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Nanopore Formation by Self-Assembly of the Model Genetically Engineered Elastin-like Polymer $[(VPGVG)_2(VPGEG)(VPGVG)_2]_{15}$

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Elastin-like polymers (ELPs) are a new class of synthetic polypeptides whose composition has been inspired by the repeating sequences found in natural elastin.[1] These polymers are gaining a strong reputation as models for the understanding of the folding, self-assembly, and function of natural proteins. Furthermore, ELPs have shown a wide set of significant properties of interest in fields such as biomaterial science, biomedical engineering and nanotechnology. Among their most outstanding characteristics are their almost ideal elastomeric behavior,[2] extraordinary biocompatibility[3] and biofunctionality,[4] acute smart nature,[1,5] and self-assembly;[6] all of these are of interest for bottom-up nanofabrication of advanced devices.

Very recently, the use of recombinant DNA technologies has revolutionized the design and production of novel ELPs. These techniques provide a tool for polymer tailoring with an absolute control of the architecture, lack of randomness in amino acid stereochemistry and sequence, comonomer arrangement, and exact molecular weight (MW). Different methods and examples have been summarized, for example, in ref 7.

Poly(VPGVG) is the most important member of the elastin-like family,[1] where G stands for glycine, V for L-valine, and P for L-proline. This polymer can be considered a predominantly hydrophobic polypeptide, where the only polar groups are the peptide moieties themselves. Derivatives are obtained by systematic substitutions of the Val amino acid residues of the pentamer by any natural amino acid. In some cases, these amino acids can be further chemically modified by conjugating a new chemical group. In this manner, light[1,5,8] or redox[1] sensitive polymers have previously been obtained.

Both the smart and self-assembly characteristics of ELPs are based on hydrophobic association and apolar−polar repulsion.[4] The polymer can switch between the folded and unfolded state of the polymer chain as a consequence of a given signal of the kind described above. In the unfolded state (in aqueous solutions), the free polymer chains remain disordered, random coils that are fully hydrated,[9] mainly by hydrophobic hydration.[4,10,11] In the folded state, the polymer folding and assembly is driven by hydrophobic forces. In the initial stages, the polymer chains form filaments made of three chains that further aggregate into nano- and microstructures. This results in a phase-separated state[12,13] in which the polymer chains adopt a dynamic, regular, nonrandom structure, called a β-spiral, stabilized by hydrophobic contacts.[1]

The recombinant ELPs used in this work, $[(VPGVG)_2(VPGEG)(VPGVG)_2]_{15}$ and $(VPGVG)_{48}$, have been prepared as reported previously.[14] The first is based on the model (VPGVG) pentapeptide of the elastin, in which the second valine has been regularly replaced by an L-glutamic acid (E) in one pentapeptide out of five. This leads to a regular distribution of E residues, which are equally spaced every 25 amino acids. This particular molecular design has shown an acute pH responding smart behavior in a previous work.[14] E has been used instead of D because, in the protonated state, E shows a hydrophobicity more similar to V than D, which is more polar, according to the hydrophobicity scale given by Urry for the natural amino acids.[1] The presence of the glutamic acid makes the polymer sensitive to the pH of the medium; modification of the pH induces large polarity changes in the free γ-carboxyl group. At acid pHs, the protonated carboxyl shows a low polarity state which is congruent with the hydrophobic nature of the rest of the polymer. However, at basic or neutral pHs the deprotonated carboxylate shows a polarity considerably higher than the surrounding environment. In this state, the polymer shows a regular heterogeneous distribution of polar and apolar segments along the polymer chain. An exciting possibility is that the regular distribution of the polymer amphiphilicity along the polymer chain could drive the formation of nanometer scale topographic structure.

To test this possibility, solutions of $[(VPGVG)_2(VPGEG)(VPGVG)_2]_{15}$ and $(VPGVG)_{48}$ were deposited on hydrophobic surfaces by spin coating. The depositions were made at room temperature, i.e., below T_t. This has been done so as to avoid the spontaneous formation of particulate aggregates on the surface. In addition, above T_t the elastin-like polymers tend to form fibrillar structures,[15,16] based on β-spirals, that we wanted to avoid in this work. The hydrophobic surfaces were obtained by immersion of silicon wafers in a bath of NH_4F/HF (7:1).[17] The solutions prepared were 10 mg mL^{-1} of 0.02 M HCl in water (acid solution) and 10 mg mL^{-1} of 0.02 M NaOH in water (basic solution). AFM analysis (tapping mode) was performed in air using a Digital Instruments Multimode (Santa Barbara, CA) using Si cantilevers (spring constant 40N/m, resonance frequency of 150−190 kHz) working in tapping mode (TMAFM).

When the E-containing polymer is deposited from the basic medium on the hydrophobic substrate, the formation of a quite homogeneous and periodic nanopore distribution is a noteworthy topological feature. Figure 1 shows a 3-dimensional AFM image of the nanopores. The nanopores are approximately 70 nm width, 8 nm depth (which is very likely underestimated due to the finite radius of curvature of the AFM tip), and they are separated by approximately 150 nm. Each experiment was repeated at least 3 times and other samples were prepared under different spin coating conditions to ensure reproducibility. The results obtained were similar in all cases. Figure 2 shows the result of the spin coating of an acidic solution of the same polymer. Under this acid pH, the polymer shows just a flat surface without any topologically remarkable features.

From the molecular point of view, the different behavior found under basic or acidic conditions can be explained by the different

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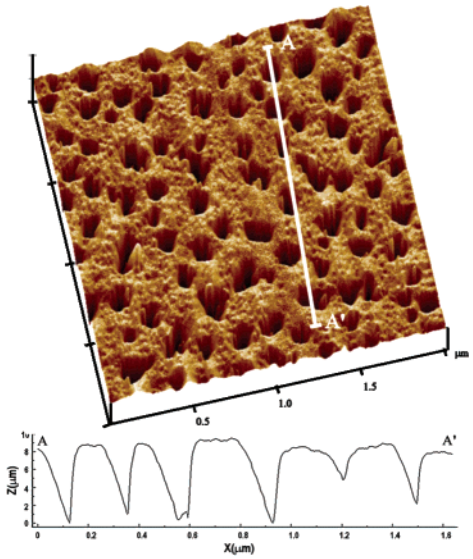

Figure 1. Top: 3D TMAFM image of $[(VPGVG)_2(VPGEG)(VPGVG)_2]_{15}$ deposited from a basic medium. Bottom: section analysis of the nanopores.

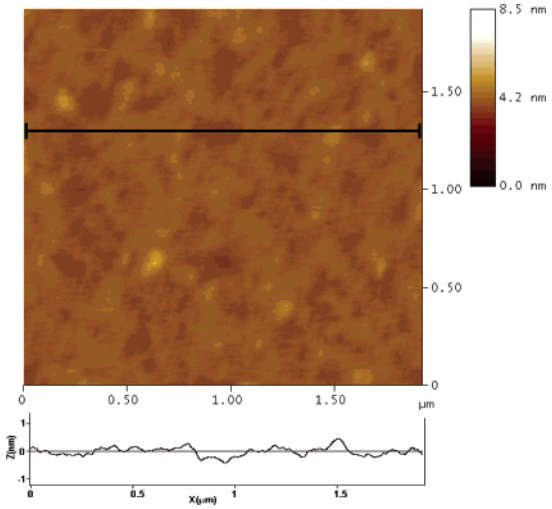

Figure 2. TMAFM 2D-image of $[(VPGVG)_2(VPGEG)(VPGVG)_2]_{15}$ deposited from an acid medium, and profile of the image.

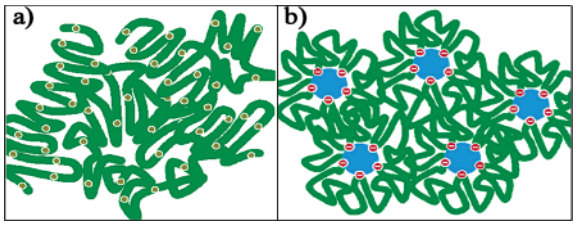

Figure 3. Schematic cartoon of the polymer distribution on a hydrophobic substrate: (a) in an acid medium, (b) in a basic medium. Counterions have been not drawn for clarity.

polarity state exhibited by the free γ-carboxyl groups of E (Figure 3). When dissolved in basic media, the negatively charged carboxylate, due to the quite apolar nature of the rest of the amino acids in the polymer, becomes the point of highest polarity along the chain, contrasting with the quite hydrophobic surroundings. This yields a heterogeneous polymer structure with a predominantly apolar chain showing highly polar domains equally spaced along it. These domains, due to their charge, impede in their vicinities both hydrophobic contacts between chains and hydrophobic interaction with the substrate. Since the position of the polar domains is well controlled to be equally spaced within nanometer distances along the polymer chain, these domains and their hydration water are finally segregated from the predominantly hydrophobic environment giving rise to the formation of pores of nanometer dimensions. However, in the acidic solution, the free γ-carboxylic group is protonated, and in this state, this group shows a low polarity, yielding a quite homogeneous polymer in polarity. The pH values used in this work are well inside the range of full protonation and deprotonation as determined in a previous work where the apparent pK_a of this polymer has been determined.[14] Therefore, in this protonated state, spin coating of the solution over a hydrophobic substrate yields a flat and homogeneous deposition with no particular topographical features.

When the control polymer, $(VPGVG)_{48}$, was deposited under the same conditions as those used for the E-containing polymer, a total lack of topologically remarkable features was observed across a wide range of pH. These results again point to the role of the free γ-carboxyl groups of E in developing the nanopore structures observed.

In conclusion, a genetically engineered ELP has been induced to form nanoporous films. This process is controlled by the pH of the surrounding medium in the sense that the pores are formed only when the deposition is made from a basic solution. Furthermore, due to the polypeptide nature of the polymer and the manner in which it has been produced, the inclusion of different (bio)-functionalities along the polymer chain is easily feasible. Future work will focus both on the dependence of the pore structure on the type of solvent and substrate and on the self-assembly capabilities of different molecular designs based on this same general composition but with different content of the free carboxyl groups. Finally, we plan to synthesize more complex hydrophilic−hydrophobic architectures and biofunctionalities, such as, for example, diblock and triblock polymer designs and specific sequences for cell attachment or biomineralization.

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