pH-Activated Fusogenic Transmembrane LV-Peptides†

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ABSTRACT: LV-peptides mimic the in vitro fusogenicity of synthetic fusion protein transmembrane domains. The original versions of these peptides consist of a variable hydrophobic core (containing leucine and/or valine residues (LV)) that is flanked by invariant lysine triplets at both termini. Previously, peptide fusogenicity was correlated with the structural plasticity of their hydrophobic cores. Here, we examined the functional importance of positively charged flanking residues. To this end, we determined the fusogenicities of peptide variants that contain terminal His and/or Lys triplets. Interestingly, liposome fusion by peptides with His triplets was triggered by acidic pH. The pH dependence of fusion is reflected by a sigmoidal titration curve whose midpoint is close to the pK_a value of histidine. Thus, only peptides with positively charged residues at both termini are fusogenic. The previously established dependence of fusogenicity on the sequence of the hydrophobic peptide core of Lys-flanked LV-peptides was preserved with the His-flanked versions at low pH. We propose that the structural flexibility of the core region as well as positive terminal charges are required for LV-peptide function in lipid mixing. In a potential practical application, the pH-dependent LV-peptides might prove to be useful in the lipofection of eukaryotic cells.

Lipid bilayers undergoing fusion transiently rearrange in a process that is supported by membrane-spanning fusogenic proteins. These fusogenic proteins include members of the SNARE[1] (soluble NSF (*N*-ethylmaleimide-sensitive factor) attachment protein receptor) protein family that drive the intracellular fusion of eukaryotic membranes as well as a diverse group of proteins from enveloped viruses (*1*−*3*). Although one function of the fusogens is to mediate the apposition of cognate membranes via their soluble domains, their single transmembrane domains (TMDs) directly contribute to lipid mixing. The functional importance of the TMDs is indicated by the fact that their replacement by lipid anchors, their truncation, or mutation affects the mixing of outer and/or inner membrane leaflets (*4*−*11*).

Another line of evidence pointing to the functional role of the TMDs is based on the observation that synthetic peptides representing the TMDs of SNAREs (*12*) or the vesicular stomatitis virus (VSV) G-protein (*13*, *14*) are capable of driving liposome−liposome fusion in vitro. Because these TMD-peptides are devoid of membrane-extrinsic domains thought to mediate membrane apposition prior to fusion, it is believed that their presence in the membrane increases the likelihood by which randomly colliding liposomes enter fusion. The ability of these TMD-peptides to initiate fusion has been related to their conformational flexibility because mutations that stabilize the helical structure in solution also compromize fusion (*13*, *14*). This is consistent with an unusual amino acid composition of fusion protein TMDs because β-sheet-promoting β-branched amino acids and/or helix-destabilizing Gly residues are overrepresented in fusion protein TMDs (*10*, *12*).

In order to investigate the structure/function relationships of fusogenic TMD-peptides by de novo design, a series of hydrophobic model sequences, termed LV-peptides, was previously developed to serve as a low-complexity model system. LV-peptides contain hydrophobic core sequences that are composed of residues with different secondary structure propensities, that is, helix-promoting Leu and sheet-promoting Val residues at different ratios (*15*). The different helix-forming propensities of these amino acids are thought to reflect the different degrees by which the side-chain entropy of Leu or Val is lost upon helix formation (*16*). Consistent with the proposed relationship between fusogenicity and structural flexibility, the fusogenicity of these peptides increases with their Val/Leu ratio and is augmented by the incorporation of Gly/Pro or Pro/Gly pairs that are known to destabilize helices. That fusogenicity and conformational flexibility of LV-peptides are related is supported by the finding that peptides that are more fusogenic can be readily refolded from the α-helical conformation to β-sheet or vice versa by changing solution polarity (*15*).

The original LV-peptide core sequences are flanked by Lys triplets. In the present study, we examined the functional importance of these terminal Lys residues. To this end, we have replaced Lys by His and investigated whether the fusogenicity of His-flanked peptides depends on solution pH.

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[1] Abbreviations: SNARE, soluble NSF (*N*-ethylmaleimide-sensitive factor) attachment protein receptor; VSV, vesicular stomatitis virus; TMD, transmembrane domain; P/L, peptide/lipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; NBD-PE, *N*-(7-nitro-2-1,3-benzoxadiazol-4-yl)hexadecylphosphatidylethanolamine; Rh-PE, *N*-(lissamine rhodamine B sulfonyl)hexadecylphosphatidylethanolamine.

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The results indeed suggest that charged side chains are required for the fusogenic function of LV-peptides.

EXPERIMENTAL PROCEDURES

Peptide Synthesis. Peptides were synthesized by Boc chemistry (PSL, Heidelberg, Germany) and were >90% pure as determined by mass spectrometry. Concentrations were determined via tryptophan absorbance.

Preparation and Fusion of Small Unilamellar Liposomes. Liposomes with or without peptides were prepared as described (*12*) from mixtures of egg phosphatidylcholine (PC)/brain phosphatidylethanolamine (PE)/brain phosphatidylserine (PS) (PC was from Serva, Heidelberg, Germany; PE and PS were from Avanti Polar Lipids) at a ratio of 3:1:1 (w/w/w, final lipid concentration = 3 mM). Donor liposomes contained 0.8% (w/w) of *N*-(7-nitro-2-1,3-benzoxadiazol-4-yl)hexadecylphosphatidyl-ethanolamine (NBD-PE) and *N*-(lissamine rhodamine B sulfonyl)hexadecylphosphatidyl-ethanolamine (Rh-PE) (Molecular Probes). Briefly, dried lipid or lipid/peptide mixtures were hydrated using buffers of different pH values (pH 4.0−5.0: 20 mM Na-Citrate; pH 5.0−7.5: 20 mM Tris-maleate). All buffers contained 150 mM NaCl, 5 mM DTT, and 0.1 mM EDTA. The hydrated mixtures were subsequently sonicated to form small unilamellar liposomes. Peptide/lipid ratios were determined as described (*15*). Fusion assays were done by a fluorescence dequenching assay (*17*) in the buffers also used for hydration and evaluated as described (*15*). Standard fusion assays were performed at total lipid concentrations from 2.5 to 2.8 mM. Total lipid mixing was tested as described (*18*) by bleaching NBD-PE in the outer monolayer of labeled liposomes by treatment with 20 mM dithionite for 30 min. All values were corrected for the peptide-independent, spontaneous fusion of pure liposomes and for detergent quenching of NBD fluorescence (<3% of the total values). For pH-induced fusion, His-flanked peptides were reconstituted into liposomes at pH 6.5 in fusion buffer (150 mM NaCl, 20 mM Tris-maleate, 5 mM DTT, and 0.1 mM EDTA) and mixed with donor liposomes containing NBD-PE and Rh-PE at a 4:1 ratio. After 10-fold dilution in buffer, fusion was monitored in a 1 cm quartz cuvette at 37 °C using a Shimadzu RF-1501 Spectrofluorimeter (Shimadzu Corp., Kyoto, Japan). Buffer pH was decreased to a value of 4 by adding 0.5 M HCl (final concentration: 18 mM).

Circular Dichroism Spectroscopy. Peptides were reconstituted into liposomes as described above in buffers of 10 mM dimethylglutaric acid/NaOH (pH 4.0, 5.5, or 7.0) and 10 mM NaCl. Lipids used were palmitoyl-oleoyl-phosphatidylcholine (POPC), dioleoyl-phosphatidylethanolamine (DOPE), and dioleoylphosphatidylserine (DOPS) at a 3:1:1 ratio. After the dilution of liposomes with buffer at a 1:1 ratio, CD spectra were recorded on a J-810 automatic recording spectral polarimeter (Jasco, Easton, MD) from 200 to 260 nm in a 1 mm cuvette. Spectra were averaged after accumulating 10 scans, each, and the background (liposomes without peptide) was subtracted. Curve smoothing was performed. After determining peptide concentrations as described (*15*), mean molar ellipticities and secondary structure contents were calculated by deconvoluting the spectra using the CDNN/PEPFIT algorithm (*28*). The experiments were carried out in triplicate.

A

His-L16	HHHWLLLLLLLLLLLLLLLLHHH
His-LV16	HHHWLVLVLVLVLVLVLVLVHHH
His-LV16-G8P9	HHHWLVLVLVLGPVLVLVLVHHH

B

His-LV16	HHHWLVLVLVLVLVLVLVLVHHH
His/Lys-LV16	HHH.....KKK
Lys/His-LV16	KKK.....HHH
LV16	KKK.....KKK

FIGURE 1: Design of fusogenic peptides. (A) Peptides are flanked by His residues and contain hydrophobic core sequences that were previously shown to exhibit different degrees of structural flexibility (*15*). A Trp residue is included for quantification. (B) Peptides with a hydrophobic core of alternating Leu-/Val-residues flanked by either His and/or Lys triplets. The dots represent residues of the parental core sequence.

Sucrose Gradient Centrifugation. The association of peptides with liposomes was examined upon separating unbound peptides from proteoliposomes by density gradient centrifugation (*12*). Briefly, 300 μL of liposome preparations were mixed with 700 μL of 60% (w/v) sucrose, and overlaid with 2.5 mL of 30% (w/v) sucrose followed by 0.5 mL of fusion buffer (pH 7.5). Upon centrifugation (56,000 rpm, 20 h, 20 °C, Beckman SW60 rotor), >99% of the loaded lipids were found in the top fraction. Peptides were found to quantitatively comigrate with the lipids because they were not detectable in the bottom fraction by analyzing their Trp fluorescence.

RESULTS

Design of His-Flanked LV-Peptides. The original Lys-flanked peptides L16, LV16, and LV16-G8P9 were previously characterized (*15*). Here, we examined the fusogenicities of peptides where the Lys triplets at both termini had been exchanged for His triplets such that the charge state of the terminal side chains could be varied by adjusting the pH value of the solution (Figure 1A). Additionally, the Lys triplets of LV16 were exchanged to His triplets at either the N- or the C-terminus (Figure 1B).

Fusogenic Activity of His-Flanked Peptides Depends on pH Value. The novel His-flanked peptides were reconstituted into liposomal membranes composed of PC/PE/PS (at a 3:1:1 weight ratio) by sonication. Liposome−liposome fusion was examined by a standard fluorescence dequenching assay (*17*) upon rapidly shifting the temperature to 37 °C. This assay is based on the fluorescence resonance energy transfer between the membrane-bound fluorophores NBD-PE and Rh-PE, which are present at quenching concentrations in the membranes of donor liposomes. Upon the fusion of donor membranes with peptide-containing unlabeled acceptor membranes, the average distance between the fluorophores, and thus NBD-fluorescence, increases over time; this is taken as a measure of lipid mixing. The extent of fusion at each time point was calculated by normalizing the respective fluorescence values to the maximal fluorescence dequenching seen upon detergent lysis of the liposomes.

Figure 2A compares the membrane fusogenicity of the different His-flanked TMD-peptides in liposomes at a peptide/lipid (P/L) ratio of 0.005 after reconstitution at pH 4.0 or pH 7.5. At these pH values, the His side chain (pK_a

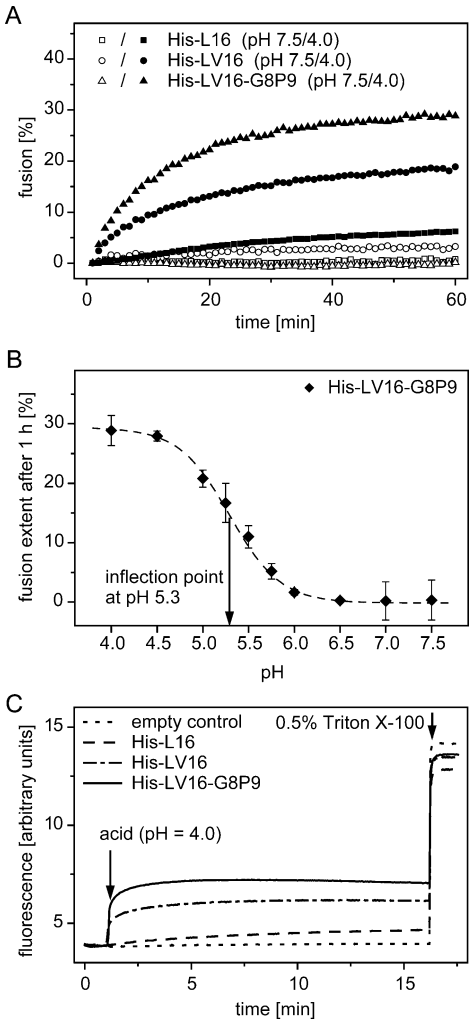

FIGURE 2: pH dependence of liposome fusion driven by His-flanked peptides. (A) Typical fusion kinetics that were recorded with liposomes formed at different pH values reveal that the peptides are fusogenic at pH 4.0 (filled symbols), but not at pH 7.5 (open symbols). The previously (*15*) determined dependence of fusogenicity on the hydrophobic core sequence is maintained. Traces represent the means of three to six independent experiments. The P/L ratios were determined to be 0.0032 to 0.0056. For better comparison of the data obtained with the different peptides, the values were scaled to P/L = 0.005. This does not introduce error because the dependence of the extent of fusion on the P/L ratio is linear (*15*). (B) Extent of fusion, as seen after 1 h, of peptide His-LV16-G8P9 (P/L = 0.005) as a function of pH. The midpoint of the titration curve (pH 5.3) is indicated by an arrow. Data points represent the mean ± SE ($n = 3-6$ independent experiments). All values in A and B were corrected for the background fusion seen without the peptide (<3% after 1 h). (C) pH-activated liposome fusion. After 2 min of incubation at pH 6.5, the pH was lowered to a value of 4.0 by adding HCl (final concentration of 18 mM). The ensuing rapid fluorescence dequenching indicates that fusion depends on the respective core sequence. Liposome lysis by 0.5% (v/v) Triton X-100 results in maximal NBD dequenching. Experiments were done in duplicate. The experimentally determined P/L ratios were 0.0052, 0.0045, and 0.0035 for His-L16, His-LV16, and His-LV16-G8P9, respectively.

= 6) is expected to be positively charged or uncharged, respectively. The fusion kinetics reveal that none of the peptides is fusogenic at pH 7.5 (Figure 2A, open symbols). At pH 4.0, however, marked fusogenicities are seen that follow the rank order His-LV16-G8P9 > His-LV16 > His-L16. The same rank order and comparable fusogenicities were previously observed for the respective Lys-flanked versions at pH 7.4 (*15*).

The pH dependence of the most fusogenic variant, His-LV16-G8P9, was now analyzed in greater detail. By plotting the extent of fusion, seen after 1 h, against pH, we obtained a sigmoidal relationship that appears to reflect the titration curve of His (Figure 2B). Its midpoint at pH 5.3 represents the pK_a value of His in the membrane head group region that is thought to deviate from the solution pK_a for the reasons discussed below.

In all experiments described above, liposomes were formed at the pH value where fusion was subsequently examined. In an alternative experiment, we examined whether the fusogenicity of the peptides can be switched on by lowering the pH value subsequent to liposome formation. Here, we reconstituted the peptides at pH 6.5, where His-LV16-G8P9 shows no fusogenicity (compare Figure 2B), mixed the samples with donor liposomes, and started to record NBD fluorescence. As shown in Figure 2C, lowering the pH to a value of 4.0 by acid addition at $t = 2$ min resulted in rapid fluorescence dequenching indicative of liposome fusion. Acid-induced fusion exhibited the same dependence on the core sequence as seen in the previous experiments. Thus, low pH is not required at the time of liposome formation but, rather, can activate His-flanked peptides after reconstitution. These results show that the fusogenicity of the peptides depends both on the charge of the terminal residues and on the composition of the core sequence.

Furthermore, we assessed whether liposome fusion driven by His-LV-peptides induces mixing of both membrane leaflets, rather than being arrested at hemifusion. This intermediate stage is characterized by the fusion of outer bilayer leaflets, whereas inner leaflets remain unfused. Hemifusion can be experimentally distinguished from complete fusion upon dithionite treatment of the fluorescent donor liposomes. Dithionite converts the NBD moiety present in the outer membrane leaflet to a nonfluorescent derivative while leaving NBD of the inner leaflet unaffected. Accordingly, only complete fusion that includes inner leaflet mixing can now result in fluorescence dequenching (*18*). The efficiency of bleaching has previously been shown by an experiment in which treatment with 20 mM dithionite reproducibly eliminated about two-thirds (~67%) of NBD fluorescence, which reached a plateau after 30 min (*11*). This reveals preferential bleaching of NBD present in the outer monolayer that contains about two-thirds of the total lipid of our about 30 nm large, unilamellar liposomes (*12*). Fusion kinetics recorded with His-TMD-peptides at pH 4.0 is virtually unchanged upon dithionite treatment of donor liposomes (data not shown). Consequently, liposome fusion by His-flanked peptides is not arrested at hemifusion. In this respect, they behave like the original Lys-flanked LV peptides (*15*).

Secondary Structure of His-TMD-Peptides in Membranes. To assess whether the pH dependence of fusogenicity is due to pH-dependent peptide secondary structure in the liposomal membrane, we determined the circular dichroism (CD) spectra of His-L16, His-LV16, and His-LV16-G8P9 in liposomes formed at pH 4.0, 5.5, or 7.0. The natural phospholipids that are routinely used in our fusion assays have significant absorbance in the UV region and thus lead to unacceptable background signals. We, therefore, used a

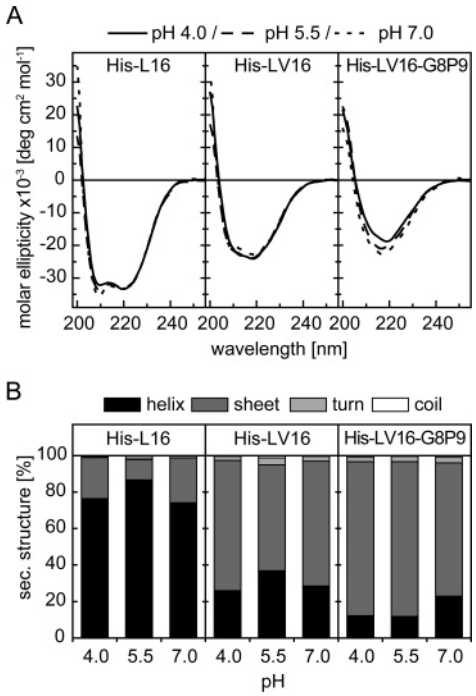

FIGURE 3: Secondary structure of peptides in liposomal membranes. (A) CD spectra of liposomes holding His-L16, His-LV16, and His-LV16-G8P9 (nominal P/L = 0.01) were recorded at the indicated pH values. (B) Secondary structure contents of peptides at indicated pH values determined by deconvolution of CD spectra from A using the CDNN/PEPFIT algorithm.

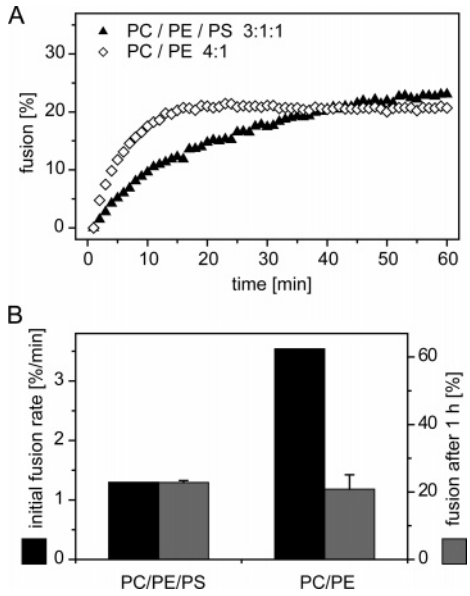

FIGURE 4: Dependence of fusion on liposome net surface charge. (A) Typical fusion kinetics of donor and acceptor liposomes consisting of PC/PE/PS (3:1:1) or PC/PE (4:1). Acceptor liposomes contained LV16 at a nominal P/L = 0.005. (B) Comparison of mean initial fusion rates and the extents of fusion, as seen after 1 h. Data represent the mean ± SE (n = 3). The mean initial fusion rates were determined from averaged kinetics and, therefore, do not have error bars. Exemplary kinetics (A) and mean values (B) were corrected for the background fusion of liposomes without the peptide.

mixture of synthetic lipids (Experimental Procedures) that supports fusion as well as the natural mixture (data not shown). Sucrose gradient centrifugation showed that peptides were quantitatively associated with the liposomes (data not shown). The shapes of the CD spectra given in Figure 3A show that the peptides adopt secondary structures that depend on their hydrophobic core sequences. A quantitative evaluation of the spectral data by a curve-fitting algorithm, which is based on peptide reference spectra, reveals ~80% α-helix plus ~20% β-sheet for His-L16, ~30% α-helix plus ~70% β-sheet for His-LV16, and ~15% α-helix plus ~85% β-sheet for His-LV16-G8P9 (Figure 3B). Although some variation with pH is seen, there is no systematic dependence of secondary structure on the pH value. The data indicate that the ratio of α-helix to β-sheet structures decreases in the following rank order: His-L16 > His-LV16 > His-LV16-G8P9. Thus, helicity is inversely correlated to fusogenicity.

Because the spectra are largely independent of pH value, we conclude that low pH does not activate peptide fusogenicity via a pH-triggered change of secondary structure in the membrane.

Role of Lipid Head group Charge in Peptide-Induced Fusion. To examine whether positively charged peptide termini trigger membrane fusion by interaction with net negatively charged lipid head groups, we investigated the effect of lipid head group charge on fusion. These experiments were done with the Lys-flanked peptide LV16 at pH 7.5. Liposomal membranes composed of our standard lipid mixture (PC/PE/PS at a 3:1:1 ratio) have a net negative surface charge due to the presence of PS. First, LV16-mediated fusion of these standard liposomes was compared to that of liposomes devoid of PS. For this comparison, we evaluated the dequenching kinetics both in terms of initial fusion rate and extent of fusion. The initial fusion rate is influenced by the probability by which a random liposome−liposome collision turns into an actual fusion reaction, whereas the final extent after 1 h reports the sum of fusion events within that time period. If fusion depended on the interaction of peptides with PS, then the omission of PS would decrease the rate and extent of fusion. In contrast to that expectation, we find that the omission of PS leads to a significant increase of the initial rate, whereas the extent of fusion is virtually unchanged (Figure 4). These results exclude the fact that the positively charged peptides require interaction with PS for fusion. Circumstantially, the data suggest that the absence of a net negative surface charge accelerates the initial fusion rate by increasing the probability of fusion after collision.

Relevance of the N- and C-Terminal Location of Charged Side Chains. Finally, we asked whether positively charged amino acids have to be present on both peptide termini to confer fusogenicity. To this end, we tested LV16 variants with Lys and/or His triplets at one or both termini (Figure 1B). These peptides were reconstituted into liposomal membranes at pH 4.0 or 7.5 and tested with our standard fusion assay. At pH 4.0, His triplets as well as Lys triplets hold positive charges. At this pH, all peptides show comparable fusion kinetics (Figure 5A), as expected. At pH 7.5, however, only the peptide with Lys triplets at both termini exhibits substantial fusogenicity (Figure 5B). An evaluation of the data confirmed that the mean extent of fusion, as seen after 1 h, are similar for each peptide variant at pH 4.0, whereas only the Lys-flanked LV16 is strongly fusogenic at pH 7.5 (Figure 5C). We thus conclude that positively charged side chains are required at both termini

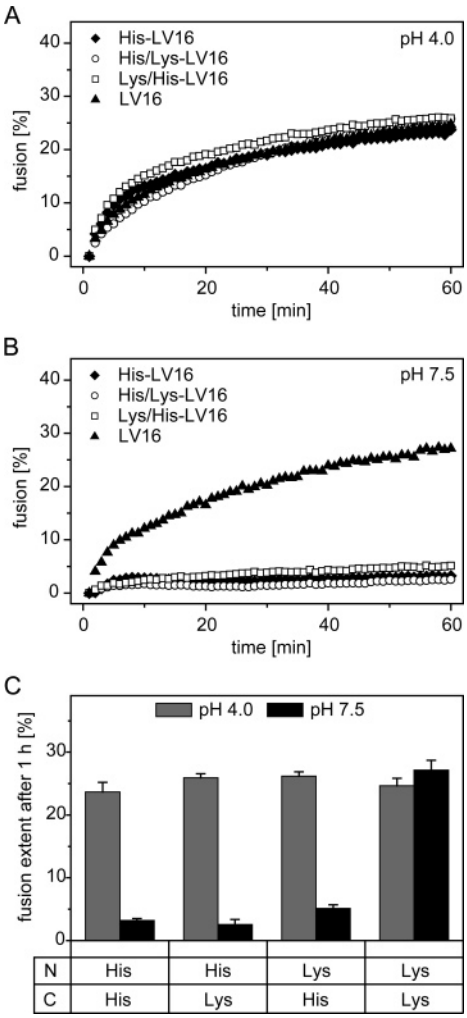

FIGURE 5: Liposome fusion driven by LV16 variants bordered by His and/or Lys triplets. (A) All peptides induce virtually identical exemplary kinetics at pH 4.0. (B) At pH 7.5, only LV16 with Lys triplets at both termini induces fusion. The P/L-ratios were determined to be 0.0031 to 0.0049. For a better comparison of the data obtained with the different peptides, we scaled to P/L = 0.005. (C) Comparison of the mean extent of fusion after 1 h at pH 4.0 and 7.5. Note that strong membrane fusogenicity at pH 7.5 requires Lys at both termini. The bars represent the mean ± SE (n = 3−5). Exemplary kinetics (A and B) and mean values (C) were corrected for the background fusion of liposomes without the peptide.

of a peptide with a conformationally flexible hydrophobic core sequence to render the peptide membrane-fusogenic.

DISCUSSION

Our results demonstrate that exchanging terminal Lys residues for His renders the membrane fusogenicity of different LV-peptides pH-dependent. Because the His-flanked variants are activated by acidic pH, the activity of the peptides appears to require protonated His residues. The titration curve that reflects the pH dependence of His-LV16-G8P9 reveals a His pK_a value of 5.3, which is somewhat lower than the one seen in water (pK_a = 6). This difference is readily explained by the lower polarity of the membranes' lipid head group region, where the peptide termini are likely to be localized. That low polarity reduces the pK_a of His has previously been demonstrated by NMR spectroscopy for a His residue that is buried inside the hydrophobic protein interior of cyclophilin (*19*). We conclude that the fusogenic function of our TMD-peptides depends not only on the conformational flexibility of their hydrophobic core sequences, as demonstrated previously (*15*), but also on the positively charged side chains at both termini.

We considered various potential mechanisms to explain why charged terminal side chains are required for fusion. First, the charge state could determine the structure and/or orientation assumed by the peptides in the membranes at the time of liposome formation. CD spectroscopy indicates that the α-helix content of membrane-associated peptides decreases in the order His-L16 > His-LV16 > His-LV16-G8P9, while β-sheet contents increase. These different helix/sheet ratios roughly reflect the abilities of the respective Lys-flanked variants to undergo helix/sheet transitions as previously seen upon changing the polarity of isotropic solutions (*15*). However, it is most likely that a conformationally flexible α-helix represents the fusogenic conformation because even the strongly helical Lys-flanked L16 can be rendered fusogenic by calcium addition (Poschner, B., and Langosch, D., unpublished work). Notably, the conformations of the His-flanked peptides are largely independent of pH. Protonated termini, therefore, seem not to be required for a fusion-active peptide conformation. Although we did not determine the relative orientation of the helical fractions in the bilayer, they are likely to traverse the membrane as previously determined for Lys-flanked L16 (*12*) or other hydrophobic model peptides (*20*, *21*) by Fourier transform infrared spectroscopy of oriented bilayers. Second, we tested whether positively charged peptide termini would initiate fusion by interaction with the negatively charged PS head group. Fusion initiation by electrostatic attraction indeed seems to play a role when liposomes of opposite net surface charge are mixed as previously observed (*22*). Our results show, however, that the use of neutral liposomes without PS did not reduce fusion. We thus exclude the functionally relevant interaction between the peptides and the PS head group. Third, charged peptide termini could affect bilayer structure by the induction of a negative hydrophobic mismatch. Negative hydrophobic mismatch results when a membrane-spanning α-helix is too short to fully span the hydrophobic core of the membrane (*23*). The hydrophobic 16-residue core of our peptides corresponds to a length of 2.4 nm in an idealized α-helical state. This helix is unable to span the undistorted hydrophobic core of liposomal membranes whose lipids contain mostly saturated and monounsaturated C_{18} lipid acyl chains (∼2.7 to 3.0 nm in the liquid crystalline state) (*24*). Thus, the charged peptides may locally deform the bilayer and thereby prime it for fusion. Although the direct demonstration of bilayer distortion by our LV-peptides is currently lacking, our present results with His/Lys- and Lys/His-flanked LV-peptides are consistent with this idea. Fusogenicity of LV16 requires that the hydrophobic peptide core is bordered by charged residues at both termini, which is consistent with a role of negative hydrophobic mismatch in fusion. This hypothesis is also consistent with our observation that extending the hydrophobic core of LV-peptides from 16 to 20 or 24 residues significantly decreases fusogenicity (Hofmann, M. W., and Langosch, D., unpublished results). It is clear, however, that the proposed

hydrophobic mismatch by itself is insufficient to trigger fusion because L16 peptides display very little fusogenicity.

Taken together, the functional importance of the basic peptide termini may be related to peptide/lipid interaction in that positively charged side chains may interact with the phosphate moiety of the lipid head groups. Interaction between basic TMD-flanking residues and lipid phosphate groups has recently been reported to account for packing interactions between aquaporin tetramers in a crystalline array (*25*).

In the future, the pH-triggered His-LV-peptides might prove to be useful for the transfection of eukaryotic cells with liposomal preparations. Liposomes that are destined to carry cargo molecules into cells are first delivered to endosomes by endocytosis (*26*, *27*). Because the endosomal lumen is slightly acidic (pH 5−6), it is conceivable that liposomes holding His-LV-peptides are triggered for fusion with the endosomal membranes. His-LV-pepides may thus increase the efficiency of cargo delivery into cells.

REFERENCES

1. Tamm, L. K., Crane, J., and Kiessling, V. (2003) Membrane fusion: a structural perspective on the interplay of lipids and proteins, *Curr. Opin. Struct. Biol. 13*, 453−466.
2. Ungermann, C., and Langosch, D. (2005) Functions of SNAREs in intracellular membrane fusion and lipid bilayer mixing, *J. Cell Sci. 118*, 3819−3828.
3. Jahn, R., and Scheller, R. H. (2006) SNAREs-engines for membrane fusion, *Nat. Rev. Mol. Cell Biol. 7*, 631−643.
4. Grote, E., Baba, M., Ohsumi, Y., and Novick, P. J. (2000) Geranylgeranylated SNAREs are dominant inhibitors of membrane fusion, *J. Cell Biol. 151*, 453−466.
5. Rohde, J., Dietrich, L., Langosch, D., and Ungermann, C. (2003) The transmembrane domain of Vam3 affects the composition of cis- and trans-SNARE complexes to promote homotypic vacuole fusion, *J. Biol. Chem. 278*, 1656−1662.
6. Xu, Y., Zhang, F., Su, Z., McNew, J. A., and Shin, Y. K. (2005) Hemifusion in SNARE-mediated membrane fusion, *Nat. Struct. Biol. 12*, 417−422.
7. Giraudo, C. G., Hu, C., You, D., Slovic, A. M., Mosharov, E. V., Sulzer, D., Melia, T. J., and Rothman, J. E. (2005) SNAREs can promote complete fusion and hemifusion as alternative outcomes, *J. Cell Biol. 170*, 249−260.
8. Melikyan, G. B., Lin, S., Roth, M. G., and Cohen, F. S. (1999) Amino acid sequence requirements of the transmembrane and cytoplasmic domains of influenza virus hemagglutinin for viable membrane fusion, *Mol. Biol. Cell 10*, 1821−1836.
9. Armstrong, R. T., Kushnir, A. S., and White, J. M. (2000) The transmembrane domain of influenza hemagglutinin exhibits a stringent length requirement to support the hemifusion to fusion transition, *J. Cell Biol. 151*, 425−437.
10. Cleverley, D. Z., and Lenard, J. (1998) The transmembrane domain in viral fusion: essential role for a conserved glycine residue in vesicular stomatitis virus G protein, *Proc. Natl. Acad. Sci. U.S.A. 95*, 3425−3430.
11. Hofmann, M. W., Peplowska, K., Rohde, J., Poschner, B. C., Ungermann, C., and Langosch, D. (2006) Self-interaction of a SNARE transmembrane domain promotes the hemifusion-to-fusion transition, *J. Mol. Biol. 364*, 1048−1060.
12. Langosch, D., Crane, J. M., Brosig, B., Hellwig, A., Tamm, L. K., and Reed, J. (2001) Peptide mimics of SNARE transmembrane segments drive membrane fusion depending on their conformational plasticity, *J. Mol. Biol. 311*, 709−721.
13. Langosch, D., Brosig, B., and Pipkorn, R. (2001) Peptide mimics of the vesicular stomatitis virus G-protein transmembrane segment drive membrane fusion in vitro, *J. Biol. Chem. 276*, 32016−32021.
14. Dennison, S. M., Greenfield, N., Lenard, J., and Lentz, B. R. (2002) VSV transmembrane domain (TMD) peptide promotes PEG-mediated fusion of liposomes in a conformationally sensitive fashion, *Biochemistry 41*, 14925−14934.
15. Hofmann, M. W., Weise, K., Ollesch, J., Agrawal, P., Stalz, H., Stelzer, W., Hulsbergen, F., deGroot, H., Gerwert, K., Reed, J., and Langosch, D. (2004) De novo design of conformationally flexible transmembrane peptides driving membrane fusion, *Proc. Natl. Acad. Sci. U.S.A. 101*, 14776−14781.
16. Chellgren, B. W., and Creamer, T. P. (2006) Side-chain entropy effects on protein secondary structure formation, *Proteins 62*, 411−420.
17. Struck, D. K., Hoekstra, D., and Pagano, R. E. (1981) Use of resonance energy transfer to monitor membrane fusion, *Biochemistry 20*, 4093−4099.
18. Hoekstra, D., and Duzgunes, N. (1993) Lipid mixing assays to determine fusion in liposome systems, *Methods Enzymol. 220*, 15−32.
19. Yu, L., and Fesik, S. W. (1994) pH titration of the histidine residues of cyclophilin and FK506 binding protein in the absence and presence of immunosuppressant ligands, *Biochim. Biophys. Acta 1209*, 24−32.
20. Zhang, Y. P., Lewis, R. N., Hodges, R. S., and McElhaney, R. N. (1995) Peptide models of helical hydrophobic transmembrane segments of membrane proteins. 2. Differential scanning calorimetric and FTIR spectroscopic studies of the interaction of Ac-K2-(LA)12-K2-amide with phosphatidylcholine bilayers, *Biochemistry 34*, 2362−2371.
21. van der Wel, P. C., Strandberg, E., Killian, J. A., and Koeppe, R. E., II. (2002) Geometry and intrinsic tilt of a tryptophan-anchored transmembrane alpha-helix determined by (2)H NMR, *Biophys. J. 83*, 1479−1488.
22. Pantazatos, D. P., Pantazatos, S. P., and MacDonald, R. C. (2003) Bilayer mixing, fusion, and lysis following the interaction of populations of cationic and anionic phospholipid bilayer vesicles, *J. Membr. Biol. 194*, 129−139.
23. Killian, J. A., and von Heijne, G. (2000) How proteins adapt to a membrane-water interface, *Trends Biochem. Sci. 25*, 429−434.
24. de Planque, M. R., and Killian, J. A. (2003) Protein-lipid interactions studied with designed transmembrane peptides: role of hydrophobic matching and interfacial anchoring, *Mol. Membr. Biol. 20*, 271−284.
25. Gonen, T., Cheng, Y., Sliz, P., Hiroaki, Y., Fujiyoshi, Y., Harrison, S. C., and Walz, T. (2005) Lipid-protein interactions in double-layered two-dimensional AQP0 crystals, *Nature 438*, 633−638.
26. Ulrich, A. S. (2002) Biophysical aspects of using liposomes as delivery vehicles, *Biosci. Rep. 22*, 129−150.
27. Hubbell, J. A. (2003) Enhancing drug function, *Science 300*, 595−596.
28. Poschner, B. C., Reed, J., Langosch, D., and Hofmann, M. W. (2007) An automated application for deconvolution of circular dichroism spectra of small peptides, *Anal. Biochem.*, in press.

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