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Structure and dynamics of the lipid modifications of a transmembrane α-helical peptide determined by ^{2}H solid-state NMR spectroscopy

Anja Penk [a], Matthias Müller [a], Holger A. Scheidt [a], Dieter Langosch [b,c], Daniel Huster [a,*]

[a] Institute of Medical Physics and Biophysics, University of Leipzig, Härtelstr. 16-18, D-04107 Leipzig, Germany
[b] Lehrstuhl für Chemie der Biopolymere, Technische Universität München, Weihenstephaner Berg 3, D-85354 Freising, Germany
[c] Munich Center For Integrated Protein Science (CIPS^M), Munich, Germany

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ABSTRACT

The fusion of biological membranes is mediated by integral membrane proteins with α-helical transmembrane segments. Additionally, those proteins are often modified by the covalent attachment of hydrocarbon chains. Previously, a series of *de novo* designed α-helical peptides with mixed Leu/Val sequences was presented, mimicking fusiogenically active transmembrane segments in model membranes (Hofmann et al., Proc. Natl. Acad. Sci. USA 101 (2004) 14776–14781). From this series, we have investigated the peptide LV16 (KKKW LVLV LVLV LVLV LVLV KKK), which was synthesized featuring either a free N-terminus or a saturated N-acylation of 2, 8, 12, or 16 carbons. We used ^{2}H and ^{31}P NMR spectroscopy to investigate the structure and dynamics of those peptide lipid modifications in POPC and DLPC bilayers and compared them to the hydrocarbon chains of the surrounding membrane. Except for the C2 chain, all peptide acyl chains were found to insert well into the membrane. This can be explained by the high local lipid concentrations the N-terminal lipid chains experience. Further, the insertion of these peptides did not influence the membrane structure and dynamics as seen from the ^{2}H and ^{31}P NMR data. In spite of the fact that the longer acyl chains insert into the membrane, they do not adapt their lengths to the thickness of the bilayer. Even the C16 lipid chain on the peptide, which could match the length of the POPC palmitoyl chain, exhibited lower order parameters in the upper chain, which get closer and finally reach similar values in the lower chain region. ^{2}H NMR square law plots reveal motions of slightly larger amplitudes for the peptide lipid chains compared to the surrounding phospholipids. In spite of the significantly different chain lengths of the acylations, the fraction of gauche defects in the inserted chains is constant.

1. Introduction

Fusion of biological membranes is an intensely investigated yet very complex process mediated by integral membrane proteins, e.g. the SNARE machinery operating within the secretory pathway [1]. Although the general patterns underlying fusion events are known, no complete biophysical picture of the cellular fusion mechanism can be sketched. Therefore, model systems still provide valuable information about molecular details of the fusion event. Several studies have highlighted the influence of transmembrane domains on fusion proteins in this process [2]. For instance, *de novo* designed model peptides, like KALR [3] or LV peptides [4], which mimic the transmembrane domains of fusion proteins, have been shown to promote *in vitro* fusion and used as simplified models to systematically investigate structure/function relationships without the influence of membrane-extrinsic domains. Such low-complexity model systems allow to study one specific aspect of a complicated biological problem with atomistic detail. LV peptides mimic a basic aspect of transmembrane domains of natural fusion proteins in that they fuse model membranes, presumably by increasing the likelihood that randomly colliding liposomes undergo fusion [5]. Further, studying a series of variants indicated the importance of helix-destabilizing residues for backbone dynamics and fusogenicity [4].

LV peptides consist of a hydrophobic core, comprising leucine (L) and valine (V) residues in varying ratios as the β-branched amino acids Ile and Val are overrepresented in SNARE transmembrane domains [6], which are flanked by three lysines (K) on each side and completed by tryptophan (W) at the N-terminal interface between the K and the L/V residues. The secondary structure of reconstituted LV peptides as well as their fusion-promoting effect has been investigated as a function of the length of the hydrophobic core, the L/V ratio, the insertion of helix breakers [7], and the influence of charge [8]. Furthermore, the interaction of the peptides with membranes was studied [9]. LV peptides can give rise to pronounced macroscopic lipid rearrangements. Specifically, the more fusogenic variants induce unaligned lipid phase and phase

* Corresponding author. Tel.: +49 341 97 15701; fax: +49 341 97 15709.
E-mail address: daniel.huster@medizin.uni-leipzig.de (D. Huster).

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separation [9]. It appears that enhanced dynamics of the helix backbone in terms of local and transient unfolding of the chain may affect the membrane structure and thereby favorably influence the fusion event [4].

Another aspect of fusion proteins that has rarely been investigated is the fact that these proteins often carry covalently attached lipid chains [10,11]. Lipidation is known as a commonly occurring membrane anchor in many membrane-associated proteins and a number of structural motifs such as isoprenylation, myristoylation, or palmitoylation have evolved [12]. Research on model peptides of varying origins has indicated that only the concerted action of at least two lipid modifications provides stable membrane insertion [13,14]. However, lipid modifications on membrane-spanning fusion proteins should not be required to anchor these molecules to the membrane. Rather, a different task could be envisioned, for instance, a fusion-promoting function. Recent molecular dynamics simulations have shown that stalk formation is initiated by a localized hydrophobic contact between the opposing membranes, formed by fully or partially splayed lipids [15]. This agrees with the experimental finding that lipid tail-to-head contacts are frequently observed in membranes [16,17]. A possible involvement of backfolded and thus water exposed protein lipid modifications in membrane fusion events appears particularly interesting. Thus, the lipid chain of a fusion protein could indeed be highly flexible and partially water exposed, such that it could insert into the opposing membrane during fusion. This would facilitate the molecular contact between these membranes and the protein lipid chain would act as the splayed lipid that initiates fusion [15].

In contrast to this rationale, experimental studies of N-terminally lipidated LV16 peptides revealed a decrease in fusogenic activity, which was more pronounced for the longer lipid modifications [18]. However, the lipid modifications appeared to stabilize global helicity without affecting backbone dynamics [19]. This may suggest that the lipid modification of LV16 would not promote the approach of opposing membranes, but influence fusion by a stabilization of the transmembrane domains. Nevertheless, an influence of acylation on the hemifusion-to-fusion transition has been reported for viral envelope proteins [20] and other fusion proteins [21–23].

To understand the role of covalent lipid chains in the process of fusion structural and dynamical aspects of the lipid modifications of fusion proteins or their transmembrane domains are essential. Although lipid modification is relevant for up to 10% of all cellular proteins, there is only little biophysical data available. In particular, the structural aspects [12] of lipid modifications along with the thermodynamics of membrane insertion [13,24] are of interest for the understanding of their role in biological function. Since membrane-inserted protein lipid chains are composed of the same atomic groups as the chains of the phospholipids, spectroscopic techniques that are sensitive to the hydrogen/deuterium isotope effect, such as neutron scattering or FTIR spectroscopy, are particularly useful [25]. In addition, ^{2}H NMR has proved very useful to analyze the structure and dynamics of lipid-modified peptides and proteins [12,26–28]. These methods allow to determine segment-specific chain order parameters [29,30], which can be converted into geometrical parameters such as lipid chain length and area per molecule [31]. Further, dynamic aspects of lipid chains can be assessed by measurement of nuclear relaxation rates and their analyses by motion models [27,32].

Here, we have studied transmembrane LV16 peptides that were N-terminally acylated with saturated C2, C8, C12, and C16 chains, respectively. In contrast to previous findings on other lipid-modified proteins [28], we find that the peptide lipid chains are much less influenced by the host membrane and show properties that are largely independent of those of the surrounding phospholipids. This could indeed support a structure stabilizing function of these chains.

2. Materials and methods

2.1. Materials

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC), as well as the analogs of these phospholipids with perdeuterated acyl chains, POPC-d_{31} (1-palmitoyl(d_{31})-2-oleoyl-*sn*-glycero-3-phosphocholine) and DLPC-d_{46} (1,2-dilauroyl(d_{46})-*sn*-glycero-3-phosphocholine), were obtained from Avanti Polar Lipids (Alabaster, AL) and used without further purification.

The LV16 peptides (sequence: acyl-KKKWL VLVLV LVLVL VLVLV KKK) with (LV16ac) or without (LV16) a covalently attached N-terminal saturated acyl chain were synthesized by standard Fmoc chemistry. Either protonated or perdeuterated acyl chains containing 2, 8, 12, or 16 carbons were used. Synthesis products were purified to >95% by HPLC as confirmed by mass spectrometry.

2.2. Sample preparation

Lipids prepared in chloroform were combined with peptides solved in 2,2,2-trifluoroethanol (TFE) at a molar ratio of 30:1 (the lipid to peptide mixing ratio considers the mass of lyophilized peptide powder used, which also includes approximately 30% counterions, TFA, etc.), diluted with cyclohexane, and vortexed, and after freezing in liquid nitrogen, samples were lyophilized overnight. Afterwards, samples were dissolved in cyclohexane and lyophilized overnight for a second time to obtain a fluffy powder. Multilamellar proteoliposomes were prepared by hydrating the sample to a water content of 50 wt.% with aqueous buffer (150 mM NaCl, 0.1 mM EDTA 25 mM Tris–HCl, pH 7.4) Several freeze–thaw cycles interrupted by gentle centrifugation were performed to homogenize the sample.

2.3. Deuterium solid-state NMR spectroscopy

^{2}H NMR spectra were acquired on a Bruker Avance 750 MHz NMR spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) operating at a resonance frequency of 115.1 MHz for ^{2}H. A single-channel solids probe equipped with a 5 mm solenoid coil was used. The ^{2}H NMR spectra were accumulated with a spectral width of ±250 kHz using quadrature detection. A phase-cycled quadrupolar echo sequence was used [33]. The length of a 90° pulse varied from 2.2 to 3.9 μs, and a relaxation delay of 1 s was applied. For the measurement of the T_1 spin-lattice relaxation times, a phase-cycled inversion recovery quadrupolar echo pulse sequence with 11 time delays between 1 ms and 2.5 s was used. The relaxation delay was 2.5 s; all other parameters were identical as for recording the ^{2}H NMR spectra. All measurements were conducted at a temperature of 310 K.

The ^{2}H NMR powder spectra were de-Paked using the algorithm of Mc Cabe and Wassall [34] and the order parameter profiles of the lipid chains were determined by a numerical spectral fitting procedure from the observed quadrupolar splitting in the dePaked spectra $\Delta\nu_Q(n)$:

$$\Delta\nu_Q(n) = \frac{3}{2}\frac{e^2qQ}{h}S(n),$$

where e^2qQ/h is the quadrupolar coupling constant (167 kHz for ^{2}H in a C-^{2}H bond) and $S(n)$ the chain order parameter for the n^{th} carbon position in the chain.

The length of the acyl chain L_c^* (also referred to as chain extent), the mean interfacial area (A) and the hydrocarbon thickness (D_c) were calculated according to the mean torque model [26,31]. For a given lipid chain with a specific length, the number of gauche conformers can be estimated by subtracting the measured chain length from the length of the all trans chain (assuming a projected