Orientation of Fusion-Active Synthetic Peptides in Phospholipid Bilayers: Determination by Fourier Transform Infrared Spectroscopy†

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ABSTRACT: A group of synthetic peptides having an amino acid sequence related to the N-terminal region of the influenza virus hemagglutinin HA-2 chain can induce phospholipid membrane fusion in a pH-dependent manner. These peptides bind to membranes to form α-helices even at pH's where no fusion activity is seen. We determined the orientation of these α-helical peptides in lipid multibilayers using attenuated total reflection infrared spectroscopy and found that the peptide α-helices took a preferential orientation, the helix axis being about 70° from the normal of the membrane plane, or in other words rather parallel to the membrane plane. The orientation was almost independent of pH and a modification of the N-terminal amino group which reduced the fusion activity of the peptides. The determination was carried out for peptides in lipid multibilayers in dry or hydrated (membranes equilibrated with D_2O vapor) conditions. Although a slight decrease in the helix orientation angle from the membrane normal was noticed for a hydrated system, the difference between the results for dry and hydrated conditions was small.

Membrane fusion or fission is an essential biological process crucial in viral transfection, cellular endo- or exocytosis, protein translocation between intracellular organelles, and other processes (Stegman et al., 1989; Marsh & Helenius, 1989; Hoekstra, 1990; Waters et al., 1991; White, 1992). Excluding the possibility of Ca^{2+}-induced fusion, these processes are triggered by specific proteins, such as HA[1] for influenza virus infection (White, 1990, 1992). In the amino acid sequences of those proteins a cluster of hydrophobic amino acids is usually found and is thought to be responsible for the interaction with the hydrophobic interior of lipid bilayers (Ohnishi, 1988). It has been shown that peptides having an amino acid sequence similar to a hydrophobic stretch of influenza virus HA (for example, amino acid sequence 1–20 of the HA-2 chain) can trigger fusion or vesiculation of small or large unilamellar artificial vesicles with a viral protein-like pH dependence (Lear & DeGrado, 1987; Murata et al., 1987, 1991a, 1992; Düzgünes & Gambale, 1988; Wharton et al., 1988; Burger et al., 1991). The relevance of peptide-induced membrane fusion as a model of protein-induced fusion has been discussed (Düzgünes & Shavnin, 1992) since the peptides have not yielded a full reproduction of the protein-mediated process at the present stage. For example, those model peptides could not induce cell-to-cell fusion, but the failure is reasonable since cell membranes are backed by rigid cytoskeletons such as actin–spectrin complexes. However, the fact that those peptides can induce one of the elementary steps of the biological membrane fusion process is important, and this is the reason we extend the study of peptide and lipid membrane interactions.

A direct interaction between these fusogenic peptides and lipid bilayers has been observed even at a pH where the fusion activity was negligible (Lear & DeGrado, 1987; Düzgünes & Gambale, 1988; Takahashi, 1990). Therefore, pH-dependent triggering of membrane fusion by these peptides must be ascribed to a difference between the states of the peptide–lipid interaction at the pH's for which the peptide is active or inactive. There is a possibility that the peptides may change their orientation within a lipid bilayer according to a change of pH. Since the peptides adopted mainly an α-helical conformation in membranes irrespective of pH (Takahashi, 1990), a drastic change of the orientation of the helix within a lipid bilayer, if it occurred, might be considered as a trigger of membrane fusion. We determined the orientation of a membrane fusion-active α-helical peptide in phospholipid bilayers using an ATR infrared spectroscopy technique and would like to describe the results in this paper.

MATERIALS AND METHODS

Materials. Peptides III, IV, and VI (Figure 1) were prepared according to a procedure previously described (Takahashi, 1990) and purified finally by high-performance liquid chromatography (Cosmosil 5C18 (10 × 250 mm)), eluted with a 0.05% aqueous trifluoroacetic acid:acetonitrile gradient from 100:0 to 40:60. Egg PC, which was purchased from Sigma, was chromatographed on silica gel before use (Singleton et al., 1965). DMPC was the product of Avanti Polar Lipids, Inc., and was used without further purification. Lipid SUV and LUV were prepared as described (Murata et al., 1987; Szoka & Papahadjopoulos, 1978).

ATR Measurements. ATR spectra were obtained with a Nicolet 6000C FTIR spectrometer coupled with an MCT detector at 4-cm^{-1} resolution with 2000 scans. A mixture of phospholipid SUV (10 mM) and peptide (1 mM) in a ratio of 5:2 (4 mg of phospholipid) was adjusted to the desired pH and deposited on a Ge ATR prism. Buffers were not used to avoid a potential perturbation of membranes during drying. The deposited material was first dried in a stream of nitrogen and then hydrated at room temperature (egg PC) or 30 °C (DMPC) for 12 h under a water-saturated atmosphere. The sample was dried again in a stream of nitrogen, and then measurements under "dry" conditions were carried out. While measurements were carried out, the ATR prism was set on a holder which was maintained at 10 °C (for egg PC) or 30

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[1] Abbreviations: ATR, attenuated total reflection; DMPC, dimyristoyl-L-α-phosphatidylcholine; eggPC, egg yolk phosphatidylcholine; FTIR, Fourier transform infrared; HA, hemagglutinin; LUV, large unilamellar vesicles; SUV, small unilamellar vesicles.

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III H-Gly-Leu-Phe-Glu-Ala-Ile-Ala-Glu-Phe-Ile-Glu-Gly-Gly-Trp-Glu-Gly-Leu-Ile-Glu-Gly-OH

IV H-Gly-Leu-Leu-Glu-Ala-Leu-Ala-Glu-Leu-Leu-Glu-Gly-Gly-Trp-Glu-Gly-Leu-Leu-Glu-Gly-OH

VI H-Gly-Leu-Phe-Lys-Ala-Ile-Ala-Lys-Phe-Ile-Lys-Gly-Gly-Trp-Lys-Gly-Leu-Ile-Lys-Gly-OH

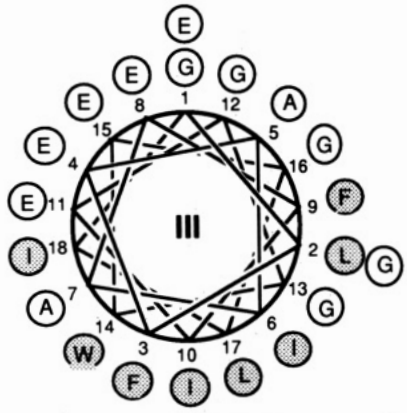

FIGURE 1: Amino acid sequences of peptides III, IV, and VI and a helical wheel representation of peptide III. Hydrophobic residues are shaded.

°C (for DMPC) by circulating water supplied from a thermostated bath, and the sample side of the ATR cell was enclosed in a casing for measurements under dry conditions or for those under saturation with D_2O vapor. The saturation was achieved by introducing about 0.1 mL of D_2O into a small dish placed near the ATR prism inside the casing. The spectra under the D_2O atmosphere ("wet" conditions) were measured 6 h after the equilibration was started, because the changes of absorptions at 2500 cm^{-1} (increase due to D_2O absorption) and at the lipid antisymmetric peak (decrease due to membrane expansion with hydration, which induced a reduction of the number of lipid molecules within the penetration depth of an incident light beam) reached a stationary state 4 h after the D_2O was placed inside the casing.

An obstructive contribution of the H_2O spectrum when the sample was equilibrated with D_2O vapor was subtracted as follows. At first, a broad peak due to H_2O (generated by hydrogen–deuterium exchange) around 3400 cm^{-1} was subtracted by adjusting a factor for the pure H_2O spectrum which was obtained by measurement of H_2O vapor on an ATR prism to give a baseline as possible as flat except for an amide A peak near 3300 cm^{-1}. After this correction, a residual H_2O spectrum, if observed in the amide I region (this was observed as unevenness of the amide I peak), was similarly subtracted to give a smooth amide I absorption. When dry samples were measured, the contribution of the H_2O spectrum was negligible.

Polarization data were obtained with a silver wire grid polarizer on KRS-5, and the same amount was subtracted for the H_2O contribution for both measurements. The observed amide I region was analyzed with the aid of a factory-supported FOCAS program for least-squares curve-fitting and Fourier self-deconvolution for resolution enhancement under the assumption of Lorentzian curves.

The electric fields of incident light in an ATR sample were calculated according to Harrick (1967) as

$$E_x = \frac{2(\sin^2\theta - n_{21}^2)^{1/2}\cos\theta}{(1-n_{21}^2)^{1/2}[(1+n_{21}^2)\sin^2\theta - n_{21}^2]^{1/2}}$$

$$E_y = \frac{2\cos\theta}{(1-n_{21}^2)^{1/2}}$$

$$E_z = \frac{2\sin\theta\cos\theta}{(1-n_{21}^2)^{1/2}[(1+n_{21}^2)\sin^2\theta - n_{21}^2]^{1/2}}$$

where θ is the angle of a light beam to the prism normal at the point of reflection, and $n_{21} = n_2/n_1$ (n_1 and n_2 are the refractive indices of Ge and the membrane sample, respectively).

RESULTS

Polarized ATR: Data Analysis. In the present case, the thickness of the lipid multibilayer (or lipid–peptide) sample on a Ge crystal was about 4 μm, which was much thicker than the penetration depth of reflecting light (0.17 and 0.83 μm for 3800 and 800 cm^{-1}, respectively; Harrick, 1965). Under these conditions, the dichroic ratio, R, was calculated for absorptions of parallel (to a membrane plane), A_p, and perpendicularly polarized incident light, A_s:

$$R = A_p/A_s$$
$$= (E_x^2/E_y^2) + (E_z^2/E_y^2)\{f\cos^2\alpha + (1-f)/3\}/\{1/2(f\sin^2\alpha) + (1-f)/3\}$$

where E_x, E_y, and E_z were 1.40, 1.52, and 1.64, respectively (the angle of incidence was 45°; the refractive indices of Ge and lipid (no anisotropy assumed) were 4.03 and 1.50, respectively). $E_{x,y,z}$, f, and α are the electric fields of incident light [the coordinate system is the same as in Fringeli and Günthard (1981)], an orientational order parameter, and the angle between the transition moment of the amide I vibration of an α-helix and the helix axis, respectively [for details, see Fringeli (1977), Fringeli and Günthard (1981), and Brauner et al. (1987)]. Although an orientational order parameter, f, corresponds to each chromophore in a molecule, the calculated f from the observed spectra is a value averaged over the molecules. We consider the observed f value to represent an orientation of a whole molecule, especially since an α-helix is a structure formed by intramolecular cooperative interactions and is rigid enough to assume that all amide chromophores in a molecule have the same f value. We then correlated the orientation of α-helices as $f = (3\cos^2\gamma - 1)/2$, assuming that the helices were evenly oriented with angle γ,

Table I: Polarized ATR Analysis of Lipid Multibilayers and Lipid–Peptide Complexes[a]

	lipid chain		peptide α-helix		
	R of $\nu_{antisym(CH_2)}$	f	R of amide I	f	γ_{pept},[b] deg
eggPC (from SUV),[c] dry	1.52	0.43			
eggPC (from $CHCl_3$),[d] dry	1.38	0.32			
DMPC (from SUV), dry	1.16	0.64			
IV–eggPC (pH 2.5), dry[e]	1.57 ± 0.02	0.29 ± 0.01	1.38 ± 0.05	−0.32 ± 0.03	69.8 ± 1.8
IV–eggPC (pH 2.5), wet[f]	1.51 ± 0.07	0.33 ± 0.05	1.45 ± 0.15	−0.28 ± 0.09	68.5 ± 5.0
IV–eggPC (pH 5.0), dry	1.39	0.42	1.31	−0.36	72.0
IV–eggPC (pH 5.0), wet	1.48	0.35	1.50	−0.25	65.7
IV–eggPC (pH 8.0), dry	1.38 ± 0.11	0.43 ± 0.09	1.43 ± 0.07	−0.29 ± 0.03	67.9 ± 2.5
IV–eggPC (pH 8.0), wet	1.46 ± 0.02	0.37 ± 0.01	1.80 ± 0.20	−0.10 ± 0.09	58.9 ± 4.0
III–eggPC (pH 2.5), dry	1.62	0.24	1.32	−0.36	72.0
III–eggPC (pH 2.5), wet	1.74	0.16	1.40	−0.31	69.0
III–eggPC (pH 5.0), dry	1.48	0.35	1.40	−0.31	69.0
III–eggPC (pH 5.0), wet	1.40	0.42	1.50	−0.25	65.7
III–eggPC (pH 8.0), dry	1.58	0.27	1.50	−0.31	69.0
III–eggPC (pH 8.0), wet	1.59	0.27	1.50	−0.25	65.7
VI–eggPC (pH 6.0), dry	1.49 ± 0.04	0.34 ± 0.03	1.38 ± 0.0	−0.32 ± 0	69.7 ± 0
VI–eggPC (pH 6.0), wet	1.41 ± 0.01	0.41 ± 0.01	1.65 ± 0.05	−0.17 ± 0.02	61.8 ± 1.2
VI–eggPC (pH 10.0), dry	1.55 ± 0.15	0.31 ± 0.10	1.64 ± 0.02	−0.18 ± 0.01	62.2 ± 0.6
VI–eggPC (pH 10.0), wet	1.58 ± 0.20	0.28 ± 0.15	1.90 ± 0.2	−0.05 ± 0.04	56.6 ± 1.9
Ac-IV–eggPC (pH 2.5), dry	1.38	0.43	1.33	−0.35	71.6
Ac-IV–eggPC (pH 2.5), wet	1.28	0.52	1.40	−0.31	69.0
Ac-IV–eggPC (pH 5.0), dry	1.42 ± 0.03	0.40 ± 0.02	1.34 ± 0.01	−0.35 ± 0.01	71.2 ± 0.4
Ac-IV–eggPC (pH 5.0), wet	1.42 ± 0.04	0.40 ± 0.03	1.60 ± 0.10	−0.20 ± 0.05	63.2 ± 2.5
Ac-IV–eggPC (pH 8.0), dry	1.62	0.24	1.80	−0.10	58.5

[a] Deviations mean the upper and lower limits of observed or calculated values for multiple measurements. [b] The angle of the axis of the peptide α-helix from the membrane normal. [c] Lipid multibilayers were prepared from SUV suspension. [d] Lipid multibilayers were prepared from chloroform solution. [e] The peptide–lipid complex was prepared from SUV solution at the pH indicated in parentheses. [f] Membrane multibilayers on an ATR prism were equilibrated with an atmosphere saturated with deuterium oxide vapor.

which was the one between the membrane or Ge surface normal and the helix axis (or, in the case of a lipid molecule, the molecular axis, which was the direction of the hydrocarbon chain in the fully extended all-trans conformation).

Lipid Film. Since the symmetric ($\nu_{sym(CH_2)} \sim 2850$ cm^{-1}) and antisymmetric ($\nu_{antisym(CH_2)} \sim 2920$ cm^{-1}) vibrations of lipid methylene C-H bonds are perpendicular to the molecular axis of an extended fully trans conformation of a hydrocarbon chain, we estimated the ordering of lipid molecules from polarized ATR analysis of these absorptions. As perturbations from other vibrations on these absorptions were small (Brauner et al., 1987), we employed the stronger $\nu_{antisym(CH_2)}$ band to calculate R after confirmation that the dichroism of each band for some of the samples actually gave the same R value within experimental error. The observed band position for $\nu_{antisym(CH_2)}$ at 2922 cm^{-1} (eggPC, DMPC) suggested that the multibilayered membrane was in the liquid crystalline phase (Cameron et al., 1980). This was expected since the measurements were carried out at a temperature higher than the gel to liquid crystalline phase transition temperature (Mabrey & Sturtevant, 1976). Due to an intermixing of gauche and trans conformation in the liquid crystalline phase, a lipid hydrocarbon chain is not expected to be as rigid as an α-helical structure. However, we can conventionally assign an f parameter as a molecular average, as in the case of an α-helix, and can calculate the apparent inclination, γ_{lipid}, of a lipid hydrocarbon chain to compare the results to the values in the literature. The results (Table I) showed that the hydrocarbon chain of eggPC multibilayers deposited from SUV solutions had orientation factors f in the range 0.25–0.43 (0.37 was the mean), which would correspond to $\gamma_{lipid} = 40°$. The value 40° is close to those observed for bilayers deposited from a chloroform solution (Fringeli & Günthard, 1981) and lipid orientation in purple membrane (42°, Yang et al., 1987), and the values of f (about 0.4) were close to those estimated from a deuterium NMR study [Seelig & Seelig, 1977, 1980; also see the discussion in Frey and Tamm (1991)]. γ_{lipid} for DMPC was estimated as ~25–30°, also in accord with literature values (for example, ~22–34°; Janiak et al., 1976). From these observations we concluded that lipid bilayer formation on a Ge prism was satisfactory, although slight disorientation of the bilayers might be present (Goormaghtigh et al., 1991).

Orientation of Peptides. When peptides were included in membranes, dichroism measurements of the peptide amide I region were carried out for dry or hydrated samples. The hydration was achieved with D_2O vapor. By this treatment, the amide protons in ramdom-coil conformation were substituted with deuterons to shift the random coil amide I absorption to the lower frequencies. Dichroism, R, was calculated from the absorbance at the peak of the α-helix amide I absorption at 1650–1660 cm^{-1} which was obtained after Fourier self-deconvolution followed by curve-fitting of the peptide absorption in the 1600–1700 cm^{-1} region (Figure 3). Due to the presence of carbonyl absorption in the 1700 cm^{-1} region, it was necessary that actual curve-fitting be extended to 1800 cm^{-1} to analyze the higher amide I frequencies. For the value of α, the angle between the α-helix axis and the transition moment of the amide I transition, we used 27° (Rothschild & Clark, 1979; Brauner et al., 1987).

We studied membrane fusion activities of peptides III, IV, and VI and their derivatives with eggPC liposomes (Murata et al., 1987, 1991a,b, 1992). Attempts to use DMPC as the membrane lipid produced unsatisfactory results, possibly due to the phase separation of the peptide and the lipid in hydrated samples. A synthetic amphiphilic peptide was shown previously to induce the formation of micelles from DMPC bilayers (Subbarao et al., 1988).

The results obtained in the present study are summarized in Table I. The effects of pH upon orientation of α-helices were almost negligible. However, the orientation in wet (equilibrated with water vapor or covered with an aqueous

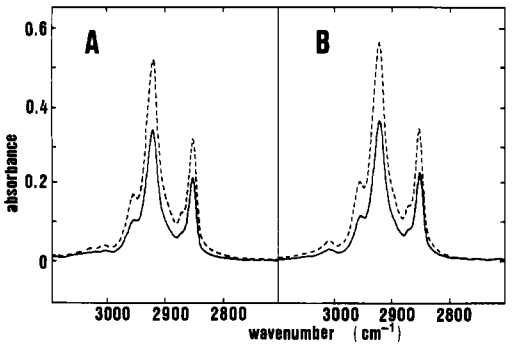

FIGURE 2: ATR dichroism in the 3000-cm^{-1} region for eggPC (A) and peptide IV–eggPC (B) under dry conditions. The membrane was prepared from SUV suspension. The solid line is for the S component of polarized incident light (an electric vector oscillates parallel to the Ge prism or membrane plane) and the broken line is for the P (perpendicular) component. The observed peak positions for $\nu_{antisym(CH_2)}$ were 2921.8 cm^{-1} for S and 2921.7 cm^{-1} for P.

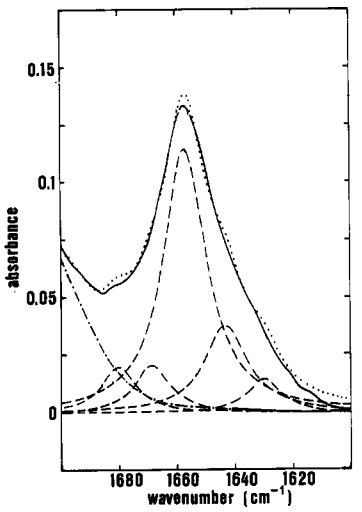

FIGURE 3: Curve-fitting of the amide I region in a polarized ATR absorption spectrum of peptide IV–eggPC for the P component of polarized light under wet conditions: observed spectrum (—), component bands of the amide I absorption after least-squares curve-fitting (– – –), contribution of the carbonyl absorptions (– • –),and summarized line shape (···). Curve-fitting was carried out after Fourier self-deconvolution of the observed spectrum to yield approximate positions of the component bands. The final positions of the amide I components were 1629.9, 1643.0, 1656.9, 1668.6, and 1680.2 cm^{-1}. Absorption at the highest peak at 1656.9 cm^{-1} (α-helix) was used to calculate the dichroism factor, R.

buffer) membranes was lower (mean, 65°) than that observed in dry membrane (70°). We cannot conclude whether the change comes from an actual decrease of inclination of α-helices or can be ascribed to increased contribution of randomly oriented molecules, which would give an angle of 54.7° as γ_{pept}, in the wet state. The orientation of the N-terminal-modified and fusion-inactive peptide α-helices was not different from that of the unmodified and fusion-active peptides.

DISCUSSION

The peptides studied in this paper have amino acid sequences related to that of the putative fusion peptide of the influenza virus HA-2 chain of strain A/PR/8/34. Modification of the original amino acid sequence was so designed as to preserve an amphiphatic α-helical structure and the relative bulkiness of the amino acid residues (Takahashi, 1990). Peptides III and IV had pH-dependent membrane fusion activities similar to that of the virus. The transition from the fusion-inactive form to the active one occurred over a narrow pH range (inactive at pH 6 or higher and fully active at pH 5, for both peptides). Basic peptide VI showed a residual fusion activity at neutral and acidic pH's and full activity at pH 10 (Murata et al., 1991, 1992). Peptides III, IV, and VI bind to neutral phospholipid bilayers to form α-helices even at pH's where the peptides are fusion-inactive (Takahashi, 1990). At pH lower than 6 (peptides III and IV) or higher than 9 (peptide VI), these peptides showed maximum helix content. Under such conditions, the ellipticities at 222 nm were 20 000, 22 000, and 20 000 for III, IV, and VI, respectively, in the presence of lipid, and these values nearly equaled or exceeded the limited value (21 500) for Glu_{20} α-helix (Rinaudo & Domard, 1976). At neutral pH, the assumed α-helices carry negative (peptides III and IV) or positive (peptide VI) charges on half of the helical surface (Figure 1). When the peptides were bound to lipid membranes, the most favorable arrangement of the peptide α-helix was considered as that in which the helix floats on the bilayer, dipping the hydrophobic side into the nonpolar interior of the membrane and exposing the charged hydrophilic side to the polar head group moieties of the lipids or to the solvent. At the fusion-active pH, one may expect more free arrangement of the peptide helices inside the membranes with neutralization of the carboxylate or ε-ammonium groups. Determination of peptide orientation in lipid membranes must be accomplished to get an insight into the mechanism of membrane fusion.

As the transition moment of the amide I absorption of an α-helix lays nearly parallel to the helix axis and is oriented symmetrically to the helix axis, measurements of the dichroism of amide I absorption induced by polarized infrared light might reveal the orientation of the helix. Of the two methods popularly employed to measure dichroism, linear dichroism (Rothschild et al., 1982) and ATR measurements (Fringeli, 1977), the latter was considered to be advantageous and was adopted in the present study owing to its enhanced sensitivity and the possibility of solution measurements. ATR-FTIR spectroscopy has been widely used for studies of phospholipid membranes and molecules interacting with lipid bilayers. Orientation of α-helical peptides in membranes has been successfully assigned for several instances by this method, for example, melittin (Weaver et al., 1992), Na^+/K^+-ATPase (Fringeli, et al., 1989), pulmonary surfactant proteins (Pastrana et al., 1991; Vandenbussche et al., 1992), colicin A fragment (Goormaghtigh et al., 1992), δ-hemolysin (Brauner et al., 1987), bacteriorhodopsin (Yang et al., 1987; Frey & Tamm, 1991), and a synthetic peptide (Goormaghtigh et al., 1991).

The present results showed that the peptide α-helices took neither a transmembrane ($\gamma = 0°$) nor a random ($\gamma = 54.7°$) arrangement but rather a lying ($\gamma \sim 70°$) position irrespective of pH (in other words, independent of the membrane fusion activity). In this orientation, the polar groups on the hydrophilic face of a peptide α-helix, either dissociated or undissociated, can interact with ions or water molecules in an aqueous phase. When membrane fusion takes place with this peptide, half-exposed peptide α-helices embedded in a bilayer might interact with other membranes or peptides in other membranes. Efficient fusion between LUV carrying separately the two kinds of peptides, III and VI, which are complementary in the sense of their electric charge distributions to each other (Murata et al., 1991a), suggests that the latter possibility is plausible. The evidence that the hydrophobic face of the α-helix is in the hydrophobic nonpolar interior of a lipid bilayer comes from the fact that the

tryptophan fluorescence shifts to ca. 330 nm in the presence of SUV or LUV from ca. 355 nm in the absence of lipids, and also was supported by a decrease of the nitrate-induced quenching of tryptophan fluorescence (Murata et al., 1993).

α-Carboxyl groups of peptides III and IV have no influence on the fusion activity of these peptides. C-terminal amide derivatives of these peptides showed a behavior quite similar to that of the parent peptides in the aspects of fusion activities and properties. On the contrary, acetylation of the α-amino group of peptide III or IV greatly reduced the membrane fusion activity of these peptides (Murata et al., 1991b). Acetimidylation of the α-amino group of the peptides, a modification to reserve a positive charge, also blocks the activity. Nevertheless, these modified and fusion-inactive peptides interacted strongly with membranes and formed a stable α-helix. Our present results (Table I) showed that the α-helices of these modified peptides oriented quite similarly to the parent peptides. Therefore a nearly parallel arrangement of α-helices to a membrane plane is a necessary but insufficient condition to show fusion activity for closely related peptide derivatives as seen in the present case. A mutational replacement of the N-terminal glycine in HA-2 by other amino acids also resulted in abolishment of the fusion activity of hemagglutinin (Gething et al., 1986). We do not know at the present stage why the N-terminal residue is so important in triggering membrane fusion.

Various orientations of a peptide α-helix in membranes have been reported: a perpendicular arrangement to the membrane plane was assigned for melittin [Brauner et al., 1987; Frey & Tamm, 1991 (in a dry membrane); Weaver et al., 1992], pulmonary surfactant proteins (Pastrana et al., 1991; Vandenbussche et al., 1992), bacteriorhodopsin (Yang et al., 1987), and a synthetic peptide (Goormaghtigh et al., 1991); a parallel orientation was also assigned for melittin [Frey & Tamm, 1991 (in a wet lipid bilayer)]; and a random orientation was assigned for δ-hemolysin (Brauner et al., 1987). Brasseur et al. (1990) studied the membrane insertion geometry of viral fusion peptides and concluded that the peptide α-helices inserted in membranes took neither a perpendicular nor a parallel orientation but an oblique position ($\sim$30–40° was proposed as γ). Their conclusion came from a theoretical consideration, and our present results may support a part of their view.

No significant change in α-helix orientation in membranes at active and inactive pH's or for the active and inactive peptide derivatives means that the fusion activity is not simply correlated to peptide static orientation. Presumably we must consider a dynamic aspect of peptide–lipid interactions. We proposed the view that a distribution of bulky side chains on the helix surface was an important factor in determining peptide fusion activities. In the present series of peptides, an uneven distribution of hydrophobic bulky and small residues was necessary to induce membrane fusion (Murata et al., 1993). Also we found that various kinds of HA-related peptides induced pH-dependent contents-leakage from lipid vesicles. The simplest idea to explain the leakage phenomenon must be channel formation with peptides occupying a transmembrane position; however, the present result may not allow such a simple view for the reason of leakage. Although leakage capacity does not necessarily mean fusion activity of a peptide, both processes must be deeply correlated as perturbation processes of membrane structures, and further analysis of the dynamic properties of peptides in membranes is required.

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