Design of an Amphiphilic Di-helix and Its Vectorial Orientation at the Air-Water Interface


1 Department of Chemistry and 2 Department of Biochemistry/Biophysics, University of Pennsylvania, Philadelphia, PA, U.S.A.
3 Department of Physics, Brookhaven National Laboratory, Long Island, NY, U.S.A.
4 Argonne National Laboratory, Argonne, IL, U.S.A.

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, including vectorial electron transfer. The first family of these artificial peptides was based on amphipathic di-helices (where each helix possesses two faces, each composed of either nonpolar or polar residues extending over the entire length of the helix). These 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]. For these designed artificial peptides to realize any potential device applications, it is necessary to vectorially orient an ensemble of them at the air-water interface. In prior work, the di-helices were rendered amphiphilic via the covalent attachment of a C16 hydrocarbon chain to their amino terminus [2]. Electron density profiles derived from specular x-ray reflectivity (XRR) showed that these amphiphilic di-helices could be vectorially oriented at an air-water interface in a Langmuir monolayer, with their helical axes normal to the interface only at relatively high surface pressures, either in pure form or in binary mixtures with phospholipids [3]. Any function exhibited by the peptide’s prosthetic group (e.g., vectorial electron transfer) would then necessarily occur in the aqueous phase; namely, on one side of the air-water interface.

In this work, each of the helices is again designed to be amphiphatic, with one face composed of polar residues and the other of nonpolar residues. However, part way along the length of the helix, these faces switch their polarity. This results in di-helices that have a nonpolar interior and a polar exterior for more than two-thirds of their length, and for the remaining one-third, have a polar interior and nonpolar exterior. Thus, one end of each di-helix will prefer a hydrophilic environment, while the other end will prefer a hydrophobic environment such as that found within the hydrocarbon-chain region of a phospholipid Langmuir monolayer. This should help to orient the peptide vectorially at the air-water interface.

Methods and Materials

The peptide was prepared by solid-phase synthesis, purified by reverse-phase high-pressure liquid chromatography (HPLC), and lyophilized. It was designed to be an α-helix 45 amino acids long (and subsequently verified by circular dichroism), with the first four heptad repeat units having a larger polar face and smaller nonpolar face, and the last two heptad repeat units having a reverse arrangement, with more of the surface being nonpolar and less being polar. A cysteine residue at the C terminus permits the helices to dimerize by formation of a disulfide bond to form the di-helices.

Monolayers of the peptide were spread from methanol/chloroform solutions — both pure and in mixtures with the phospholipid dilignoceroyl phosphatidylcholine (DLgPC) — onto an aqueous subphase (1 mM phosphate buffer, 10 mM KCl, pH 8) 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) sector at APS. Specular XRR was collected with 8-keV x-rays.

Results

Electron density profiles 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 refinement formalism and the model-independent box-refinement algorithm for phasing the data. They show that the di-helices of the pure peptide lie in the plane of the interface at low surface pressure and that they become only modestly oriented out of the plane when surface pressure is applied. In contrast, when sufficient phospholipid is present to solvate the nonpolar end of the amphiphilic peptide, the orientation of the peptide’s dihelices normal to the interface is greatly enhanced, even at low surface pressure (10 mN/m).

Discussion

These results show that the synthesis of amphiphilic peptides that insert vectorially via self-assembly into both phospholipid monolayers and bilayers holds considerable
promise. Appropriate positioning of metalloporphyrin prosthetic groups via bis-histidyl coordination along the peptide’s di-helices should provide for electron transfer across the nonpolar/polar interface present in such systems.

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References