Assembly of a Vectorially Oriented Four-helix Bundle at the Air-Water Interface

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Introduction

α-helical bundles can provide a structural framework for binding specific prosthetic groups at selected locations within the structure of an artificial peptide. They are thereby designed to mimic a number of functions exhibited by biological proteins. The first family of these artificial peptides designed *de novo* was based on amphipathic di-helices that self-assembled in aqueous solution to form four-helix bundles with a polar exterior and nonpolar interior. They were shown to be of *anti* (or antiparallel) topology [1]. In order to realize any potential device applications, the peptides must be vectorially oriented in an ensemble (e.g., at an interface). The di-helices were rendered amphiphilic via the attachment of C16 hydrocarbon chains to their amino terminus [2]. Specular x-ray reflectivity (XRR) showed that these amphiphilic di-helices can be vectorially oriented at an air-water interface in a Langmuir monolayer with their helical axes normal to the interface [3]. But off-specular XRR indicated that these di-helices did not associate to form four-helix bundles, possibly because they were constrained to be of parallel topology. To achieve four-helix bundles vectorially oriented at the interface, we relaxed this constraint via a 1:1 association of the amphiphilic di-helices with their water-soluble counterparts (without the C16-chain modification).

Methods and Materials

Peptides were prepared by solid-phase synthesis, purified by reverse-phase high-pressure liquid chromatography (HPLC), and lyophilized. Two different pairs of soluble and amphiphilic peptides were used. The first peptide consisted of a 31-mer that mostly forms an amphipathic α-helix except for a Gly_3Cys flexible loop at the amino terminus that allows two such units to dimerize covalently via disulfide bond formation. The amphiphilic peptide has a single saturated C16 hydrocarbon chain bound to the free amino group of each cysteine residue. Except for the region between the two helices, the peptide is composed of polar residues with an even balance of positively charged lysine and negatively charged glutamate residues. The second peptide used [4] was synthesized as a 62-mer helix-loop-helix in which each helix has the same length as that in the previous peptide, but the one helix has only positively charged lysine residues while the other has only negatively charged glutamate residues. In its amphiphilic derivative, this peptide has a single C16 chain attached to its amino terminus.

Monolayers of the peptides were spread from methanol/chloroform solutions of 1:1 mixtures of each peptide and its corresponding amphiphilic derivative. Isotherm data gave indications about whether the soluble peptide was retained in the monolayer as surface pressure was applied. For structural evidence, we collected specular XRR from the monolayers with the phospholipid dilaurylphosphatidylethanolamine (DLPE) added as a spacer to make the method sensitive to the presence or absence of the soluble peptide. The subphase (1-mM TRIS buffer, pH 8) was contained in a Langmuir trough mounted on the sample stage of the liquid surface spectrometer of the Complex Materials Consortium Collaborative Access Team (CMC-CAT) at APS.

Results

Electron density distributions for the monolayers — namely, the electron density distribution as projected onto the coordinate normal to the interface — were obtained from the reflectivity data by using both the slab-model formalism and the model-independent box-refinement algorithm for phasing the data. They show that the first peptide system, with an even arrangement of positively and negatively charged residues, does not retain the soluble peptide as surface pressure is applied. In contrast, the other peptide system, with its asymmetric arrangement of charges, does retain the soluble component as surface pressure is applied.

Discussion

Electrostatic interactions are sufficient to promote the formation of four-helix bundles at the air-water interface. This is important for developing these peptides as model systems for studying the binding of small molecules, such as general anesthetics, since di-helices are too small to provide the binding pocket believed to be necessary for their action [5]. Future work will characterize the

peptide's association by in-plane x-ray scattering and its topology by neutron reflectivity from deuterium-labeled peptides.

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