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Impact of peptide clustering on unbinding forces in the context of fusion mimetics

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ABSTRACT

Coiled-coil zipping and unzipping is a pivotal process in SNARE-regulated membrane fusion. In this study we examine this process mediated by a minimal model for coiled-coil formation employing force spectroscopy in the context of membrane-coated surfaces and probes. The interaction forces of several hundred pN are surprisingly low considering the proposed amount of molecular bonds in the contact zone. However, by means of high-resolution imaging employing atomic force microscopy and studying the lateral mobility of lipids and peptides as a function of coiled-coil formation, we are able to supply a detailed view on processes occurring on the membrane surfaces during force measurements. The interaction forces determined here are not only dependent on the peptide concentration on the surface, but also on the regional organization of lateral peptide clusters found prior to coiled-coil formation.

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1. Introduction

Membrane fusion is an essential process in eukaryotic cells transporting e.g. messenger molecules from one cell compartment to another [1]. The SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) motif mediates fusion processes of synaptic vesicles with the synaptic cleft by forming coiled-coil structures consisting of four α-helices with an eight-heptad repeat recognition domain [2]. Due to a zipper mechanism the SNARE core complex formation brings membranes into close contact and therefore overcomes the hydration barrier, hence fusion can occur [3]. The release of free energy upon coiled-coil formation in the context of lipid bilayers has only sparsely been addressed. Bornschlögl et al. proposed a detailed energy landscape of coiled-coil forming proteins suggesting a quasi-reversible unbinding under external force [4]. Moy and coworkers recently managed to measure fusion forces of single SNARE complexes using membrane-coated colloidal probes [5] while Jahn and coworkers proposed that single SNARE complexes might be sufficient to induce fusion [6].

Against this background, we synthesized cysteine-terminated heptad repeat peptides similar to the helix region of the peptide-lipid structures developed by Litowski et al. [7] in order to form lipopeptide constructs in lipid bilayers [8]. As shown previously, a parallel coiled-coil formation of the peptides *i*-E3Cys and *i*-K3Cys shows decent fusogenicity in particular lipid mixing rendering it a suitable minimal model for SNARE mediated fusion [9]. We found that heterodimerization correlates with a free energy release of $-10.6\ k_BT$ on lipid bilayers strongly influenced by a loss of translational entropy due to potential peptide immobilization on the surface. Here, we analyze the impact of lipopeptide organization or clustering on coiled-coil formation between two membranes using force spectroscopy measurements similar to the setup introduced for SNARE proteins by Abdulreda et al. [5] and Lorenz et al. [10]. With this so called membrane probe spectroscopy (MPS) setup, we studied the interaction between SSM doped with *i*-K3Cys and *i*-E3Cys constructs, respectively, in order to extract interaction force values of *i*-K3Cys/*i*-E3Cys heterodimers (Fig. 1A). By adjusting the applied peptide surface density combined with dwell time studies, we were able to measure interaction forces for ensemble and single molecule events. At this juncture, a detailed view of docking mediated by coiled-coil peptides could shine light on the impact of lateral clustering in fusion mimetics.

2. Material and methods

2.1. Materials

All chemicals were of HPLC grade and used without further purification. POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) and MCCDOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[4-(*p*-maleimidomethyl)cyclohexane-carboxamide]) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Fluorescently labeled lipid BY (BODIPY C12-HPC; 2-(4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine) and Oregon Green 488 maleimide (OG) were purchased from Invitrogen (Darmstadt,

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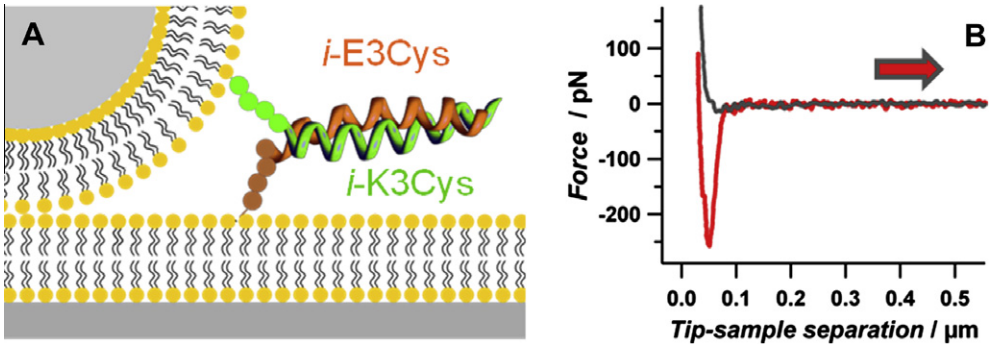

Fig. 1. Coiled-coil interactions lead to increased membrane interaction forces. (A) Schematic drawing of experimental setup employed to analyze membrane interaction mediated by *i*-K3Cys/*i*-E3Cys coiled-coil formation. (B) Force–distance curves (retraction) recorded for interaction between neat POPC membranes (grey) and POPC membranes decorated with 3 mol% *i*-K3Cys and *i*-E3Cys peptides (red). Forces of ≈250 pN act on the membrane probe upon peptide interaction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Germany). All chemicals for peptide synthesis were purchased from Novabiochem (Darmstadt, Germany).

2.2. Peptide synthesis

Peptides *i*-E3Cys (Ac-$(KELAAIE)_3$GWGGGC-NH_2) and *i*-K3Cys (Ac-WG$(EKLAAIK)_3$GGGGC-NH_2) were synthesized manually as described elsewhere [9]. The peptide sizes could be estimated to be $2.7 \times 1.6\ nm^2$ (height × diameter) by the usage of software UCSF Chimera [11].

2.3. Preparation of solid supported membranes (SSM) and lipopeptide formation

Solid supported membranes (SSM) were prepared on hydrophilized silicon oxide substrates (MPS) or glass (MPS and FRAP) (for hydrophilization protocols see [10]), and freshly cleaved mica (AFM imaging). For all measurements, small unilamellar vesicles (SUV) were prepared by sonication using PB 5.9 (50 mM Na_2HPO_4, pH = 5.9). For bilayer spreading, surfaces were incubated with SUV at room temperature (0.1–0.5 mg/mL, 15–60 min). Lipid compositions were POPC/MCCDOPE/BY on probe and silicon wafer with MCCDOPE concentrations ranging from 0.1 to 10 mol% supplemented with 0.5 mol% of fluorescent lipid. For AFM imaging on mica, SSM prepared from POPC/MCCDOPE were used. After spreading, samples were rinsed with PB 6.8 (50 mM Na_2HPO_4, pH = 6.8) and incubated with *i*-K3Cys (1 h, 30 µM; on colloidal probe: 15 min, 100 µM) or with *i*-E3Cys (2 h, 30 µM), respectively. After rinsing, coiled-coil forming peptides could be added (30 µM, 30 min) to allow for the formation of heterodimers on the surface.

2.4. FRAP (fluorescence recovery after photobleaching)

For FRAP experiments, bilayers were spread on glass and are subsequently functionalized with the corresponding peptides (for a detailed description of experiments and analysis see [9]). For determination of membrane mobility, SSM were labeled with 1 mol% BY, while for peptide mobility, coiled-coil forming peptides were labeled with OG maleimide after binding to preformed lipopeptides.

2.5. AFM (atomic force microscopy) imaging

AFM images of peptide-functionalized SSMs were acquired in liquid (PB 6.8) at room temperature using tapping mode on mica as solid support (NanoWizard II, JPK Instruments, Berlin, Germany). MSCT cantilever (Bruker AXS, Camarillo, CA, USA) exhibiting nominal spring constants of 0.05 N/m were employed.

2.6. MPS measurement with i-K3Cys/i-E3Cys-functionalized membranes

Colloidal probe cantilevers were prepared by attaching a borosilicate glass microsphere with a diameter of 15 ± 1 µm (borosilicate glass 9015, Duke Scientific Corporation, Palo Alto, CA, USA) to a tipless MLCT-O10 cantilever (Bruker AXS, Camarillo, CA, USA) (for details see [10]). For force-distance measurements, we used a commercial atomic force microscope (MFP3D, Asylum Research, Santa Barbara, CA, USA) and colloidal probe cantilevers with a nominal spring constant of 0.01 N/m. Spring constants were calibrated using the thermal noise method and found to range from 0.01 to 0.03 N/m [10]. If not stated otherwise, force-distance cycles were performed with a dwell time of 1 s, a force load of 200 pN, and a velocity of 1 µm/s, corresponding to a loading rate of 10 nN/s. The experiments concerning pulling velocity dependence were carried out with a constant approach velocity of 1 µm/s and variable relaxation speed. All measurements were performed in PB 6.8 at room temperature in a home-made PTFE measuring chamber [10,12].

3. Results

Interaction forces between E-peptides and their corresponding K-peptides in the context of fluid lipid bilayers were assessed by means of membrane probe spectroscopy (MPS). MPS permits to acquire force-distance curves between a membrane-coated bead (∅ = 1–20 µm) and membrane on a flat support. Force-distance curves comprise approach of the probe to the underlying SSM surface thereby revealing fusion events as mechanical instabilities [5] and retraction of the probe from the contact zone. Adhesive forces are obtained from retraction curves due to formation of bonds in the contact area. In the presence of complementary peptides on the membrane surfaces (*i*-K3Cys on the membrane probe, *i*-E3Cys on the flat support) interaction forces are significantly increased in contrast to control experiments with unfunctionalized, neat membranes (Fig. 1). A very broad distribution of interaction forces ranging from ≈80 pN to ≈600 pN was found for 3 mol% of lipopeptides (Fig. 3C). In contrast, neat membranes show most probable interaction forces of ≈20 pN which are only slightly above the experimental noise [12].

Bornschlögl et al., investigated the unfolding mechanics of single coiled-coil structures and found that unzipping forces are independent of the superhelical length and in a range of ≈12 pN [4].

The authors clearly show that unzipping under external force occurs in quasi-equilibrium being largely independent of pulling velocity. Furthermore, Pähler et al., determined a coiled-coil interaction free energy of $-10.6\ k_BT$ for the employed peptides, which translates into an interaction force of 17 pN, assuming a helix length of 2.7 nm [11]. Hence, interaction forces must evolve from a large number of heterodimers formed between the membrane surfaces during contact. Calculating the contact area and therefore estimating the number of peptide molecules available for coiled-coil formation between the two membranes by using the Hertz model [13], we obtain ≈470 lipopeptides available for dimerization considering bilayers doped with 3 mol% MCCDOPE (contact area: 0.011 μm^2; Young's modulus 10 MPa; area per lipid: ≈0.7 nm^2 [14]). Assuming an interaction force of 17 pN, a maximum adhesion force of ≈8 nN should be observed, which is 8 times higher than the highest interaction forces found in our experiments. Hence, not all peptide molecules on the surfaces are available for coiled-coil formation suggesting e.g. steric hindrance due to a potential peptide clustering or a limited contact area due to a finite roughness.

To examine whether a clustering of lipopeptides on the surface is responsible for the low adhesion force, AFM imaging was carried out (Fig. 2A and B) in conjugation with FRAP measurements. We found that both, K-lipopeptides as well as the E-lipopeptides (prefix LP-) form distinct structures with a height of ≈2–3 nm on a membrane consisting of POPC/MCCDOPE 97:3. While the LP-*i*-E3Cys clusters show a rather scattered pattern, LP-*i*-K3Cys display well-defined borders. Employing grain analysis, the mean area A of the membrane occupied by clusters was determined, resulting in $A_{i\text{-K3Cys}} = 30 \pm 2\%$ for K-lipopeptides and $A_{i\text{-E3Cys}} = 23 \pm 7\%$. Considering that a full coverage of the surface with lipopeptides is achieved with 10 mol% MCCDOPE, these values are in good agreement with the expectations for a concentration of 3 mol% of receptor lipids in the SSM [9]. Furthermore, the mean equivalent disc radius r of the clusters is calculated. Due to the scattered and diffuse arrangement of E-lipopeptides, the clusters are not well described by a circular shape; hence, no equivalent disc radius can be defined here. For K-lipopeptides the mean radius of clusters was found to be $r_{i\text{-K3Cys}} = 21 \pm 8$ nm, resulting in the mean number of clusters per area $n_{i\text{-K3Cys}} = 145 \pm 80$ cluster/μm^2.

AFM images suggest that clustering of lipopeptides reduce the ability to form coiled-coil dimers with the opposing membrane thereby reducing the overall adhesion strength. Furthermore, FRAP measurements were employed to determine the lateral mobility of coiled-coil structures and that of the surrounding matrix membrane. The aim was to study the maximum effect of peptide coupling to membrane integrity, hence 10 mol% of MCCDOPE as receptor lipid were used [9]. Neat SSM consisting of POPC/MCCDOPE/BY 89:10:1 served as a reference, which showed a mobile fraction F_{mob} of 74 ± 9% and a diffusion coefficient D of 5.5 ± 2.5 μm^2/s, which is in good agreement with literature values (Fig. 2E) [15]. Formation of lipopeptides and coiled-coil dimers had no impact on the mobile fraction. However, the diffusion coefficient of the membrane decreased by a factor of two for LP-*i*-K3Cys coupled covalently to the SSM ($D_{\text{LP-}i\text{-K3Cys}} = 2.4 \pm 1.6$ μm^2/s), while samples covered with LP-*i*-E3Cys showed no influence on lateral mobility ($D_{\text{LP-}i\text{-E3Cys}} = 5.8 \pm 3.4$ μm^2/s). After coiled-coil formation on the surface, however, the diffusion coefficient decreased substantially below 1 μm^2/s for LP-*i*-K3Cys + *i*-E3Cys and for LP-*i*-E3Cys + *i*-K3Cys. These results imply that peptide coupling, particularly dimerization, leads to a reduced lateral mobility of the membrane lipids. To analyze the specific lateral mobility of the actual coiled-coil dimers, a second FRAP study with fluorescently labeled peptides was carried out. We found that the diffu-

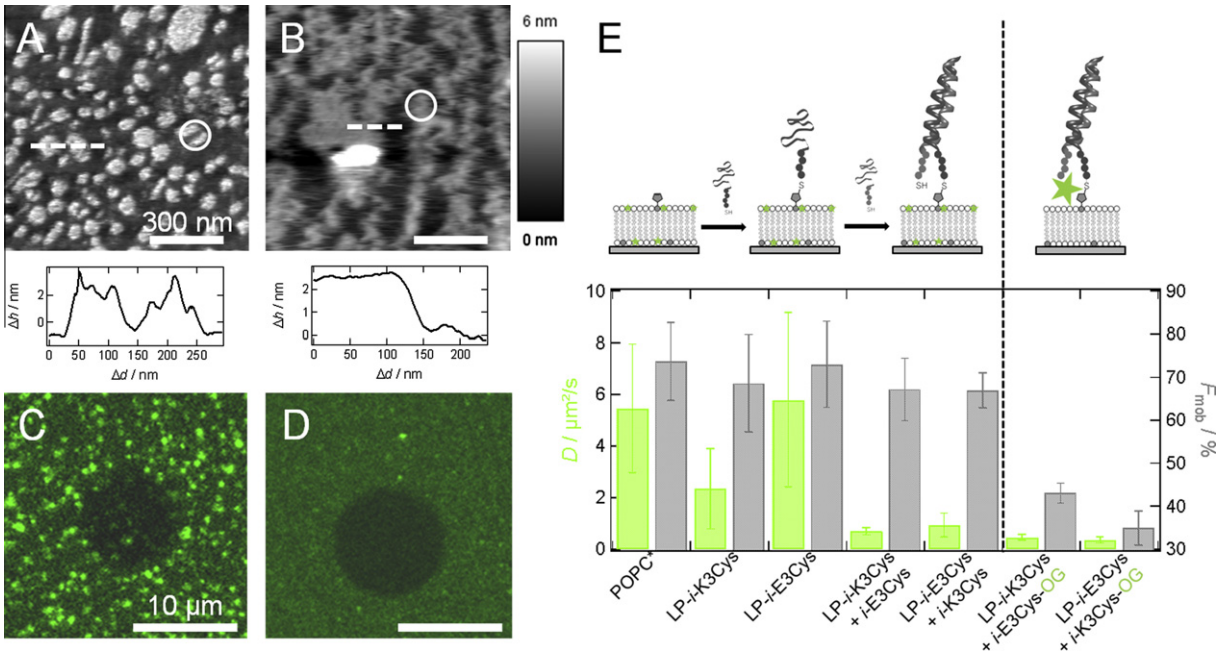

Fig. 2. Lipopeptide and subsequent coiled-coil formation affects lateral mobility of the membrane. (A, B) AFM images of peptides bound to POPC/MCCDOPE 97:3 (scale bar: 300 nm). Scattered lines show position of height profiles presented below. Rings simulate contact area between colloidal probe and silicon wafer. In (A) *i*-K3Cys was attached covalently to the lipid bilayer, in (B) *i*-E3Cys is shown as lipopeptide (prefix: LP-). (C, D) Fluorescence micrographs after bleaching in FRAP experiments of OG-labeled coiled-coil structures on SSM (scale bar: 10 μm). (C) LP-*i*-K3Cys + *i*-E3Cys-OG, (D) LP-*i*-E3Cys + *i*-K3Cys-OG. (E) Schematic drawings and results from FRAP experiments to determine lateral mobility of matrix lipids and of peptides. Determined diffusion coefficients (green bars) and mobile fractions (grey bars) are presented. Left of scattered line, mobility of lipid matrix was measured using POPC/MCCDOPE/BY 89:10:1 bilayers. Plain bilayer (POPC*), lipopeptide decorated SSM (LP-*i*-K3Cys and LP-*i*-E3Cys) as well as coiled-coil decorated SSM (LP-*i*-K3Cys + *i*-E3Cys and LP-*i*-E3Cys + *i*-K3Cys) were examined. Right of scattered line, lipopeptides were formed on POPC/MCCDOPE 90:10 and afterwards OG-labeled coiled-coil forming peptides were added. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

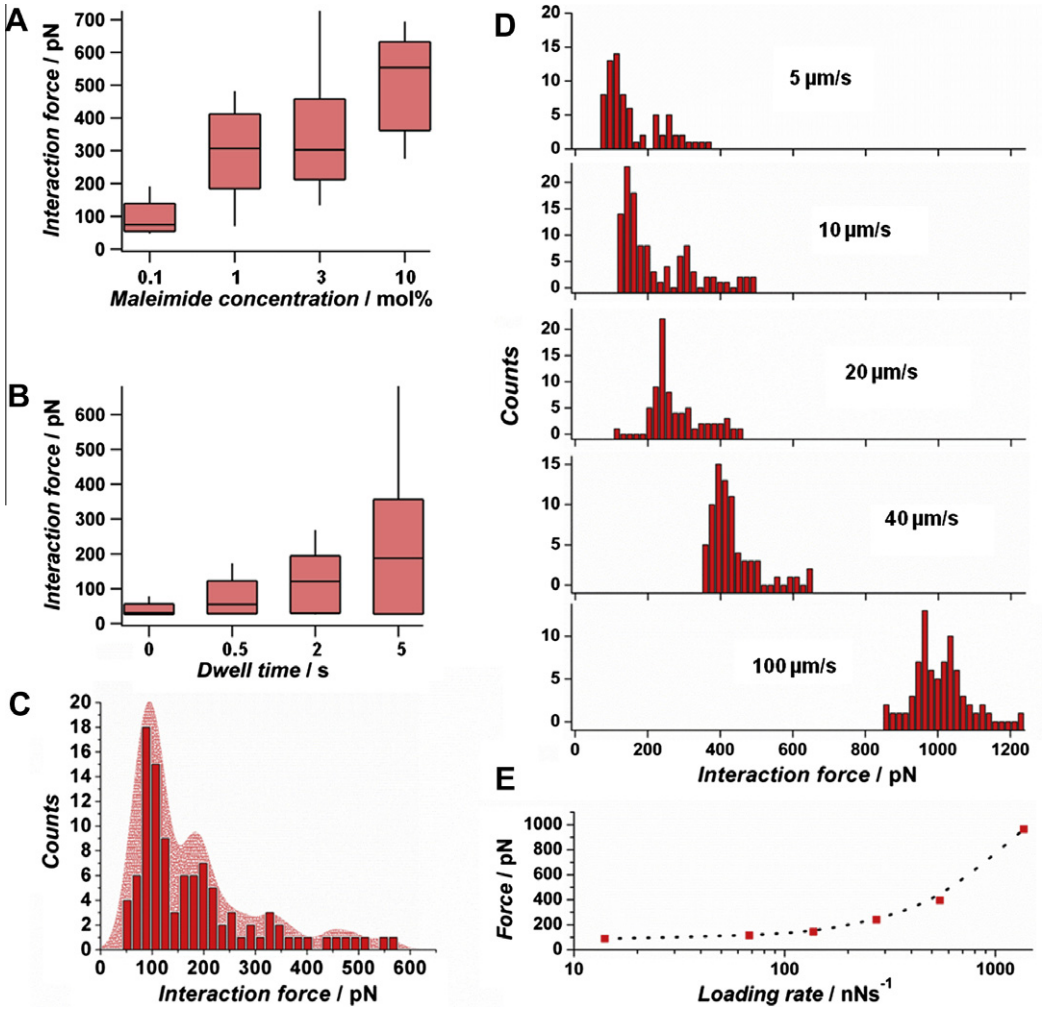

Fig. 3. Impact of peptide concentration, interaction time and pulling velocity on *i*-K3Cys/*i*-E3Cys-mediated membrane interaction forces. (A) Peptide surface concentration dependency: median of membrane interaction force values increases from ≈80 pN (0.1 mol%) to ≈550 pN for 10 mol% lipopeptides in POPC membrane. (B) Membrane interaction time dependency on interaction forces (3 mol% lipopeptides): median of interaction forces increases from ≈40 pN (no additional dwell time) to ≈200 pN at a dwell time of 5 s. (C) Most probable interaction forces between *i*-K3Cys and *i*-E3Cys decorated membranes are ≈100 pN, ≈190 pN, ≈330 pN, and ≈480 pN (3 mol% lipopeptides). Multiple force peaks can be interpreted in terms of interaction between several *i*-K3Cys/*i*-E3Cys clusters. Kernel density estimation (bright red) shows maximum forces at 100 pN, 196 pN, 342 pN, 475 pN, and 560 pN. (D) Velocity dependence of determined interaction forces. With increasing retraction speed (stated in graph), main peak force increases from ≈100 pN to ≈1000 pN. (E) Most probable rupture forces (corresponding to peak maxima in (D)) are increasing with applied loading rate determined for velocity dependent measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

sion coefficients for the peptides were similar compared to the ones obtained from a labeled matrix, namely 0.5 ± 0.1 µm^2/s for LP-*i*-K3Cys + *i*-E3Cys-OG, and 0.4 ± 0.1 µm^2/s for the inverted coiled-coil consisting of LP-*i*-E3Cys + *i*-K3Cys-OG. In contrast to the experiments with a labeled lipid matrix, a clear impact on the mobile fraction was detected, which decreased to 43 ± 2% and 35 ± 4%, respectively. Therefore, we can conclude that the clustering of peptides leads to an immobilization of the receptor lipids in the membrane. Furthermore, the formed lipopeptides act as obstacles for the surrounding matrix lipids, essentially freezing the membrane. This can also be directly seen in the fluorescence micrographs displaying a pronounced clustering (Fig. 2C and D). Especially, the coiled-coil dimers consisting of LP-*i*-K3Cys + *i*-E3Cys show an inhomogeneous fluorescence distribution.

In summary, the results from diffusion measurements imply that coiled-coil lipopeptides are virtually immobile in comparison to the surrounding membrane. While in any case peptides are virtually immobile after coiled-coil formation only K-lipopeptides form distinct clusters within a lipid bilayer hampering diffusion of lipids. Hence, in the following we consider K-lipopeptides as virtually immobile and organized in clusters, neglecting a potential fraction of free K-lipopepitdes in the membrane, whereas E-lipopeptides are considered to be mobile and freely accessible to form coiled-coil dimers. Using the calculated clusters per µm^2 (145 ± 80) and the estimated size of the contact zone (0.011 µm^2) [13], around 1.6 *i*-K3Cys clusters are assumed to be in the contact area (indicated in Fig. 2 A and B by the ring). Furthermore, it is conceivable that preferably peptide molecules in the outer cluster-shell undergo coiled-coil formation, due to steric hindrance and the determined low mobility of lipopeptides in the inner part of the cluster. Assuming a circular shape of the found clusters, it can be shown that only 18% of LP-*i*-K3Cys in a membrane consisting of POPC/MCCDOPE 97:3 are located in the outer shell of formed clusters and are therefore assumed to be able to undergo heterodimerization.

To assess the impact of clustering on peptide interaction forces and to gain insights in how many bonds participate in cluster formation, we performed membrane probe spectroscopy measurements with varied amount of peptide on the surface (0.1–10 mol%), resulting in mean interaction forces of ≈80 pN at the

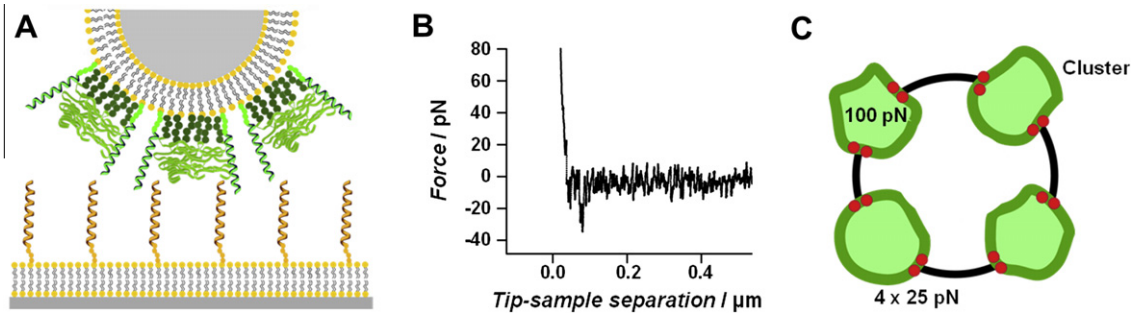

Fig. 4. Membrane interaction scenario involving *i*-K3Cys/*i*-E3Cys peptides and spatial reduction of contact area. (A) *i*-K3Cys (green) clusters get in contact with *i*-E3Cys (yellow) decorated membrane. Due to steric hindrance of coiled-coil formation in the inner part of the cluster, solely *i*-K3Cys molecules in the outer ring of the cluster (helical shape) are available for heterodimerization. (B) Force-distance curve (retraction) for interactions between membranes with 0.1 mol% LP-*i*-K3Cys and LP-*i*-E3Cys. Curve displays a single worm-like chain unbinding event with an interaction force of ≈25 pN. (C) In combination with the contact zone defined by the probe geometry (black ring), coiled-coil formation (red dots) is only possible in intersection points of contact zone and clusters (green), with detected forces of ≈100 pN per cluster. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

lowest surface concentration increasing to ≈550 pN for 10 mol% of lipopeptides (Fig. 3A). This increase of interaction forces is non-linear indicating a progressive steric hindrance of peptides on the surface leading to constraints of peptide interactions. Since we assume that LP-*i*-E3Cys are still mobile in the SSM and therefore can diffuse into the contact area to form additional coiled-coil heterodimers with K-lipopeptides, the dimerization process should be time-dependent. By analyzing different membrane contact times for a given peptide surface concentration, interaction forces increased by a factor of five from a median of ≈40 pN at a dwell time of 0 to a median of ≈200 pN at a contact time of 5 s (Fig. 3B).

Furthermore, we analyzed the interaction force distribution for 3 mol% of receptor lipids present in both membranes and found a multi-peak profile (Fig. 3C) with a most probable interaction force of ≈100 pN. Further maxima can be found at peak forces of ≈190 pN, ≈340 pN, ≈480 pN, and ≈560 pN. Force spectroscopy data (Fig. 3D/E) confirms the unbinding scenario proposed by Rief and coworkers who found that unzipping of coiled-coil complexes occurs speed independent similar to B-S transition of double-stranded DNA [16]. Only at higher velocities, the process becomes speed-dependent entering non-equilibrium conditions. This distinct pattern of rupture forces suggests that each peak represents an additional cluster participating in the contact. We identified five separate force peaks being indicative for a maximum number of five peptide clusters of differing interaction strength. When comparing these findings to the membrane probe contact area, it seems reasonable to claim that repositioning of the probe between individual measurements allows for different interaction scenarios involving varying numbers of peptide clusters (see Fig. 4A). Assuming that each cluster is characterized by interaction strengths of ≈100 pN and that a single coiled-coil bond displays interaction strengths of ≈15–50 pN, we arrive at two to six unbinding events per cluster. Actually, this is still considerably lower than the expected adhesive forces considering that 18% of LP-*i*-K3Cys are still accessible, while the rest is bound and inactivated due to complexation inside the clusters. However, assuming that interactions and hence coiled-coil formation is only possible in the intersections of the outer rings of K-clusters on the probe and the outer ring of the contact area, this would lead to only two point-shaped intersections (red dots in Fig. 4C) where heterodimerization could occur, decreasing the number of possible coiled-coil structures formed per cluster to two to four. Additionally, it should be noted that the glass bead serving as probe displays a significant roughness, which has usually a decreasing impact on the size of the contact area [17]. However, in this specific case with cluster formation on the probe, the effect is not exactly tractable and was therefore neglected here.

By reducing the peptide density on the membrane surface down to 0.1 mol%, we were able to extract unbinding events characterized by forces down to 25 pN and presenting a typical shape following the worm-like chain model indicative for the detection of unbinding of a single pair of coiled-coil peptides in a POPC bilayer (Fig. 4B). This means in turn that the cluster being characterized by the main peak at 100 pN is most likely composed of four *i*-K3Cys/*i*-E3Cys heterodimers and that the maximum at 340 pN is generated by around 14 heterodimers. In accordance with the membrane topography depicted in the presented AFM images, we consider that peptide clusters of different sizes and of different availability for coiled-coil formation contribute to the overall membrane interaction force.

The dramatic reduction of binding sites deviating from the expected several hundred events to 4–14 coiled-coil heterodimers, is not exclusively explainable with K-lipopeptide clustering presented in this paper. Since in the outer ring of lipopeptide-clusters 18% of LP-*i*-K3Cys are available for heterodimerization, a decrease by a factor of five would be reasonable. However, the low determined forces can be explained assuming that interactions and hence coiled-coil formation is only possible in the intersections of the two outer rings of K-clusters on the probe with the contact area of the probe. With on average 1.6 clusters in the contact area, the two main peaks found in Fig. 3C at ≈100 pN and ≈196 pN can be correlated with binding of one and two cluster(s), respectively. With the here determined interaction force for one heterodimer (25 pN), which is good accordance to the results found by Bornschlögl et al., (12 pN) and our calculated estimations (17 pN), this leads to four to six unbinding events per cluster. Hence, at one intersection point, two to three unbinding events are likely to occur.

4. Discussion

In summary, we found that lipopeptides organize into small lateral clusters embedded in solid supported membranes that impair with the formation of coiled-coil dimers. This is in accordance with membrane probe spectroscopy, lateral diffusion studies and AFM imaging. Recently, it was shown that positively charged peptides can induce lipid demixing if negatively charged lipids are present [18]. Since *i*-K3Cys carries a net charge of + 3, the peptides might be interacting with remaining non-reacted receptor lipids exhibiting a negative charge, resulting in cluster formation.

In 2001, Lang et al., showed that syntaxin was found to be arranged in cholesterol dependent nanodomains. Their presence is crucial for fusion involving SNARE complex formation [19]. In a later study employing high resolution fluorescence imaging (STED,

stimulated emission depletion microscopy) in cells, the diameter of these domains could be defined to be 50–60 nm [20], which is very similar to the size of our *i*-K3Cys clusters. However, in model systems it was shown that changes in cluster size can be generated by increasing the protein concentration by a factor of 100, leading to a strongly reduced docking and thus fusion efficiency [21]. Generally, it can be speculated why only few SNARE zippers are sufficient to induce fusion if cluster formation impairs with formation of coiled-coil complexes.

5. Conclusions

Formation of lateral clusters consisting of lipopeptides prior to heterodimerization into coiled-coil complexes impairs with efficient docking and might thereby explain the lack in fusion efficiency compared with more native systems.

In order to provide the necessary amount of free energy to overcome the barrier for fusion two strategies are generally conceivable. Either longer superhelices are formed to provide enough docking strength or a larger number of opposing coiled-coil dimers is formed. Our data suggests that due to potential lateral clustering the number of putative bonds is limited explaining why longer coiled-coil peptides are realized in native systems. Our study also reveals that short E- and K-peptides are of limited use to mimic SNARE-mediated fusion due to clustering as well as non sufficient release of free energy per dimer.

Acknowledgments

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