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Surface-driven first-step events of nanoscale self-assembly for molecular peptide fibers: An experimental and theoretical study

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ABSTRACT

New experimental results are reported on the self-assembling behavior of EAK16-II, the first discovered ionic self-complementary peptide, incubated at ultralow concentration (10^{-6} M) at neutral pH onto differently charged surfaces. It is found that strongly negatively charged surfaces promote the self-assembly of flat, micrometer-long mono-molecular fibers of side-on assembled sequences, lying onto a continuous monolayer of flat-on EAK16-II molecules. These results suggest that the monomolecular EAK16-II self-assembly is driven by the peculiar matching condition between peptide and surface electrostatic properties.

Molecular Mechanics simulations of the basic bimolecular interactions confirmed the experimental inferences, showing that the flat-on state is the most stable arrangement for two interacting EAK16-II sequences onto strongly negatively charged surfaces, where indeed EAK16-II β-sheet conformation is stabilized, while the weak electrostatic interactions with mildly charged substrates promote an "entangled" EAK16-II geometry.

Molecular Dynamics simulations further showed that the mobility and diffusional freedom of the peptides from the surfaces are ruled by the relative strength of peptide-surface electrostatic interactions, so that desorption probability for the peptide sequences is negligible from strongly-charged surfaces and high from mildly-charged surfaces. Furthermore, it has been found that an oligopeptide sequence lying onto two flat-on EAK16-II molecules, gains a remarkable lateral mobility, while remaining weakly bound to the surface, thus allowing the further molecular self-alignment responsible for the micrometer-long fiber formation. The reported results pave the way to the understanding and control of the subtle peptide-surface structural motifs matching enabling the formation of micrometer-long, but nanometer-wide monomolecular fibers.

1. Introduction

Peptides are generally considered among the most versatile and intrinsically biocompatible building blocks for supramolecular architectures fabrication [1,2]. A particular interest has been devoted to the study of the family of so-called ionic self-complementary peptides [3,4], due to their unprecedented ability to adopt specific secondary structures, tunable by appropriate selection of their amino acid sequence [5–7] and solution conditions, including pH [8] and charge distribution along the peptide sequence [7]. These materials have been shown to form a unique platform for the design of self-assembling biomaterials with hierarchical three-dimensional architectures and tunable physical properties. To date, synthetic membranes, multilamellar structures, amphiphilic micelles, tubules and fibrillar networks have been obtained from the self-assembly of various peptide motifs [9]. The outstanding properties of these peptide families prompted the opening of two complementary routes to produce increasingly complex biomaterials for biomedicine and bionanotechnology, taking profit of their self-assembling properties. The first route can be schematically described as aiming to construct highly bioactive self-standing materials, in the form of large 3D-fibrous networks [3,10], also giving efficient water-entrapping hydrogels [11] for the delivery of bioactive therapeutics [12] and biological scaffolds in regenerative medicine [1,13–15]. The second route is based on the use of these peptide families to obtain the functionalization

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of solid surfaces, including flat or nanostructured synthetic surfaces and fibers, to be employed in tissue engineering strategies able to drive and boost the processes of cell seeding, spreading and proliferation at biomaterial surfaces [16–20]. Accordingly, the self-assembly properties of the ionic self-complementary peptides and, more in particular, of the first discovered ionic self-complementary sequence, i.e., the EAK16 peptide [3], have been mostly studied and discussed in a three-dimensional context as the one of aqueous solutions, i.e., without spatial and orientational constrains, as the most appropriate to understand the solution parameters and conditions leading to the formation of self-standing fibers, tubules, membranes, etc. [5–9].

In fact, these conditions substantially enhance the role played by the peculiar two distinct hydrophilic (ionically charged) and hydrophobic side chains per each molecule, which are assumed to prompt the alternate hydrophobic–hydrophobic and charge-matched binding, assumed to drive the observed massive fiber formation [3,4,7,9,21]. A few recent theoretical papers, reporting Molecular Dynamics simulations of the proposed process of EAK16 nanofiber formation in solution, also show the role of hydrogen bonds to mediate the hydrophobic–hydrophobic molecule side interaction and the role of the self-complementary charge distribution to stabilize the ß-sheets stacks [22,23].

On the other hand, also if the interest for the functionalization of surfaces with amino acids and peptides is continuously growing (see for instance [24]), only few papers have been dedicated till today to the study of the interactions and structure of ionic self-complementary peptides adsorbed onto solid surfaces. These include an experimental and theoretical study on the interaction of dipeptides belonging to the EAK16 sequence [25], some experimental studies on the characterization of EAK16 and EAK16-scrambled sequences interacting with Au and TiO_2 [26,27], a theoretical study dealing with the interactions of EAK16 sequences with hydrophilic and charged SiO^- surfaces and hydrophobic neutral $SiCH_3$-terminated surfaces [28] and, more recently, a theoretical study of the behavior of EAK16 molecules onto hydrophobic graphite [29].

All these papers have been essentially limited to the study of the molecular arrangement of single EAK16 sequences onto the various surfaces.

In view of all the above, the present paper is aimed to study the early events of the self-assembly processes for a simple ionic complementary peptide model system at a controlled surface properties and structure.

Accordingly, the EAK16-II sequence, i.e., the first ionic complementary peptide discovered from a yeast protein, zuotin [3], has been chosen as the most suitable model systems, largely employed as inspiring system to design a large class of self-assembling construction motifs [9]. The sequence consists of 16 amino acid residues, AEAEAKAKAEAEAKAK, where A = alanine, E = glutamic acid, and K = lysine alternate hydrophobic (alanine (A)) and hydrophilic (glutamic acid (E) and lysine (K)) groups. The peptide folds into ß-sheet secondary structures with distinct hydrophobic and hydrophilic surfaces, where the hydrophobic alanines have been proposed to promote the coupling of the hydrophobic sides of two adjacent molecules in solution, while positive and negative charges of adjacent glutamic acid and lysine residues (on the hydrophilic side) are suggested to pack together, through intermolecular ionic interactions in a checkerboard-like manner [7,15]. This coupling mechanism in solution has been reported to provide nano- and mesoscale fibers, which have been reported to be, in average, 29.65 ± 2.37 nm wide [6], corresponding to an initial building block of about 5 aligned molecules, growing by the successive coupling of the ß-sheets stackings.

We report new experimental results for EAK16-II self-assembly onto strongly negatively charged mica, finding that the strongly charged surfaces may act as templating scaffolds, resulting in the formation of monomolecular fibers, approximately 6.0 nm wide, lying on a uniform monolayer of underlying EAK16-II molecules. The paper focus on the first-step events of peptide-surface interaction by using a very short incubation time of 60 min to highlight the basic short-term assembly processes. Quantum Mechanical and Molecular Dynamics simulations of the basic molecular interactions showed that the self-assembly of EAK16-II at surfaces is actually a charge-assisted process, demanding peculiar relative surface and peptide charges to allow the nanoribbon growth, as a results of the balance of molecular binding to the surface and lateral mobility.

2. Experimental

2.1. EAK-16 II sequences preparation

The EAK16-II peptide used in this study is characterized by alternating hydrophobic (Alanine) and hydrophilic (Glutamic acid and Lysine) amino acids, i.e., AEAEAKAKAEAEAKAK [3,6]. The hydrophilic residues, in turn, bear alternating negative and positive charges, yielding the following zwitterionic [–++–++] charge distribution at neutral pH. Due to the described peculiar structure, EAK16-II has been shown to form in solution stable β-sheet structures, which in turn give complex fibrillar assemblies, attributed to the extended alternating hydrophobic and electrostatic interactions [14,21].

EAK16-II sequences were synthesized with a Syro I peptide synthesizer (Multisynthec, Witten, Germany) using fluorenyl-9-methoxycarbonyl (Fmoc) solid phase chemistry. The synthesis was carried out on a 0.60 mmol Rink amide MBHA resin (Novabiochem, Langelfingen, Switzerland) using 5 eq. of side chain protected Fmoc-amino acids (Novabiochem) with 5 eq. of 2-(1Hbenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 5 eq. 1-hydroxybenzotriazole (HOBt) (Chem-Impex International, Wood Dale, IL, USA) and 10 eq. of diisopropylethylamine. The following side chain protections were used: *tert*-butyl ester (OtBu) for Glu and *tert*-butyloxycarbonyl (Boc) for Lys. The couplings were double except for 12–15 sequence. Crude peptide was detached from the resin and the protecting groups were released using a 95% trifluoroacetic acid, 2.5% triethylsilane, 2.5% water mixture (1.5 h, 25 °C). Purification of the crude products was performed through reverse phase-high performance liquid chromatography (RP-HPLC). Conditions used for the purification of crude peptide: Nova Pak C18 semipreparative column (6 μm, 60 Å, 7.8 × 300 mm, Waters, Milford, MA, USA); eluent A: 0.05% trifluoracetic acid (TFA)/water; eluent B, 0.05% TFA/CH_3CN; gradient, 0–8% of B in 2 min and from 8 to 16% of B over 32 min; detector, 214 nm; flow rate, 4 ml/min. Peptide purity was determined by analytical RP-HPLC and resulted over 98% (detection at 214 nm). Conditions used for analytical chromatography: Vydac C18 column (5 μm, 100 Å, 4.6 × 250 mm Grace Vydac, USA); eluent A: 0.05% TFA/water; eluent B, 0.05% TFA/CH_3CN; gradient, from 10% to 20% of B over 20 min; detector, 214 nm; flow rate, 1 ml/min. The identity of the purified product was confirmed by electrospray mass spectrometry using an Applied Biosystems Mariner System 5220 (theoretical value: 1614.8 Da, experimental value: 1613.8 Da).

Freshly cleaved strongly negatively-charged (hydrophilic) mica and ultrathin mildly negatively-charged (hydrophobic) polyhydroxymethylsiloxane (PHMS) films (30 nm thick) on silica, with Rq (mica) = 0.11 ± 0.00 nm and Rq (PHMS) = 0.34 ± 0.01 nm, before incubation, were immersed, for 60 min at RT, in 10^{-6} M EAK16-II solution added to a LiCl 40 mM one. The samples were then extracted from the peptide solution and gently dried in air. The peptide sequences, synthesized with a C-terminal amide, have a net

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