIV_{V2E}/POPC.

nearly cannot induce the leakage of POPC liposomes. HIV_{V2E} does not have the ability to induce either POPG or POPC liposomes leakage.

(iii) Light-scattering. Fig. 3 shows the 90° light-scattering values changing with time after adding peptide to POPG and POPC SUVs (peptide /lipid ratio was 1∶25). The light-scattering of POPG SUVs increases by 286% after adding HIV_{WT} for 10 min, whereas the light-scattering of POPC SUVs just increases by 66%. HIV_{V2E} has not any effect on the light-scattering of POPG and POPC SUVs.

(iv) Electron microscopy. Fig. 4 exhibits the transmission electron micrographs of POPG LUVs. The diameters of vesicles increase after incubation with HIV_{WT}, but no change is observed after interaction with HIV_{V2E}. Neither HIV_{WT} nor HIV_{V2E} affects the dimension of POPC LUVs (graphs omitted).

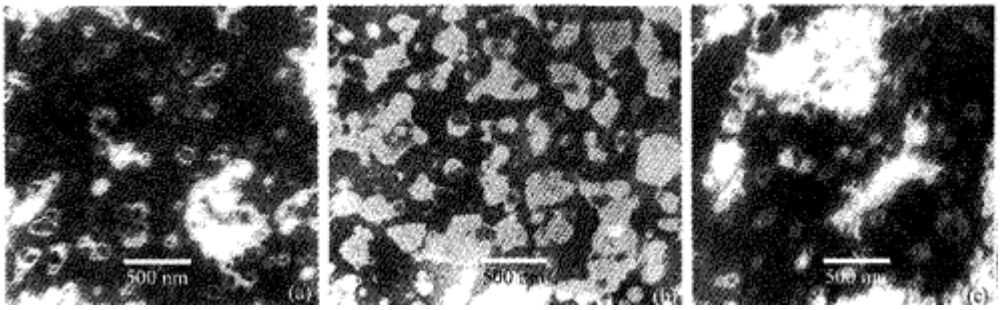


Fig. 4. Electron micrographs of negatively stained POPG liposomes. (a) POPG LUVs (100 μmol/L) alone; (b) POPG LUVs incubated with HIV_{WT} (4 μmol/L) at 37℃ for 10 min; (c) POPG LUVs incubated with HIV_{V2E} (4 μmol/L) at 37℃ for 10 min (bar=500 nm).

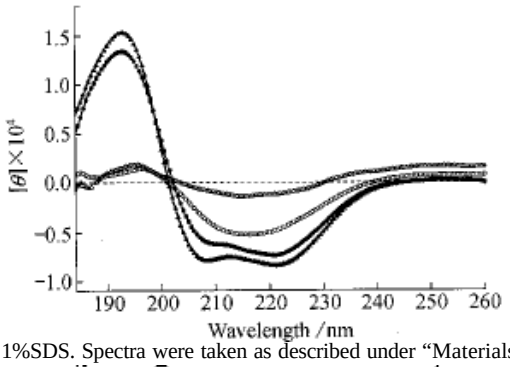
(v) Membrane insertion depth of phenylalanine residues of HIV_{WT} and HIV_{V2E}. The fluorescence emitted by phenylalanine residues could be quenched to a certain extent by the spin group doxyl which attaches covalently to the fifth and twelfth carbons of phospholipid acyl chain respectively. Table 1 shows the average depth of the two phenylalanine residues of HIV_{WT} and HIV_{V2E} inserted into POPG and POPC membrane. The values were calculated by eq. (1).

Table 1 The average depth of the Phe residues of HIV_{WT} and HIV_{V2E} inserted into POPG and POPC SUV

Sample	HIV_{WT}/POPG	HIV_{WT}/POPC	HIV_{V2E}/POPG	HIV_{V2E}/POPC
Z_{cf} /nm	0.905	1.673	1.527	1.782

(vi) CD spectra. HIV_{WT} and HIV_{V2E} dissolved in HEPES buffer exhibit predominant β-sheet conformation with a characteristic negative band peak at 217 nm and positive weak band peak at 195 nm. The membrane-mimic solvent SDS induces the secondary structure transition of HIV_{WT} and HIV_{V2E} from β-sheet to α-helix, as shown in fig. 5.

(vii) FTIR spectra. Fig. 6(a)—(f) shows the FTIR spectra and curve fitting results of amide I band of HIV_{WT} and HIV_{V2E} in HEPES buffer and in POPG or POPC liposomes. The quantitative analysis of the secondary structure is summarized in table 2.



and in 1%SDS. Spectra were taken as described under "Materials and methods (ii)(6)". ○, HIV_{WT} in HEPES buffer; △, HIV_{V2E} in HEPES buffer; ●, HIV_{WT} in 1%SDS; ▲, HIV_{V2E} in 1%SDS.

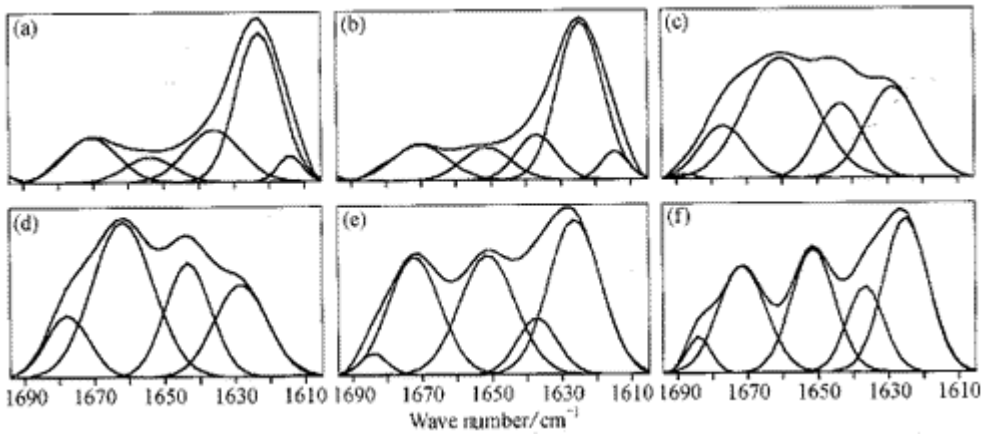


Fig. 6. FTIR spectra and curve fitting of the amide I band of HIV_{WT} and HIV_{V2E} in HEPES buffer and in liposomes. The peptides were added to SUVs at peptide-to-lipid ratio 1∶25, then incubated for 10 min at pH 7.4, 37℃. (a) HIV_{WT} in HEPES buffer; (b) HIV_{V2E} in HEPES buffer; (c) HIV_{WT} in POPG SUVs; (d) HIV_{V2E} in POPG SUVs; (e) HIV_{WT} in POPC SUVs; (f) HIV_{V2E} in POPC SUVs.

Table 2 Quantitative studies of the secondary structure of HIV_{WT} and HIV_{V2E} in HEPES buffer and liposomes by FTIR

Sample	α-helix (%)	Random coil (%)	β-sheet (%)	β-turn (%)
HIV_{WT}/HEPES	8.7	0	73.9	17.4
HIV_{V2E}/HEPES	12.2	0	73.0	14.8
HIV_{WT}/POPG	44.2	17.4	26.0	12.4
HIV_{V2E}/POPG	42.7	23.5	22.0	11.8
HIV_{WT}/POPC	28.2	0	46.0	25.8
HIV_{V2E}/POPC	25.2	0	50.9	23.9

3 Discussion

HIV_{WT} and HIV_{V2E} are two synthetic peptides representing HIV-1 gp41 N terminal fusion peptide which consists of 23 amino acids and its position 2 mutant (Val→Glu). We have made a preliminary study on the binding of HIV_{WT} and HIV_{V2E} with lipid membrane by using fluorescence spectroscopy and monomolecular layer techniques. The results show that HIV_{WT} penetrates deeply into the interior of acidic POPG membrane with negative charge, HIV_{V2E} binds to POPG membrane but cannot or just slightly peretrate into it, whereas neither HIV_{WT} nor HIV_{V2E} binds to neutral POPC membrane evidently[7]. In the work reported here, we further investigated the difference of inducing fusion and membrane insertion abilities between HIV_{WT} and HIV_{V2E} by fluorescence energy transfer, leakage of

fluorescence contents, light scattering and electron microscopy. Besides, we analyzed the conformational changes of HIV_{WT} and HIV_{V2E} before and after they interact with lipid membrane by CD and FTIR, in an attempt to explore the structure-function relationship of HIV_{WT} and HIV_{V2E}.

The results observed from fluorescence energy transfer, leakage of fluorescence contents, light scattering and electron microscopy indicate HIV_{WT} can disrupt the stability of acidic POPG lipid bilayer and induce POPG membrane fusion but cannot affect neutral POPC membrane; whereas HIV_{V2E} cannot destabilize both POPG and POPC bilayer and induce them to fuse. In order to reveal the structure basis underlying the above phenomena, we measured the depth of phenylalanine residues of HIV_{WT} and HIV_{V2E} buried in POPG and POPC lipid bilayer by the method of fluorescence quenching. The results demonstrate that the two phenylalanine residues located at positions 8 and 11 of HIV_{WT} can penetrate deeply into the hydrophobic core of POPG bilayer (the average distance between the bilayer center and the phenylalanine residues is 0.905 nm, the thickness of one leaflet of a bilayer is more than 2 nm), but only locate near the surface of POPC bilayer (1.673 nm from the bilayer center); whereas the two phenylalanine residues of HIV_{V2E} just locate close to the surface of POPG and POPC bilayer (1.527 and 1.782 nm from the center of POPG and POPC bilayer, respectively). This result is similar to that of Chang et al.[8] observed by ^{1}H-NMR. They reported that the position of FLG (8—10) motif is close to the center of SDS vesicles. Analyzing the primary structure of HIV_{WT} and HIV_{V2E}, we understand that the phenylalanine is the most hydrophobic amino acid residue which should have the strongest surface activity when interacting with lipid membrane. If the phenylalanine residues only bind to the surface of lipid membrane, we can deduce that the other hydrophobic residues of HIV_{WT} and HIV_{V2E} cannot penetrate into lipid bilayer.

Besides, CD spectra indicate that both HIV_{WT} and HIV_{V2E} adopt predominant β-sheet conformation in water solution, and predominant α-helix conformation in 1% SDS. It is difficult to study the conformation of HIV_{WT} and HIV_{V2E} when they interact with liposomes by CD spectra because of the light scattering. So we take FTIR as a complementary method to study the secondary structure of HIV_{WT} and HIV_{V2E} when they are alone or interact with POPG and POPC liposomes. The FTIR results, which are similar to CD spectra, show that HIV_{WT} and HIV_{V2E} exhibit predominant β-sheet in water solution; after interaction with POPC liposomes, their β-sheet contents decrease but still remain to be the predominant conformation, the contents of α-helix and β-turn increase; after interaction with POPG liposomes, their α-helixes become predominant conformation and random coil appears, and the contents of β-sheet decrease largely. From the results of CD and FTIR, we can get a notion that the wild type of fusion peptide HIV_{WT} and its position 2 mutant HIV_{V2E} exhibit the same conformational characteristics and tendencies of conformational transition: membrane environment can induce conformational change from β-sheet to α-helix, different liposomes result in different conformation transition. Compared to neutral POPC liposomes, acidic POPG liposomes make the conformation of HIV_{WT} and HIV_{V2E} exhibit more α-helix and random coil appear.

From the results of fluorescence quenching and the secondary structure analysis, it can be determined that only when HIV_{WT} adopts predominant α-helix conformation and penetrates into lipid bilayer can it exert the function of disrupting membrane stability and inducing membrane fusion. Although HIV_{V2E} forms predominant α-helix conformation when it interacts with POPG liposomes, it cannot destabilize membrane and induce fusion because it cannot penetrate into POPG bilayers. That HIV_{WT} cannot penetrate into POPC bilayer may be due to the neutral POPC which does not interact with positive charged Arg residues of HIV_{WT}, so that POPC cannot induce HIV_{WT} to form predominant α-helix conformation. Whereas the reason for HIV_{V2E} not penetrating into POPG bilayer may be the replacement of N terminal hydrophobic Val residue by hydrophilic Glu residue. Thus we speculates that the membrane insertion process of HIV-1 fusion peptide might start from N terminus, which leads most parts of hydrophobic amino acids forming α-helix into membrane thereby disrupting the structure of bilayer and results in membrane fusion. Other researchers studying on fusion peptide also supported the view that fusion peptide should undergo a conformational transition from β-sheet to α-helix when it interacts with membrane lipid[8,9]. But others reported that fusion peptide adopts predominant β-structure to realize its function[10]. Because we only used static methods (e.g. CD and FTIR) to study the

equilibrium conformation adopted by HIV-1 fusion peptide when it interacts with membrane lipid, we can only make preliminary deductions about the membrane fusion process according to acquired results. In our next work, we will further explore this course by way of dynamic fluorescence technique, Raman spectroscopy and polarized infrared spectroscopy.

Acknowledgements This work is supported by the National Natural Science Foundation of China (Grant No.39770181).

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(Received August 3, 1999)