Surface Structures

Adsorption and Self-Assembly of Peptides on Mica Substrates**

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Nature produces a wide variety of biocomposite materials, from merely proteins and inorganic salts, often having remarkable properties not encountered in the "inorganic world".[1] These materials are formed by a precisely controlled templating process, whereby the protein preferentially nucleates a specific crystallographic face of the seed crystals.[2–4] Conversely, inorganic substrates may act as templates to induce large-scale order in protein supramolecular structures.[5,6] An understanding of these processes requires insight into the fundamental nature of the interactions between proteins and inorganic substrates. To this end we have carried out an empirical study of the adsorption and self-assembly behavior of a model peptide, CH_3CO-Q-Q-R-F-Q-W-Q-F-E-Q-Q-$CONH_2$ (P_{11}-2),[8] at solution/mica interfaces, and find that tapes with a cross β-sheet structure, a single molecule in thickness, grow epitaxially to produce a two-dimensional network reflecting the hexagonal symmetry of the substrate. Evaporation of the solvent gives rise to a dramatic reorganization of the tapes to produce a variety of structural morphologies depending on the drying process.

Our earlier work demonstrated how peptides with a propensity to form cross-β structures can, above a particular concentration, the critical tape concentration (ca. 90 μM for P_{11}-2), undergo self-assembly in either water or polar organic solvents to form long helicoidal, tapelike objects.[7–9] At higher concentrations still, ribbons (double tapes), fibrils (stacks of four or more tapes), and fibers (entwined fibrils) are formed successively. This hierarchical self-assembly behavior has been shown to stem from the chirality of the individual peptide molecules.[10]

In the present study our working hypothesis was that, below the critical tape concentration, we can expect planar tapelike structures to self-assemble at solid/solution interfaces, provided the peptide/surface binding energy is more than sufficient to compensate for the elastic distortion energy cost of "flattening" the tape onto the surface. This should make it possible to coat a solid surface with a "self-assembled monolayer" of tapes. Just how these tapes would interact with each other and how this would influence the longer length scale self-organization was less certain.

Working with aqueous solutions at pH 6.7, we were unable to image by atomic force microscopy (AFM) any in situ adsorbed P_{11}-2 structures. We raised the peptide/surface binding energy by lowering the dielectric constant of the solvent. In particular, using a solvent mixture of 10% water in 2-propanol (v/v), dielectric constant 26.1[11] and ${}^{s}_{w}$pH 5.5,[12] we were able to image the spontaneous formation of β-sheet tapes at the surface of the mica substrate in solution (Figure 1). The peak-to-peak distance between two touching tapes was 4.9 nm. (For reference, the calculated length of an 11-residue peptide in an extended β-strand conformation is 3.7 nm.) The tapes are seen to extend across the surface until they make contact. This contact inhibition defines the length of the tapes, and their growth is restricted to the two-dimensional plane of the surface. This peptide "monolayer" has a well-defined hexagonal symmetry, reflecting that of the mica surface lattice.

The orientation of dried films prepared by the immersion method (see Experimental Section) was studied by angle-resolved X-ray photoelectron spectroscopy (XPS) (tabulated results may be found in the Supporting Information). These experiments reveal that the peptide molecules within the adsorbed β-sheet tapes are oriented with the hydrophobic moieties of the amino acid residues at positions 4, 6, and 8 oriented outwards into the solution phase as illustrated in Figure 2. We were unsuccessful in our attempts to use ATR-IR spectroscopy to directly establish how the peptide molecules are packed within the tapes. However, structural studies of P_{11}-2 tapes in solution have established previously that the tapes are packed in an antiparallel cross-β struc-

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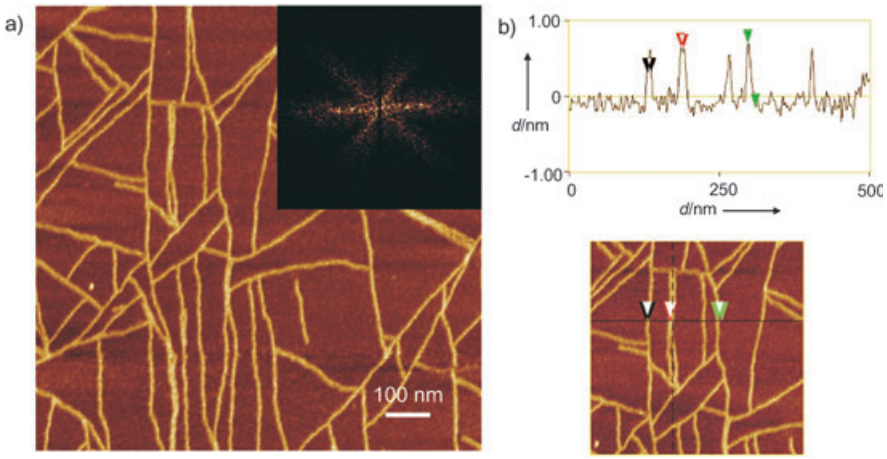

Figure 1. In situ AFM images measured by the tapping mode showing single β-sheet tapes grown on mica from 5 μM P_{11}-2 in 10% H_2O in 2-propanol; height scale 2 nm. a) Tapes aligned with the hexagonal symmetry of the underlying mica lattice with Fourier transform of the image showing hexagonal symmetry (inset, top right corner). b) Section analysis with line graph (top) showing the width (black arrows, convoluted width 6.9 nm) and height (green arrows, 0.8 nm) of the self-assembled tapes. The red arrows show the peak-to-peak distance (4.9 nm) between adjacent tapes.

ture.[7–10] The dimensions of the tapes assembled on the mica surface are very similar to those of the tapes in solution. Furthermore, molecular dynamics simulations[9] of tapes in aqueous solution have established that the antiparallel arrangement of peptide molecules depicted in Figure 2 is more stable than the alternative parallel one. These calculations also revealed how, for P_{11}-2, an intrinsic twist of three degrees per molecule along the tape axis is reduced to one degree in its corresponding dimeric ribbonlike structure. This is due largely to the cross-tape hydrophobic forces between residues at positions 4, 6, and 8. These are relatively modest forces. In the case of P_{11}-2 tapes on mica surfaces, we believe the predominant forces involved are electrostatic in origin. In particular, the positively charged δ-guanidinium of the arginine residues in position 3 displace hexagonally coordinated potassium ions on the basal layer (0001) of muscovite mica,[13] and the negatively charged γ-carboxylate of the glutamate residues at position 9 bind in turn to the K^+ ions. This directs the epitaxial growth of the β-sheet tapes.

We also studied dried films prepared on freshly cleaved mica substrates by the drop and blotting methods (see Experimental Section). In both cases a dramatic restructuring was observed upon drying. Contact-mode AFM images (Figure 3a–d) show that, as successive drops of a 10 μM aqueous solution (pH 6.7) of P_{11}-2 were applied to the surface and allowed to dry, there was a corresponding increase in the surface coverage of adsorbed peptide. The adsorbed peptide took the form of flat elongated aggregates. It appears that these objects are formed during the drying process through a combination of self-assembly and capillary interactions between the mesoscopic self-assembled β-sheet tapes. We have termed these objects, tactoids, meaning organized particles composed of smaller subunits (tapes). The tactoids have a uniform height of approximately 0.8 nm, commensurate with a monomolecular layer. The length and width of the tactoids vary from one area to another. Although generally elongated, some are needlelike and others more sheetlike in form. We believe that the variation in the dimensions of the mesoscopic tapes and consequently the tactoids is related to the drying rate for specific areas on the surface. The images shown in Figure 3 are representative of the needlelike

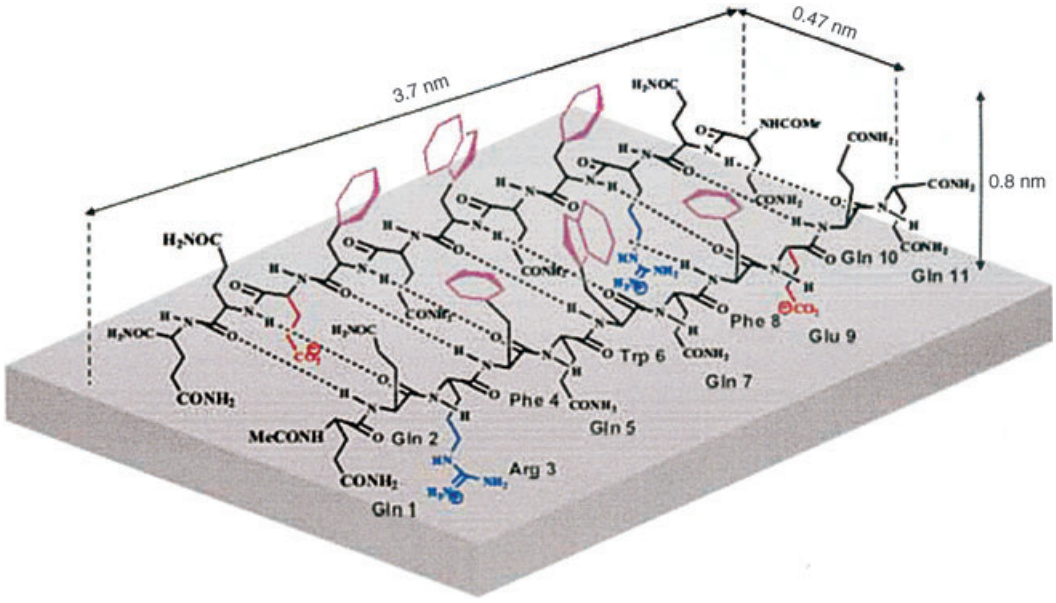

Figure 2. Schematic diagram showing the orientation and dimensions of a dimeric P_{11}-2 peptide tape in an antiparallel cross-β configuration adsorbed at the mica–water interface. XPS measurements show that the hydrophobic side chains in the residues at positions 4, 6, and 8 are directed into solution. The dimensions correspond with those obtained from modeling experiments.[9]

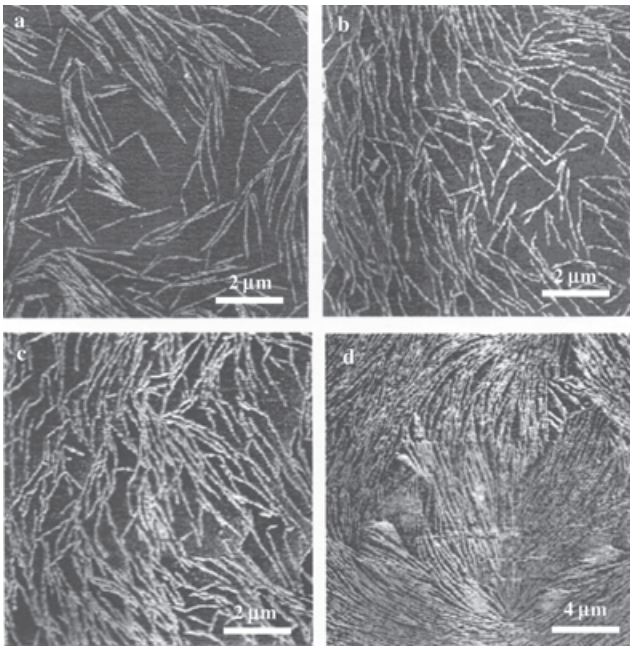

Figure 3. Contact-mode AFM images (a–d) of the tactoid distributions obtained by drying a film produced from sequential drops of 10 μM P_{11}-2 aqueous solution (pH 6.7). Height scale was 5 nm.

morphology. These tactoids were generally 100–200 nm in width and 2–3 μm in length. Interestingly, they are self-avoiding and do not overlap. This gives rise, at the higher surface concentrations, to a nematic-like long-range orientational ordering of tactoids, evident in Figure 3d, with the occurrence of a prominent disclination.[14] The monolayers obtained by the immersion method were similar in appearance to those described above for the drop method. Using this method, we were able to conduct precisely controlled adsorption studies, which will be described in subsequent publications.

In samples prepared by the blotting method it was possible to induce macroscopic uniaxial order in the adsorbed self-assembled tapes. The image in Figure 4 was obtained by allowing a 10 μL drop of 10 μM P_{11}-2 solution in water to flow over the surface of a freshly cleaved mica substrate, blotting excess fluid from the edge, then allowing the film to dry. The flow of the macroscopic drop over the surface produces a shear field that aligns the individual tapes in the direction of the receding interface. The tapes, imaged as regular corrugations, have an average width of 4.4 nm and correspond to single β-sheet tapes. They appear to extend indefinitely, suggesting that it ought to be possible, given a large enough volume of peptide solution and controlled retraction of the substrate through the air/water interface, to produce macroscopically aligned peptide monolayers on solid substrates.

In conclusion, it has been demonstrated that a peptide, P_{11}-2, designed to form twisted tapes and higher order structures in solution, can self-assemble at solution/mica interfaces into planar tapes, a single molecule in thickness, and having a cross-β structure. This is seen to occur at concentrations well below the critical concentration at which self-assembly into tapes occurs in bulk solution. Growth of planar tapes at an interface requires a peptide/surface binding energy sufficient to suppress the intrinsic twist in the tapes stemming from the chirality of the individual peptide molecules.[10] We believe, that in the system alluded to here, the predominant forces involved are electrostatic in origin. In particular, that the δ-guanidinium of Arg in position 3 displaces hexagonally coordinated potassium ions within the basal layer (0001) of the mica substrate, and the γ-carboxylate of Glu at position 9 binds to potassium ions. When the solvent is allowed to evaporate, quite different aggregate morphologies are obtained depending on the method used to prepare the initial film. One of these, a close-packed monolayer of parallel-aligned tapes, may be of practical utility as a functionalized protein-like surface.

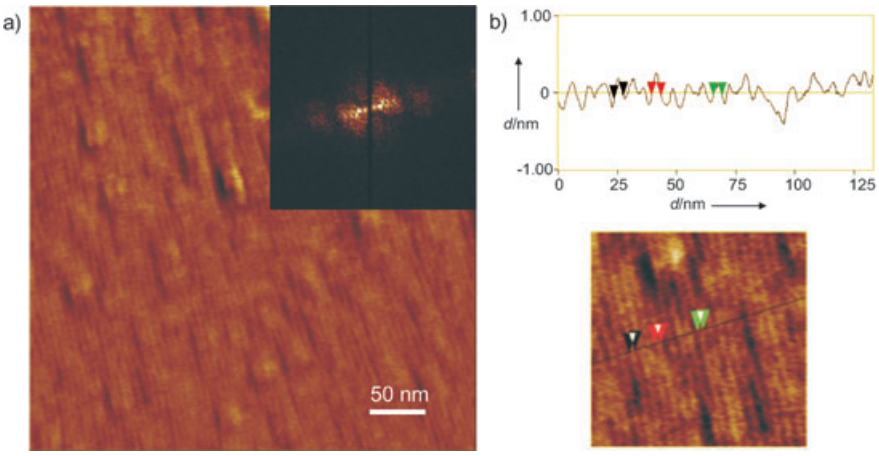

Figure 4. Tapping-mode AFM images showing aligned β-sheet tapes produced by shear flow alignment on a mica surface. a) Aligned tapes at the surface with Fourier transform of the image showing the uniaxial orientation of aligned tapes (inset, top right corner). Height scale: 5 nm. b) Section analysis and line graph (top) showing the measured widths of three single tapes imaged as corrugations within the sheet. The black, red, and green arrows show measured widths of 4.0, 4.0, and 3.8 nm, respectively.

Experimental Section

P_{11}-2 ($M_w = 1593.7$ Da by MS) was synthesized by Neosystems Groupe SNPE, France.[8] Solutions (pH 6.7) were prepared from freeze-dried P_{11}-2 with HPLC-grade water and 10% H_2O in 2-propanol. Circular dichroism (CD) spectroscopy (260–190 nm) confirmed that P_{11}-2 was present in its monomeric, random-coil state. To study the growth of equilibrium structures at solution/mica interfaces, we injected the solution of monomeric peptide into a sealed AFM fluid cell containing a freshly cleaved mica substrate. The peptide was allowed to adsorb, and the spontaneous self-assembly was imaged in solution. In the drying experiments, samples were prepared by one of three methods. Drop method: One or more drops of 10 μM peptide solution were placed onto the center of a freshly cleaved mica sheet and the resulting film allowed to dry. Immersion method: A freshly cleaved mica sheet was placed into a

flat-bottomed vial containing 10 mL of P_{11}-2 solution. After the allotted time the mica sheet was removed and blotted from the edge to remove any excess solution, and the sample left to dry. Blotting method: A 10 μL drop of peptide solution was placed on the edge of a freshly cleaved mica sheet and allowed to wash over the inclined surface. Excess solution was blotted from the edge of the sheet, and the sample was left to dry in air. Tapping-mode AFM observations were carried out using a Multimode Nanoscope IIIa Scanning Probe Microscope (Veeco, USA). Noncontact silicon probes (NCL, Nanosensors GmbH) were used for imaging in air. In situ imaging was performed using a closed fluid cell with contact-mode oxide-sharpened silicon nitride probes (NP-S, Veeco) driven at a frequency of $\approx$9.2 kHz, within the broad resonance in fluid. Contact-mode AFM observations in air were performed on a Park Scientific Instruments Autoprobe using v-shaped silicon nitride cantilevers (Veeco, USA). High-resolution X-ray photoelectron spectra for both the C 1s/K 2p and N 1s regions were recorded on a VG Scientific Sigma Probe instrument with monochromatic $Al_{K\alpha}$ radiation. XPS spectra were taken from monolayers adsorbed on mica from 22 μM P_{11}-2 solution in water using the immersion method. Additional experimental details for XPS measurements are given in the Supporting Information.

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