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Membrane binding of peptide models for early stages of amyloid formation: Lipid packing counts more than charge

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ABSTRACT

Amyloid formation is related to neurodegenerative diseases like Alzheimer's disease or Parkinson's disease. In the molecular onset of the diseases, soluble peptides adopt conformations that are rich in β-sheet and ultimately form aggregates. How this process is triggered or influenced by membrane binding, or how the membrane integrity is disturbed by the peptide binding and conformational transition is still under debate.

In the present study, we systematically examine the effects of β-sheet prone model peptides on zwitterionic and negatively charged lipids in both mono- and bilayers and in various lipid phase states by infrared reflection absorption spectroscopy, grazing incidence X-ray diffraction, and small and wide angle X-ray scattering.

No difference in the interaction of the peptides with zwitterionic or negatively charged lipids was observed. Furthermore, the interaction of β-sheet prone model peptides leaves the lipid structure largely unaffected. However, the lipid phase state decides upon the mode of interaction. Peptides insert into liquid-expanded layers and interact only with the head groups of liquid-condensed lipid layers.

Using a zoo of complementary techniques and critically examining preparation procedures we are able to obtain an unambiguous picture of peptide binding to membranes.

1. Introduction

Amyloid formation is related to a variety of diseases including Alzheimer's disease, Parkinson's disease or type II diabetes. Amyloid is formed when soluble peptides or proteins undergo conformational changes toward structures rich in β-sheet. These associate to oligomers and finally form fibrils.

In this process, lipid membranes are discussed as possible triggers for the onset of the conformational change, but also as targets for the mechanisms of toxicity.

It is not surprising that there is no agreement on the interplay of amyloid formation and lipid membranes to date, given the variety of amyloidogenic peptides and lipid model systems. There is clear evidence that amyloidogenic peptides such as Aβ (involved in Alzheimers' disease) or IAPP (islet amyloid polypeptide involved in diabetes type II) are interacting with lipid layers (reviewed in Gorbenko and Kinnunen, 2006; Thakur et al., 2009; Torres-Bugeau et al., 2011). In any case, interactions depend on the peptide fragment used and on conditions like ionic strength or pH (McLaurin and Chakrabartty, 1997; Curtain et al., 2003; Matsuzaki, 2007; Khemtemourian et al., 2011) and may require specific peptide sequences and interaction sites in lipids. Therefore, there are contrary findings in slightly different conditions regarding the charge of lipid layers. For example, in some studies, IAPP and Aβ are found to bind to negatively charged lipids only (Terzi et al., 1997; Bokvist et al., 2004; Maltseva et al., 2005; Knight et al., 2006; Lopes et al., 2007; Matsuzaki, 2007). Other work proves that binding of amyloidogenic peptides to lipid layers does not require specific charge–charge interactions (Kremer et al., 2001) and no apparent difference between interactions with zwitterionic phosphatidylcholine (PC) or negatively charged phosphatidylgylcerol (PG) was observed (Maltseva et al., 2005).

Accordingly, electrostatic interactions may not be the only factor regulating peptide binding and aggregation at lipid layers. For example, not the charge, but the phase state of the lipid can be decisive on the interactions of Aβ with lipid membranes (Wong et al., 2009; Hellstrand et al., 2010).

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Concerning the effect of lipid layers on the aggregation behaviour of amyloidogenic peptides, the influence of charges and electrostatic interactions is discussed in various studies (Terzi et al., 1997; Bonev et al., 2001; Lopes et al., 2007; Murphy, 2007; Chi et al., 2008; Caillon et al., 2013; Stoeckl et al., 2013), but a unified picture is not yet established.

Generally, zwitterionic PC layers are associated with less or slower aggregation as compared to the bare air–water interface (Jean et al., 2009; Hellstrand et al., 2010). On the other hand, PG layers are often found to enhance aggregation. One hypothesis is that electrostatic interactions with the head group may induce aggregation while immersion into the hydrophobic chain layer inhibits aggregation (Bokvist et al., 2004). However, these findings can be explained by unspecific effects as well. Zwitterionic lipid layers might shield neighboring peptides from interacting with each other, simply causing a lower nucleation rate and hence less aggregation (Sabate et al., 2005). Similarly, by screening of repelling charges, charged lipid layers may enhance interpeptide interactions, and the formation of intermediate states may be favoured (Knight et al., 2006). This may agree with an unspecific increase in peptide concentration due to electrostatic interactions, without any specific effect of the lipid molecules. The tendency to enhance aggregation of IAPP and Aβ was found to increase in the series: bulk – PC layers – bare air–water interface – PG layers (Jean et al., 2009).

Both, the effect of lipids on aggregation is widely studied, but also the effect of aggregating peptides on lipids is important. Membrane perturbation or damage may be related to the mechanism of (neuro-)toxicity (McLaurin and Chakrabartty, 1997; Kremer et al., 2001; Yip and McLaurin, 2001; Zhao et al., 2004; Engel et al., 2008). As possible mechanism, the extraction of lipids due to fibril formation (Sparr et al., 2004; Hellstrand et al., 2013), or transient pore formation, also called membrane thinning (Huang et al., 2004; van Rooijen et al., 2010; Arce et al., 2011; Last and Miranker, 2013; Stoeckl et al., 2013), are considered most likely to date. In the focus of the present study are the more subtle changes in biophysical properties of lipid membranes that may lead to an apparent thinning of the membrane hydrophobic core. This would have a drastic influence on membrane integrity.

We aim to further elucidate the role of charges, lipid packing density and the mode of interaction of amphipathic and amyloidogenic model peptides with well-established lipid model systems. The present study addresses the effects of amphipathic and amyloidogenic model peptides on various types of lipid layers. We do not aim at understanding the effects of lipids on peptide aggregation, but the effect of binding of peptide monomers and early oligomers to lipid layers. The model peptides i,i+2 and i,i+4 (also called VW29 and VW30, respectively) are designed to exhibit strong propensities for defined secondary structure such as amphipathic α-helix and β-sheet (Fig. 1) (Pagel et al., 2008). These peptides are amphipathic in their α-helical conformation due to a leucin zipper motif. They were designed and proven to transform into structures that are rich in β-sheets and subsequently form amyloid fibrils in solution (Pagel et al., 2008). Their behaviour at the air–water interface was systematically studied including factors that influence the β-sheet formation by various conditions such as concentration, presence of metal ions, orientational order (Pagel et al., 2008; Hoernke et al., 2010, 2011, 2011a). Thus, i,i+2 and i,i+4 provide a well characterized set of model peptides. Because we do not aim at specific interactions, we chose relatively simple lipids. The most common phospholipid in cell membranes of humans is phosphatidylcholine. Besides zwitterionic lipids, anionic lipids like phosphatidylglycerols are investigated as most abundant anionic lipids. Therefore, DPPC, DMPC, and DPPG are chosen for zwitterionic and negatively charged membrane models, respectively. In order to access both liquid-expanded and liquid condensed phase, monolayer studies were performed on dipalmitoyl-lipids.

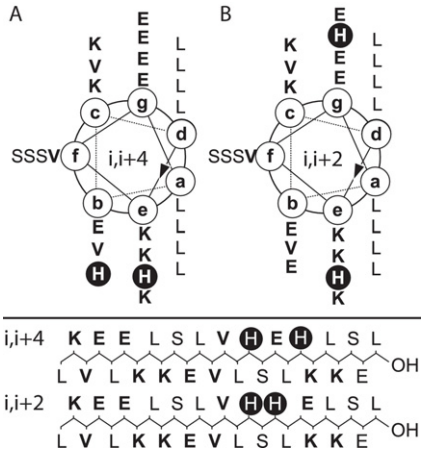

Fig. 1. (Top) α-Helix representation of i,i+4 (A) and i,i+2 (B), respectively. (Bottom) Sequences of i,i+4 and i,i+2 as elongated β-sheets. Both representations are ideal and not identical with the real conformation of the peptides (Pagel et al., 2008).

Accordingly, dimyristoyl-lipids allow the study of lipid in their fluid phase and gel phase in dispersions. Both types of lipids were investigated as monolayer films in various compression states and as lipid dispersions. The physical properties of these lipids are well established (Grigoriev et al., 1999, 2003; Aroti et al., 2004; Maltseva et al., 2006; Bringezu et al., 2007; Wagner and Brezesinski, 2007; Neville et al., 2008; Broniatowski et al., 2010), so that effects that arise from interaction with peptides can be analysed unambiguously.

The interaction of peptides with various preformed lipid monolayers as well as the resulting structure from submolecular to micron length scales are characterized by a variety of complementary techniques described later. This multitude of methods will be shown to be mandatory and unique.

2. Materials and methods

All solutions were prepared using milliQ water.

The peptides were synthesized and purified by reversed-phase HPLC according to reference (Pagel et al., 2008). After lyophilizing the peptides three times with 0.5 mM HCl and once with milliQ water (Andrushchenko et al., 2007), they were dissolved in hexafluoroisopropanol (1 mg/mL HFIP, Aldrich) to revert possible aggregation. The peptides were dried in vacuum or by a gentle N_2 stream directly before being dissolved in PBS (Fluka 10 mM, 150 mM NaCl Fluka, tempered at 600 °C before use, pH 7.4) to yield a 0.3 μM peptide solution. The solution was used immediately, shaking was avoided to prevent aggregation. In SAXS and WAXS experiments, i,i+4 with an additional p-Br-phenylalanine at the C-terminus was used. Negatively charged 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (DPPG) and 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (DMPG), as well as zwitterionic 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) were purchased from Avanti Polar Lipids (USA), stored at −20 °C and used without further purification. Before experiments, lipids were dissolved in $CHCl_3$ to a concentration of 1 mM and kept at 4 °C.

2.1. Langmuir film balance measurements

For preparing lipid films on top of peptide solutions, two different techniques were applied. Samples for IRRAS experiments were obtained by a multi-step process. First, a lipid film of the desired surface pressure π was spread from $CHCl_3$ onto the buffer subphase, left to equilibrate for 15 min and examined for reference purposes. Then, the film was transferred onto the subphase containing the