Stimuli-responsive properties of peptide-based copolymers studied via directional growth of self-assembled patterns on solid substrate

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Abstract

We studied the self-assembly of peptide-based ABA and CBC triblock-copolymers (obtained by bacterial expression) containing random coiled hydrophilic central B blocks flanked with helical A or C blocks. The A and C blocks were of different compositions with respect to the fraction of lysine residues which provided a higher pH-sensitivity of the copolymer solutions. The interchain interactions of the copolymer driven by external stimuli (pH and temperature) were explored in the process of macromolecular self-assembling in the thin films of the copolymer solutions deposited on the solid substrate. The interactions involved in the macromolecular association affected the morphology of the developed patterns. The polypeptide of the B block was not involved in the formation of the secondary structures, while the A and C blocks demonstrated helical folding responsible for the intermolecular association. The mechanism of the responsive behavior of the copolymers is based on the reversible assembling of the helices into coiled-coil structures upon the change of pH or temperature. It was found that at low pH values, when electrostatic repulsion was strong and the A/C blocks unfolded, assembling yielded fractal dendrites. Increasing the pH resulted in the recovery of the helical conformation of the A/C blocks and caused a transition from the fractal to compact structures. An elevation of temperature resulted in the disruption of the dendritic structures. The reported here approach to the evaluation of the intermolecular interactions, based on the analysis of the dendritic patterns, provides a rapid and simple method for the characterization of complex processes of self-assembling biomacromolecules.

Introduction

In self-assembled nanostructures, the occurrence of multiscale ordering promises unique optical, electrical, magnetic and mechanical properties of materials. Colloidal particles, surfactants, block-copolymers and proteins have been successfully used for assembling complex hierarchical structures (see for example Refs.1-4). Peptide-based materials are among the most promising candidates for applications in nanobiotechnology due to the precise control of the interchain interactions. Self-assembled polypeptides can be used as drug delivery hydrogels, molecular actuators, functional materials for engineering

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biointerfaces and analytical biosensors, as well as to construct complex nanostructures of different shapes and sizes for drug delivery and biomineralization.[5],[6]

The scaling behavior of the hierarchically assembled polypeptides is driven from the structural specificity of the polypeptide chains (monodispersity, stereoregularity and monomer sequence control). Depending on the constituent amino acids, polypeptides can fold into conformationally stable secondary (helices and sheets), tertiary and quaternary structures. Due to the specificity of the chain structure and conformational stability linear macromolecules are able to assemble into hierarchically ordered three-dimensional structures. A spontaneous organization of macromolecules and their blocks is driven by the formation of intra- and intermolecular bonds. Interactions within the polypeptide molecules influence the macromolecular conformation, while structural complementarity promotes their assembling. A coiled-coil structure of macromolecules represents the basic folding pattern observed in the native proteins. It originates from the helical conformations of the polypeptide segments interacting via hydrophobic and electrostatic forces. The helical structure can be characterized by the $(abcdefg)_n$ heptad, containing hydrophobic moieties in the a/d positions and charged groups in the e/f positions (for example, see in Figure 1 the structure of the polypeptides, that we used in this article).[6] Assembled coiled-coil structures composed of two or more aligned helices maintain integrity due the hydrophobic and electrostatic interactions of the a/d and e/f residues, respectively. Temperature and pH sensitivity of the interactions involved in the helical folding and association allow application of the coiled-coil crosslinkers for the formation of responsive hydrogels.[7] For the hydrogelation process, the polymer chain also has hydrophilic blocks possessing the conformation of random coil swollen in the aqueous environment. When the helical and random coiled blocks are covalently bound, the balance between the attractive and repulsive forces exerted by different blocks results in the formation of hydrogel.[8]-[10] Application of the block-copolymer peptides is not limited only to the hydrogel formation but contains also potential for the fabrication of complex nanostructures with variable morphology and functional properties.[1],[4],[5],[11] Design of macromolecules with advanced structural and compositional complementarities leads to their ordered assembling and selective formation of the shaped structures. Increasing specificity of the molecular interactions, such as a selective coiled-coil association, is used for the fabrication of structures of various geometrical complexities.[12]-[20]

Scaling analysis is commonly used for studies of hierarchical structures of colloidal assemblies.[21] Depending on the interactions involved, the assembling process can be controlled either by diffusion of clusters or forces acting between clusters and aggregates. In the diffusion-limited process, the obtained aggregates possess a highly porous structure, while a shift towards the reaction-limited process results in a decreased porosity. The fractal analysis for the assembled patterns is an efficient tool for a quantitative representation of the structures' complexity. A comparison of the fractal dimension values for the obtained patterns with those in the existing models provides valuable basic information about the mechanism involved in the assembling process.[22],[23] Previous studies have shown that the morphology of the polypeptide 2D patterns formed on the solid substrate is controlled by the diffusion of clusters and interactions involved in their assembling.[24]-[27] Reported results indicate that when interactions are strong, the kinetics of the assembling process shifts into the diffusion-controlled regime and yields patterns with low fractal dimensions. The increase in the fractal dimension values was attributed to the reaction-limited regime characterized be the weak intermolecular forces.

In this article we investigate assembling of the ABA and CBC block-copolypeptides on the mica substrate. The major attention is focused on the effect of the macromolecular interactions and the assembling conditions on the directional pattern growth. The phase

growth takes place on the solid-liquid interface. There are several basic parameters that govern this process: surface tension, molecular attachment anisotropy and diffusion of mass. Their interplay determines the morphology and structure of the obtained patterns. The effect of surface tension, related to the stability (solubility) of the curved surface, acts as a factor that opposes formation of complex patterns due to the high interfacial area. Anisotropy of the surface energy, on the other hand, promotes dendritic growth.[28] Development of the pattern morphology is controlled by the diffusion fields.[29] Inhomogeneities of the solute concentration, otherwise referred to as noise, have a major influence on the branching process. When concentration fluctuations are low, protrusions that form during the nonequilibrial growth develop uniformly. An increase of fluctuations triggers the coarsening process: some protrusions develop into independent side branches while the growth of others remains depressed.[30] The selection process is random and branching positions are not symmetrical. Small changes of the surface tension anisotropy or noise may result in the morphological transition from dendritic to densely branched structures. The latter, also called a seaweed morphology, can be described as a pattern lacking directional growth.[31] Nonetheless, it still possesses crystalline structure. Thus, analysis of the patterns' morphologies formed at the various solution conditions (pH, temperature) provides valuable information about the assembling mechanism and properties of the constituent macromolecules.

A major scope of the current paper is to find a relationship between the conditions for self-assembling in the casted polypeptide solution (pH, temperature) and morphology of the obtained patterns. The environmental conditions determine the balance between intermolecular forces and formation of the secondary structures in the peptide macromolecules. In the studied ABA copolymers, attractive interactions of the block-copolypeptides originate from the hydrophobic and electrostatic forces acting between the folded A blocks. Valine and leucine groups in the **a/d** positions provide hydrophobic interactions, while glutamic acid and lysine in the **e/g** positions exert electrostatic attraction (Figure 1). By modification of the polypeptide chain structures, the interactions between the macromolecules can be regulated. Substitution of one valine and three serine residues of the A blocks with lysine, produced block C. The structural modification changed the stability of the helical conformation and propensity to the coiled-coil formation. The latter has a direct effect on the gelation properties of the copolypeptides. The role of the introduced (via lysine) amine groups in the C blocks is to provide a better pH and temperature response than that of the A blocks.[9] Electrostatic repulsion induced by the amine functionalities at low pH values disrupts the secondary structures responsible for the physical crosslinking of macromolecules.

Thus, we hypothesized that the self-assembling of the block-copolymers via helices formed by the A and C blocks could contribute to an increasing molecular attachment anisotropy and concentration fluctuations. The former promotes directional growth of the dendritic patterns. Association of macromolecules, on the other hand, increases the concentration fluctuations responsible for the sidebranching process. Consequently, the regulation by external stimuli (pH and temperature) of the formation of the helixes could be used to affect the morphology of the self-assembling materials from compact to fractal structure and vice versa. We demonstrate here that the analysis of the fractal patterns formed by polypeptide molecules provides a simple and rapid method for the characterization of balanced interactions between the biomacromolecules at different environmental conditions.

Experimental

Materials

Recombinant triblock ABA and CBC polypeptides consisting of two terminal coiled-coil forming domains (blocks A or C) flanking a central water-soluble random coil block B were synthesized as described previously.[9] Briefly, *E. coli* BL21(DE3)pLysS competent cells were transformed with corresponding expression vectors. The polypeptides (Figure 1) were purified by immobilized metal affinity chromatography on Ni-NTA agarose resin and characterized by MALDI-TOF mass spectroscopy, analytical ultracentrifugation, and CD spectroscopy.[9] The structure of the individual blocks was as follows: Random coil block B: ASYRDPMG[(AG)$_3$PEG]$_{10}$ARMPTSADP. The presence of proline in the polypeptide backbone prevented formation of the secondary structures and preserved randomly coiled conformation of the B blocks.[8]

Coiled-coil forming A block: (VSSLESK)$_6$

Coiled-coil forming C block: [(VSSLESK)$_2$-(VSKLESK)-(KSKLESK)-(VSKLESK)-(VSSLESK).

Further details of the peptides characteristics can be found in Ref. [9].

Positions **a/d** in the heptads of the block A are occupied by the hydrophobic valine and leucine, while **e/g** are occupied by glutamic acid and lysine. In this configuration, hydrophobic interactions between **a/d** groups, as well as electrostatic interaction between **e/g** groups, provide high stability for the coiled-coil associates characterized by the midpoint transition temperature of ABA copolymer above 95°C.[9] To reduce the unfolding temperature, one valine and three serine residues of C blocks were replaced with lysine. Introducing the amine-containing groups disturbs the hydrophobic interactions at the hydrophobic interface and increases electrostatic repulsion within the coiled-coil structure. Consequently, the transition temperature for copolymer CBC dropped to 45°C.[9]

Study of self-assembly of the copolypeptides

We used the following procedure to prepare the polypeptides solutions for casting. The polypeptide was dissolved in Millipore water (1.0 g/L) and filtered through a filter with pore diameter of 0.45 μm. After dilution (10 times), the filtered solution was split into three aliquots and pH adjusted to pH 1.0, pH 4.0 and pH 7.4, respectively. The concentration barrier for the formation of macromolecular assemblies was around 10% for CBC and 30% for ABA copolymer.[9] For the samples' preparation a 20 μL droplet of 0.1g/L polypeptide solution was placed on the freshly cleaved mica (disks of 10 mm in diameter) and dried at different temperatures (20°C, 45°C, and 70°C) in air. Afterwards, the formed patterns were analyzed using AFM microscopy (Dimension 3100, Veeco, NY) equipped with silicon probe BS-TAP300 (Innovative Solutions Bulgaria Ltd., Bulgaria) with tip radius ~15 nm. AFM images were processed using WS×M software.32 For the optical imaging a camera attached to the Dimension 3100 AFM instrument was used.

The polypeptide assembling on the flat substrate involves formation of small associates (clusters) composed of several coiled-coil macromolecules followed by their diffusion and aggregation that yields branched patterns. Formation of the associates originates from non-covalent interactions between the coiled-coil domains. The development of the patterns in different areas of the droplet from the edge to the droplet center (from right to left, respectively) is shown in Figure 2. The self-assembling near the contact line is frozen because of water evaporation so that the patterns at the droplet periphery were formed for much shorter period of time as compared to those formed in the droplet center. Thus, the

image in Figure 2 provides information about the self-assembling kinetics. At the short assembling times – ca. 10 s (right), the copolymer forms small aggregates with the seaweed morphology. An increasing of the assembling time to ca. 1 min results in an increase of the size of the surface aggregates followed by a slow transformation of the pattern morphology from the seaweed (right) to dendrite (left). The kinetics of the pattern formation can be attributed to the diffusion-limited assembling of the surface-confined copolymer associates. In our analysis we used the patterns formed in the central part of the droplet. These patterns were formed for longer periods of time and less affected by concentration fluctuations present near the drying edge of the droplet

For the analysis of the obtained patterns, we used the conventional box-counting method.[33] In this method, a series of grids with different box sizes is overlaid onto the binarized image and the number of boxes occupied with the pattern's features is counted. Fractal dimension D_B is calculated using $D_B = -\lim[\log N_\varepsilon / \log \varepsilon]$, where N_ε is the number of boxes containing elements of the pattern, and ε is the grid scale. For fractal analysis we used adapted AFM images (Figures 3 and 5). To trace contours of the patterns we used Corel Photo-Paint software. Binarized contour images were then processed with ImageJ 1.40g software available from http://rsb.info.nih.gov/ij/ (National Institutes of Health, USA). ImageJ provided conventional box-counting tool for the fractal dimension analysis and with the FracLac 2.5, plugin for ImageJ, we analyzed lacunarity of the patterns.

Fractal analysis of the obtained patterns provides quantitative information about the patterns' complexity. Dendrites with low sidebranching have a fractal dimension of around 1.5. An increase of the branching density produces fractal dendrites with dimensions up to 1.7. Above this value, directional patterns can be considered as compact dendrites.[28] Fractal dimensions of the visual textures are often supported with lacunarity measurements. Sometimes, patterns have the same fractal dimensions even though their morphology is quite different. Lacunarity provides solution for this problem by measuring heterogeneity or translational invariance in digital images. These measurements take into account number of pixels in each unit area and calculate its variation for the whole image. Application of lacunarity for dendritic patterns yields numerical representation of the branching density.

Results and Discussion

Morphology of the ABA and CBC polypeptide patterns obtained by casting of 0.1 g/L solution on the mica substrate in the ambient conditions is shown in Figure 3. Dendritic morphology is a common feature for all obtained images. The directional growth character of the structures is related to the surface tension anisotropy and originates from the crystalline anisotropy. Different crystal faces have different surface energies and, thus, the latter results in variation of the molecular attachment kinetics.

The major tendency observed in the dendritic patterns shown in Figure 3 is the gradual increase of the sidebranching density with the increase of the solution pH. Multiple theoretical and experimental studies have shown that propagation of the dendritic tip growth is followed by the formation of the side arms.[34] This effect is commonly referred to as moving phase boundary instability.[35] The original protrusions' density is rather high and due to the competition in the diffusion field, only a few of them can develop into independent sidebranches.[36] The selection process is called coarsening and before the winning branches are established all the protrusions build up uniformly. Coarsening, which disturbs the uniform growth, is triggered by the concentration fluctuations or noise. When the level of fluctuations remains low the incubation period extends, thus, promoting formation of the wide trunks with few sidebranches (Figure 4a). In the opposite case a strong noise increases the sidebranching density yielding compact dendrites. In the studied system, the effect of noise can be assigned to the formation of helical structures from the A

and C blocks and interactions between them. A dynamic clustering of macromolecules, which occurs due to the reversible association of the A and C blocks, creates concentration fluctuations. These fluctuations participate in the coarsening process. Noise-induced coarsening provides a rapid selection of the dominant sidebranches amongst multiple protrusions competing in the diffusion field (Figure 4b).

Formation of the macromolecular clusters originates from the coiled-coil association of the A/C segments in ABA/CBC copolymers (Figure 1). Temperature and pH sensitive folding of the A/C blocks is driven by the hydrophobic interactions between valine and leucine groups located in the **a/d** positions and electrostatic interaction between glutamic acid and lysine in the **e/g** positions. [9] At the low pH values, protonation of the amine groups exerts strong electrostatic repulsion between macromolecular arms, which causes unfolding of both A and C blocks. Disruption of the intermolecular association results in a low noise level. Lack of noise slows down coarsening kinetics and promotes formation of the wide trunks with few sidebranches. The latter was observed at pH 1 for both polymers (Figure 3a, d). Formation of helices that occurs with increasing pH values promotes coiled-coil assembling. Intermolecular association results in an increased noise level and, consequently, in pattern growth shifts towards formation of the compact dendritic morphology. The effect of noise is even more pronounced at pH 7, when both polymers develop patterns denser than at the low pH values (Figure 3c, f).

Comparison between ABA and CBC copolymers indicates that at the given pH range the CBC copolymer reveals a wider variety of structures than the ABA polymer. The CBC copolymer has needle-shaped dendrite morphology at pH 1 and a compact dendritic structure at the neutral pH. In turn, the ABA copolymer experiences similar behavior but the amplitude of changes is rather small. A poor branching of the CBC copolymer at pH 1 can be related to the strong electrostatic repulsion of the amine groups. Compared to CBC, the dendrites formed from the ABA copolymer at pH 1 reveal denser branching. This effect can be related to the structural difference between A and C blocks. The latter has four lysine groups instead of one valine and three serine groups of the A block. Lower amount of the amine functionalities in ABA results in a reduction of electrostatic repulsion and promotion of macromolecular association, which increases noise level and, hence, promotes branching process. Unlike the low pH range, at pH 7 the CBC copolymer reveals denser branching than the ABA copolymer. This fact can be assigned to the stronger intermolecular association of the CBC copolymer. Previous studies of analytical ultracentrifugation[9] showed that in diluted solutions, polypeptides with the A blocks exist in a monomer-dimer equilibrium, while those with the C blocks form dimer and tetramer associates. Moreover, the gelation concentration of the CBC copolymer was three times lower than that of the ABA copolymer.[9] The intense clustering of the CBC copolymer resulted in denser branching at neutral pH. Fractal dimension and lacunarity of the patterns was used to evaluate the difference between branching densities of the dendritic structures of two copolymers at pH1 and pH7 (Figure 5).

The temperature-dependent pattern growth, studied for the ABA and CBC copolymers at pH 1, revealed different behaviors with varying temperatures (Figure 6). For the ABA copolymer, an increase of temperature resulted in the disruption of the directional pattern growth already at 45°C. Some structures still retained elements of the dendritic trunks with normal sidebranches but the majority formed seaweed patterns (Figure 6b). When temperature increases up to 70°C, polypeptide assembling yields a uniform array of the nanosized clusters scattered over the mica surface. The uniform deposits indicate a strong cluster-surface attraction, which hinders diffusion and assembling of the adsorbed polypeptide clusters.

CBC copolymer, on the other hand, preserved the directional pattern growth at the high temperatures. Comparing to the patterns obtained at 20°C (Figure 6d), the dendritic growth of the CBC copolymer at 45°C and 70°C revealed an increasing of the branching density with temperature (Figure 6e,f). The fractal dimensions of the obtained patterns changed from 1.47 to 1.72 at 20°C and 70°C, respectively; this indicated transformation of the dendritic structure from fractal to compact. This observation was noticed at temperatures unfavorable for the folding of helices. Thus, for this polymer we clearly observed an interplay of two different tendencies: (a) formation of dense branches, and (b) disruption of the patterns and transition to the seaweed morphology. The change of the patterns' morphologies with temperature can be assigned, from the one hand, to an increase of the evaporation rate and temperature-induced fluctuations. The high temperature promotes fast evaporation of the solvent, which, in turn, results in an increase of the supersaturation concentration. The latter creates the driving force for the solid phase growth, thus, yielding the denser patterns. On the other hand, a significant increase of the driving force may result in the morphological transition from dendritic to seaweed structures.[28] The first signs of the morphological transformation were observed at 70°C as changes of the branching angles and of the overall decrease of directional ordering of the dendritic patterns.

In contrast to the patterns obtained at pH 1, an elevation of temperature at pH 4 and pH 7 for both copolymers did not result in the directional pattern growth, but rather in formation of the polypeptide clusters deposited on the mica surface (Figure 7). This behavior indicates a strong competition between the intermolecular interactions of the copolymers and the substrate-copolymer interactions. At the elevated temperatures and at the pH values between 4 and 7, van der Waals forces between the copolymer molecules and the mica substrate are dominated so that the interaction with substrates prevents a directional growth of the patterns.

Conclusions

We show that the intermolecular forces involved in the association process and macromolecular diffusion control the assembling of the polypeptides on the solid substrate. Clustering of the solute polypeptides followed by the attachment of clusters to the surface-confined structures results in the growth of compact patterns. Strong repulsion between the assembling macromolecules, on the other hand, promotes formation of the fractal patterns characterized by wide trunks with few sidebranches. Intermolecular association of polypeptides is generally governed by the formation of secondary structures. Folded macromolecules undergo association processes that affect the morphology of the assembled patterns.

In this work we demonstrate the dependence of the polypeptide pattern morphology on the pH and temperature. We relate the variation of the obtained structures to the changes of macromolecular conformations at different pH and temperature values. A change of the electrostatic and hydrogen bonding results in the conformational transformation of the polypeptide chains and a shift in the balance in the macromolecular association process. For the ABA and CBC copolypeptides used in the self-assembling studies, the pH response is attributed to the folding propensity of the A and C blocks and their association into the coiled-coil structures. An increase of the electrostatic repulsion at the low pH values causes unfolding of the helical structures and, consequently, results in a decreased intermolecular association. The latter results in the formation of the fractal dendrites with a low branching density. At the neutral pH, however, the helical folding of the A and C blocks and their association promotes formation of the compact dendrites. Comparison between the two polymers containing A and C blocks indicates that the patterns formed by the CBC copolymer show more prominent morphological transformations with pH than those of the ABA copolymer. The stronger pH response of the C blocks is attributed to the additional

amine functionalities introduced with the lysine residues instead of the nonpolar hydrophobic valine and neutral serine residues corresponding to the A blocks.

An elevation of temperature results in the disruption of the dendritic structures of the ABA polymer and the random deposition of polypeptide clusters on the mica surface. This change of the pattern morphology indicated a strong effect of the interactions of polypeptide clusters with substrate surface on the pattern growth. Similar behavior was documented for the CBC copolymer at the pH values 4 and 7. However, at pH 1 the CBC copolymer formed compact patterns at higher temperatures. These structures were affected by the increases of the driving force for the solid phase growth as a result of the fast evaporation of the solvent.

The reported here approach to the evaluation of the intermolecular interactions, based on the analysis of the dendritic patterns, provides a rapid and inexpensive method for the characterization of complex processes of self-assembling biomacromolecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Tsai CJ, Zheng J, Zanuy D, Haspel N, Wolfson H, Aleman C, Nussinov R. Proteins: Struct. Funct. Bioinf 2007;68:1–12.

2. Jenekhe SA, Chen XL. Science 1999;283:372–375. [PubMed: 9888850]

3. Tanev PT, Liang Y, Pinnavaia TJ. J. Am. Chem. Soc 1997;119:8616–8624.

4. Wong MS, Cha JN, Choi KS, Deming TJ, Stucky GD. Nano Lett 2002;2:583–587.

5. Zhang SG. Biotechnol. Adv 2002;20:321–339. [PubMed: 14550019]

6. Kopeček J, Yang JY. Polymer Int 2007;56:1078–1098.

7. Kopeček J. Biomaterials 2007;28:5185–5192. [PubMed: 17697712]

8. Petka WA, Harden JL, McGrath KP, Wirtz D, Tirrell DA. Science 1998;281:389–392. [PubMed: 9665877]

9. Xu C, Breedveld V, Kopeček J. Biomacromolecules 2005;6:1739–1749. [PubMed: 15877401]

10. Xu C, Kopeček J. Pharmaceutical Res 2008;25:674–682.

11. Chow D, Nunalee ML, Lim DW, Simnick AJ, Chilkoti A. Mater. Sci. Eng. R-Reports 2008;62:125–155.

12. Tsai CJ, Zheng J, Nussinov R. PLoS Comput. Biol 2006;2:311–319.

13. Tsai CJ, Zheng J, Aleman C, Nussinov R. Trends Biotechnol 2006;24:449–454. [PubMed: 16935374]

14. Reches M, Gazit E. Curr. Nanosci 2006;2:105–111.

15. Zheng J, Zanuy D, Haspel N, Tsai CJ, Aleman C, Nussinov R. Biochemistry 2007;46:1205–1218. [PubMed: 17260950]

16. Zanuy D, Jimenez AI, Cativiela C, Nussinov R, Aleman C. J. Phys. Chem. B 2007;111:3236–3242. [PubMed: 17388467]

17. Zanuy D, Rodriguez-Ropero F, Haspel N, Zheng J, Nussinov R, Aleman C. Biomacromolecules 2007;8:3135–3146. [PubMed: 17854222]

18. Zhao X, Zhang S. Macromol. Biosci 2007;7:13–22. [PubMed: 17225214]

19. Capito RM, Azevedo HS, Velichko YS, Mata A, Stupp SI. Science 2008;319:1812–1816. [PubMed: 18369143]

20. Cockroft SL, Chu J, Amorin M, Ghadiri MR. J. Am. Chem. Soc 2008;130:818–820. [PubMed: 18166054]

21. Witten TA, Sander LM. Phys. Rev. Lett 1981;47:1400–1403.

22. Gosal WS, Ross-Murphy SB. Curr. Opin. Colloid Interface Sci 2000;5:188–194.

23. Pellenc D, Gallet O, Berry H. Phys. Rev. E 2005;72 Art No 051904.

24. Gan HY, Li YL, Liu HB, Wang S, Li CH, Yuan MJ, Liu XF, Wang CR, Jiang L, Zhu DB. Biomacromolecules 2007;8:1723–1729. [PubMed: 17458934]

25. Lomander A, Hwang W, Zhang S. Nano Lett 2005;5:1255–1260. [PubMed: 16178220]

26. Murr MM, Morse DE. Proc. Natl. Acad. Sci. U.S.A 2005;102:11657–11662. [PubMed: 16091468]

27. Schüler J, Frank J, Saenger W, Georgalis Y. Biophys. J 1999;77:1117–1125. [PubMed: 10423456]

28. Brener E, Müller-Krumbhaar H, Temkin D. Phys. Rev. E 1996;54:2714–2722.

29. Karma A, Rappel WJ. Phys. Rev. E 1998;57:4323–4349.

30. Langer JS. Phys. Rev. A 1987;36:3350–3358. [PubMed: 9899254]

31. Ihle T, Müller-Krumbhaar H. Phys. Rev. E 1994;49:2972–2991.

32. Horcas I, Fernández R, Gómez-Rodríguez JM, Colchero J, Gómez-Herrero J, Baro AM. Rev. Sci. Instrum 2007;78 Art No. 013705.

33. Smith TG, Lange GD, Marks WB. J. Neurosci. Meth 1996;69:123–136.

34. Langer JS. Rev. Mod. Phys 1980;52:1–28.

35. Mullins W, Sekerka R. J. Appl. Phys 1963;34:323–329.

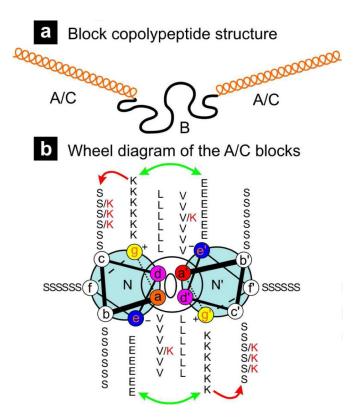36. Okerberg B, Marand H, Douglas J. Polymer 2008;49:579–587.

Figure 1.
Structure of the peptide macromolecules (**a**) and wheel diagram of the coiled-coil assembly
(**b**). Helical domains: (A) – (VSSLESK)$_6$, (C) – [(VSSLESK)$_2$-(VSKLESK)-(KSKLESK)-
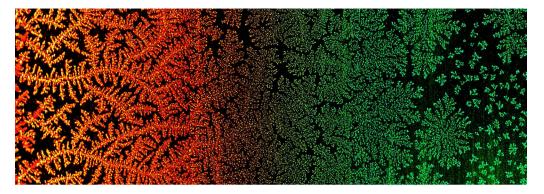(VSKLESK)-(VSSLESK)]. Random coil: (B) – ASYRDPMG-[(AG)$_3$PEG]$_{10}$-
ARMPTSADP.[9]

Figure 2.
AFM image shows gradual transformation of the seaweed morphology into dendrite from the periphery (right) to the center (left) of the solution droplet containing ABA copolymer at pH 1, 20°C. Vertical size 40 μm.
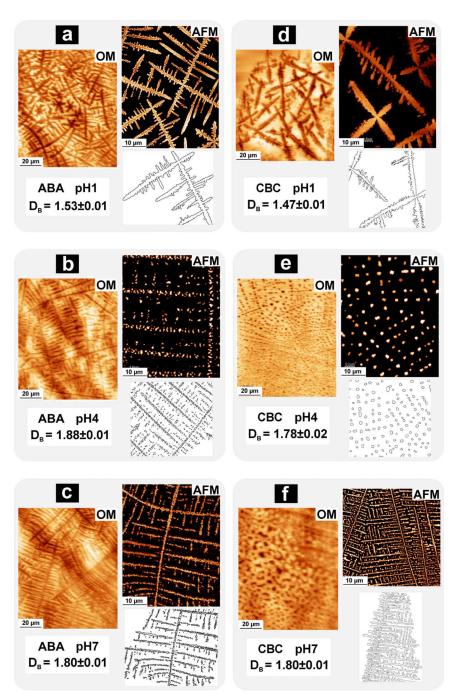
Figure 3.
AFM topography and optical images (OM) of the patterns obtained by casting of 0.1 g/L copolypeptide solutions on the mica surface at the ambient conditions for ABA (a-c) and CBC (d-f) copolymers at pH 1 (a,d), pH 4 (b,e), and pH 7 (c,f). The adapted AFM images for the evaluation of the fractal dimensions (D_B) with the box-counting method are located below the original AFM images.
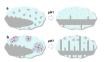
Figure 4.
Assembling of dendritic structures from the polypeptide macromolecules and their clusters. At low pH values, when electrostatic repulsion hinders macromolecular association, the assembling by the attachment of single polymer chains yields dendrites with wide trunks and few sidebranches (a). Formation of the polypeptide clusters, observed at high pH values, results in patterns with a high density of the sidebranches (b). The difference is due to the strong effect of the clustering process on the uniformity of the protrusions' growth. While single polymer chains have rather small effect on the competition between the neighboring protrusions, the arrival of the large clusters triggers coarsening of the protrusions and promotes development of the densely branched patterns.
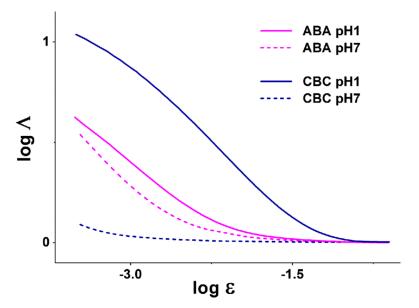
Figure 5.
Lacunarity (Λ) versus scan size (ε) of the patterns obtained by assembling of the ABA and CBC copolymers on the mica substrate at 20°C. We used lacunarity to estimate branching density of the dendritic patterns. For both copolymers patterns obtained at pH1 (solid) show lower branching (higher heterogeneity) than at pH7 (dash). Yet difference between pH1 and pH7 is much higher for CBC (blue) than for ABA (magenta) indicating higher pH sensitivity of the CBC copolymer.
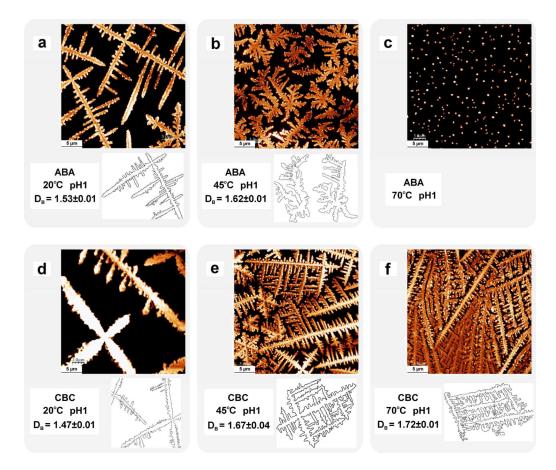
Figure 6.
AFM topography of the patterns grown by casting of 0.1 g/L ABA (a-c) and CBC (d-f) solutions at pH 1 on the mica surface at the different temperatures: 20°C (a,d), 45°C (b,e), and 70°C (c,f). The adapted AFM images for the evaluation of the fractal dimensions (D_B) with the box-counting method are located below the original AFM images.
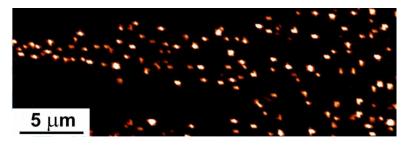
Figure 7.
AFM image of the spherical clusters of ABA deposited at pH4, 45°C. Both ABA and CBC polymers showed similar behavior at pH4 and pH7 with temperature (See Figure 1S in the Supporting Information).