Synthesis and analysis of β -peptides for the specific and non-specific aggregation on model membranes

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Introduction

In biological systems protein-membrane interactions are mediated by complex structures, which fulfill numerous functions such as signaling, regulation, domain formation, molecule transport or information storage. All these tasks are based on peptides and proteins forming stable secondary structures like β -sheets or helices. They often provide further interaction or even higher aggregation.[1] Higher aggregates are responsible for the interaction between peptides/proteins and other biomolecules and are therefore also of great importance to the functional and structural integrity of biomembranes.[2,3] For example, the cytoskeletal complexes of the BAR domain containing proteins interact with spectrin and actin, which are in contact with membranes, aggregate specifically and form cell-spanning scaffolds.[4,5] Other examples for peptide-membrane interaction affecting the functional integrity of a biomembrane are the peptides Maculatin and Citropin, that have an antibiotic effect when interacting with membranes.[6] Yet many aspects of mechanism and dynamics of protein-membrane interactions remain unknown. Therefore, artificial model systems might give an insight into the complex nature of protein-membrane interactions.[7]

In this study, a synthetic approach using β^3 -peptide scaffolds as model systems for aggregational peptide-peptide and peptide-membrane interactions is presented. These scaffolds have the advantage to form stable and well-defined secondary structures, such as 14-helices.[8,9] Key properties of the 14-helix are the three-residue repeating arrangement of the side chains, a pitch of approximately 5.0 and every third side chain being arranged alike. These pattern allows to define three sites of the helix with different properties.

Figure 1: Model system of β -peptide helices (blue) functionalized with specific (yellow and red) and non-specific recognition units (green), lipidic side chains (gray), and fluorophores (green).

The peptides were obtained *via* manual solid phase peptide synthesis (SPPS). The peptides are modified with recognition units to enable specific and non-specific aggregation depending on the kind of recognition. The specific aggregation is realized by utilizing nucleobase modified amino acids and in case of non-specific aggregation a 2,2'-bipyridyl derivative was used for metal coordination (Fig. 1). The peptide-membrane interaction is induced by lipid-like amino acid side chains like C14 or C16 alkyl chains or cholesterol, thereby spontaneously anchoring within the membrane.[10]

Results

Non-specific coordination induced by metal coordination

The aggregation and organization of β -peptide helices by bipyridyl ligand metal coordination was investigated by titration of peptide 1 with Cu^{2+} and Zn^{2+} metal solutions and analysis by UV/Vis titration (Fig. 2). Formation of stable bipyridyl metal complexes was indicated and taken as proof for the aggregation of peptide helices. So far, this β -peptide helix aggregation was only investigated in solution. Therefore, it was required to prove the anchoring of the lipid chain modified β -peptide helices within the membrane. Binding of the peptides to the surface of vesicles was indicated by a FRET assay between the NBD-peptide and labeled vesicles (Fig. 3). The

FRET pair of NBD labeled peptides and Lissamine™ Rhodamine B marked vesicles was used. Increase of fluorescence by addition of the labeled peptide is evidence for a peptide-membrane interaction, and therefore, for binding of peptide helices to the surface of the vesicle.

Specific aggregation induced by nucleobase-nucleobase recognition

For the investigation of nucleobase modified peptides, oligomers 2-10 were synthesized varying in the nucleobase sequence. Anchoring of the peptide helices was indicated by FRET measurements between NBD-peptides 2-10 and Lissamine™ Rhodamine B labeled vesicles (Fig. 4). In addition, concentration dependent FRET was performed to analyze peptide-peptide interaction driven by specific nucleobase recognition. In solution NBD-peptides were titrated with TAMRA-labeled peptides. With increasing molar fractions of the TAMRA-labeled peptide, a FRET-signal was observed for the combination of peptide sequences CTG and CAG (7 and 9, Fig. 5a), whereas minor indication for a stable interaction was obtained for the ATT/AAT recognition motif (3 and 6, Fig. 5b). The mismatch combination of peptide sequences CTG/ATT (7 and 6, Fig. 5c) serves as reference for lacking interaction. Depending on the nucleobase sequence the peptide helices can be specifically aggregated at least in duplexes. Further studies will investigate the specific peptide helix aggregation of membrane bound oligomers.

Scheme 1

Figure 2: UV/Vis titration of b-peptide 1 ($c = 30 \mu\text{M}$ in MeOH) with CuCl_2 .

Figure 3: Normalized FRET spectra of NBD labeled peptide 1 and DOPC-Rhodamine vesicles in a 1:300 ratio.

Scheme 2

Figure 4: FRET spectra of rhodamine-labelled DOPC-vesicles (0.75%) with the indicated b-peptides (5 μ m) in Tris-HCl 10 mM pH 7.5.

Figure 5: Concentration-dependent FRET experiments in Tris-HCl 10 mM pH 7.5 at 10°C with a total peptide concentration of 8 μ m and the indicated molar fractions of TAMRA-labelled peptides. (a) Fluorescence emission spectra of b-peptides 4, 5 and 7. (ATT-AAT) (b) Fluorescence emission spectra of b-peptides 8, 9 and 7. (CTG-AAT) (c) Fluorescence emission spectra of b-peptides 8, 9 and 10 (CTG-CAG).

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