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

Gesa Pähler [1], Bärbel Lorenz [1], Andreas Janshoff *

Institute of Physical Chemistry, Tammannstr. 6, University of Goettingen, 37077 Göttingen, Germany

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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 $-10.6\ k_{\mathrm{B}}T$ 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,

* Corresponding author. Fax: +49 0 551 39 14411.
E-mail address: ajansho@gwdg.de (A. Janshoff).
[1] These authors contributed equally to this work.

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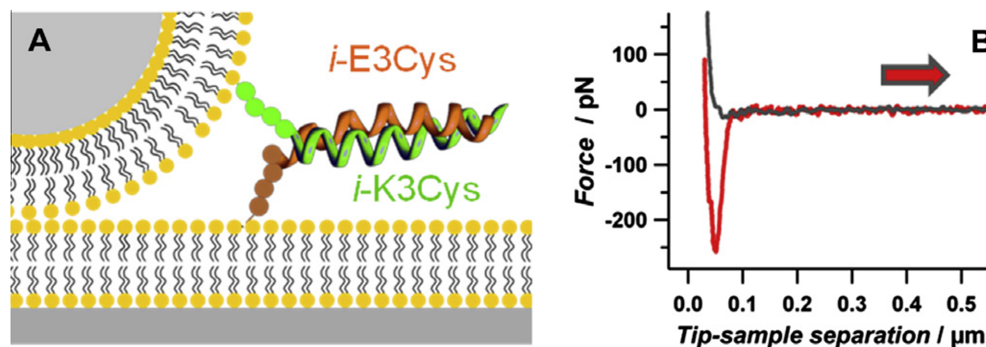

Fig. 1. Coiled-coil interactions lead to increased membrane interaction forces. (A) Schematic drawing of experimental setup employed to analyze membrane interaction mediated by *i*-K3Cys/*i*-E3Cys coiled-coil formation. (B) Force–distance curves (retraction) recorded for interaction between neat POPC 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].

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