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Tuning the Chain Helicity and Organizational Morphology of an L-Valine-Containing Polyacetylene by pH Change

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ABSTRACT

The helical chirality and self-assembling structures of an unnatural polymer, poly(*p*-ethynylbenzoyl-L-valine) (1), are readily manipulated by a simple environmental perturbation of pH change. The amino acid appendages of L-valine create an asymmetric force field, inducing the polyacetylene backbones to helically rotate, and form intra- and interchain hydrogen bonds, stabilizing the screw-sense conformation of the polymer chains. The polymer exhibits a large Cotton effect in methanol, which decreases with an increase in pH upon addition of KOH into the polymer solution. The change in the chain helicity is reversible: the unfolded polymer chains refold back to their original helical conformations when the solutions are neutralized. Natural evaporation of the methanol solutions of 1 on mica gives long, bundled nanofibers of macromolecular assemblies; in contrast, evaporation of the methanol/KOH solutions yields short, unraveled nanofibers with sizes of roughly single macromolecular chains. The ionization of the carboxy groups of the valine moieties by KOH breaks the hydrogen bonds, and the entropy-driven randomization leads to the observed chain helicity attenuation. The electrical repulsion between the polyelectrolyte chains carrying the negatively charged carboxylate ions disassembles the macromolecular association, resulting in the formation of the nanofibers of single chain dimension.

Helicity, as a special form of chirality,[1] easily reminds us of the helical conformation of proteins. The helicity of the polypeptide chains are determined by the chiral information encoded in the sequence of their building blocks of amino acids and are stabilized by the noncovalent molecular interactions such as hydrogen bonding.[2] The noncovalent interactions are, however, susceptible to external perturbations and the disturbed helixes often mutate into other forms of conformation to fit or adopt the changes in the surrounding environment.[2,3] This conformational mutation in turn affects the assembling or organizational structures of the biomacromolecules and may eventually change their biological functions.[4] Denaturation of proteins by hydrogen bond-breaking reagents such as urea is a "classic" example in this regard,[3,5] which disrupts or destroys the native conformations of the biopolymers, giving partially unfolded structures or completely denatured random coils with ultimate loss of their bioactivity. Changes in pH are also known to cause variations in the folding structures and biological functions; for example, enzymes, like other proteins, are stable over only a limited range of pH. Outside this range, changes in the charges on ionizable amino acid residues result in modifications of molecular conformation, chain helicity, packing pattern, and active-site structure,[6] which eventually leads to denaturation.[5] This is best manifested by the optimal pH windows of the enzyme activity; for example, the maximum activity of chymotrypsin occurs around pH 8, the activity of pepsin peaks around pH 2, and acetylcholinesterase works best at pH 7 or higher.[3]

Learning from nature, we sought to create unnatural polymers whose chain helicity and organizational structures can be modulated by external stimuli. We launched a research program on the development of biomimetic polymers and synthesized a wide variety of helical polyacetylenes containing naturally occurring building blocks such as amino acids, saccharides, and nucleotides.[7,8] In this paper, we demonstrate the ready tunability of the chain conformations of one of such nonbiological polymers, a poly(phenylacetylene) with

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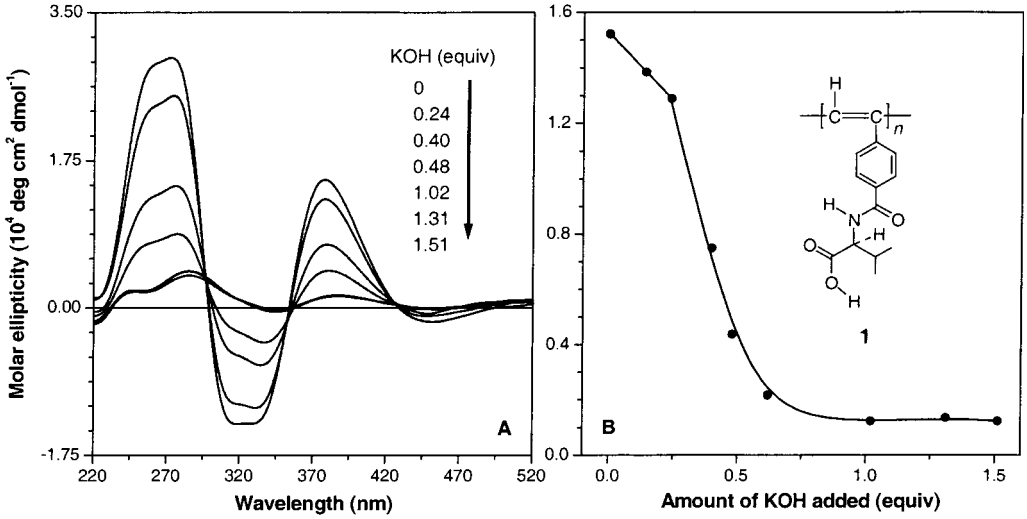


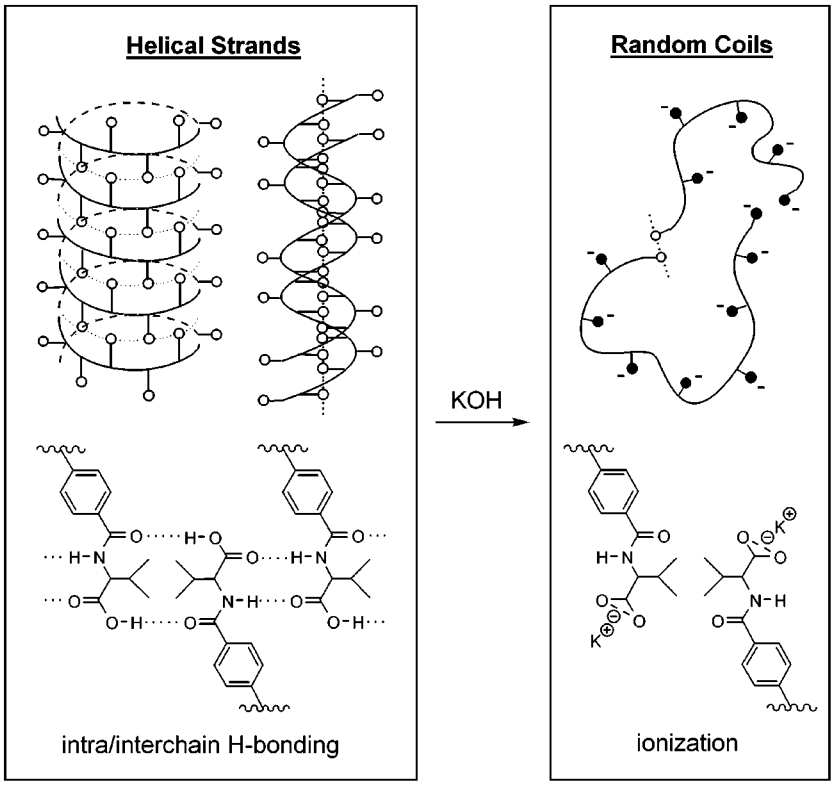
Figure 1. (A) CD spectra of methanol solutions of **1** containing different molar equivalents of KOH (recorded on a JASCO J-720 spectropolarimeter). (B) Change of the second Cotton effect with the amount of KOH added to the polymer solutions. Concentration of **1**: 1.47−1.71 mM. Temperature: ∼22 °C (room temperature).

amino acid appendages of L-valine or poly(*p*-ethynylbenzoyl-L-valine) (**1**), whose molecular structure is given in Figure 1B. Employing pH change as an external stimulus, we succeeded in manipulating the organizational structures of macromolecular assembly of the amphiphilic polyacetylene.

The synthetic procedure and characterization data of **1** have been reported in our previous publications.[7a,b,9] In the design of the molecular structure of **1**, we incorporated the L-valine moieties into the polyacetylene structure with the hope that the bulky chiral pendants would exert an asymmetric force field on the polymer backbone to induce the macromolecular chain to take a helical conformation. To check whether this is the case, we investigated the chain conformations of **1** using circular dichroism (CD) spectroscopy, a powerful tool for helical analysis.[10] As shown in Figure 1A, in methanol **1** exhibits an intense CD peak at ∼378 nm with a large molar ellipticity ([θ] ca. +15 200 deg cm^2 dmol^{-1}), while its monomer is CD-inactive at wavelengths longer than ∼280 nm. The CD peak at the long wavelength of ∼378 nm (the second Cotton effect[11]) thus must be due to the absorption of the polyacetylene backbone, unambiguously confirming that the macromolecular chain takes a handed helical conformation. The CD activity is, however, liable to the pH change of the solution: upon addition of a small amount of a base (KOH), the CD spectrum of **1** sensitively weakens. The CD intensity progressively decreases with an increase in the molar equivalent of KOH, with the second Cotton effect dropping by more than 10 times when ≥1 equiv of KOH is added to the polymer solution. Remarkably, the original CD spectrum is fully reinstalled when the alkaline solution is neutralized by an acid of HCl; that is, the tuning of the chain helicity by pH is reversible, or in other words, the polymer chains "remember" their "natural" folding conformations in the unperturbed state (memory effect[12]). Figure 1B depicts the change of the second Cotton effect with the amount of KOH added to the polymer solutions. Three (3) stages are clearly distinguishable. In the first stage (KOH < 0.24 equiv), the second Cotton effect moderately decreases. In the second stage, the Cotton effect sharply falls in a narrow range of KOH concentrations (∼0.24−0.62 equiv). In the third stage, the Cotton effect approaches a minimum molar ellipticity of ∼1360 deg cm^2 dmol^{-1} and remains unchanged when the KOH concentration is increased to over ∼0.8 equiv.

The formation of regular helical structures of polymer chains is obviously entropically unfavorable; this entropic cost may, however, be compensated by the multiple intra- and interchain hydrogen bonds of the amino acid moieties, as schematically illustrated in Chart 1. The helical structure is thus a consequence of the subtle balance of the two antagonistic effects; any external perturbation that disrupts the hydrogen bond formation will break the balance and may partially or completely randomize the polymer chains. Addition of KOH to the methanol solution of **1** would ionize the carboxy groups and cleave the hydrogen bonds. The negatively charged carboxylate ions will be solvated by the polar solvent molecules of methanol, blocking the access of the amide hydrogen for hydrogen bond formation. When a small amount of KOH is added to the methanol solution of **1**, a proportionally small fraction of hydrogen bonds will be broken, resulting in the linear decrease of the molar ellipticity (cf., the first stage in Figure 1B). The helical conformation catastrophically collapses when the amount of KOH reaches a threshold, causing quick unfolding or rapid unzipping of the helical chains accelerated by the entropy-driven chain randomization (the second stage). The CD intensity drops to a minimum value when less than 1 equiv of KOH is added to the polymer solution, further confirming the important contribution of the entropic effect to the chain randomization. Understandably, when a majority of the hydrogen bonds is broken, the entropy-driven randomization will separate the chain segments apart, thus tearing more hydrogen bonds. Further addition of KOH will further increase the pH of the solutions but will induce little change from a statistic viewpoint in the conformations of the already randomized chains (the third stage). Complete ionization of all the

Chart 1. Formation and Stabilization of Single- and Double-Stranded Helixes of **1** via Intra- and Interchain Hydrogen Bonding (••○•○ ••)[a]



[a] The helical strands can be further bundled and linked through lateral and terminal hydrogen bonding, respectively, to assemble into thick and long helical nanofibers. Ionization of carboxylic acid (●⁻) destroys the hydrogen bonds, and entropy-driven randomization leads to the formation of random coils. Some of the charged chains may still be linked by terminal hydrogen bonds of the carboxy groups that have survived the ionization due to the "polymer effect" involved in a polymer reaction.

carboxy groups may, however, be hampered by the famous "polymer effect" in a polymer reaction due mainly to the involved steric hindrance.[13] Thus, even when excess amounts of KOH are added to the polymer solutions, some small fractions of the carboxy groups may still maintain intact and exist in un-ionized form in the charged polymer chains.

In the structural hierarchy of proteins, the helix is a secondary structure, whose change often brings about variations in the higher-order structures of the biopolymers.[3,14] The changes in the chain conformations of **1** induced by the pH variation may also affect the organizational morphologies of the polymer chains, and we thus explored the possibility of manipulating the macromolecular assembly by the simple external stimulus of pH change. We first examined the morphology formed by natural evaporation of the (pure) methanol solutions of **1** (in the absence of KOH). When ~5 μL of a dilute polymer solution is placed on freshly cleaved mica, nanofibers spontaneously form upon solvent evaporation. Some of the fibrils are combined and merged in a convoluted manner while others are aligned and packed in a regular fashion; in all the cases, the helical trajectory of the fibrils is clearly imaged by the AFM tip (Figure 2). The helical polymer chains may be associated and bundled by the lateral interstrand hydrogen bonds and self-assembled into the fibrils whose constituent filaments are spirally twisting.[15] The lengths of the nanofibers reach to several tens of micrometers, which are much longer than the theoretical lengths of the single polymer chains even in their fully extended conformation (~410 nm).[16] This is due to the series connection of the polymer chains through the "sticky end" biting[17] or the interstrand hydrogen bonding of the amino acid residues at the chain terminals. It is amazing that such well-organized morphological structures are formed almost instantly, taking into account that the tiny amount (~5 μL) of methanol solvent takes a split second to evaporate in open air at ambient temperature. [In actuality, the macromolecules of high molecular weight (M_w ~ 408 000) should start to fold and precipitate at an even earlier stage, well before all the solvent molecules evaporate.] The organizational assembling of the macromolecular chains thus must be highly cooperative, much like that of biopolymers in the natural systems.[3,18]

We then checked how the pH change would affect the organizational morphology of the polymer chains. As anticipated, addition of KOH to the solutions of **1** readily changes the polymer morphology, examples of which are given in Figure 3. The fibrils shown in Figure 3A appear to be unraveled and disassembled into coiled filaments, although their ends appear to be still helically braided, which is in good agreement with the weak CD signals of the polymer solution. At the high pH, the polymer chain as a whole has almost completely lost its helicity, whose precipitate thus would not rotate in any preferred direction but would coil in a random fashion. The nanofibers shown in panels B and

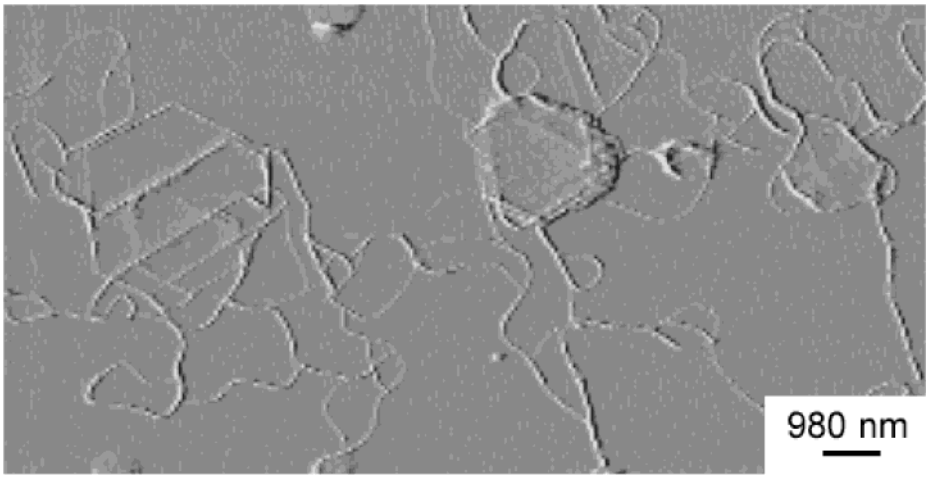


Figure 2. Helical nanofibers formed by **1** upon natural evaporation of its methanol solution (concentration: 7 μg/mL) on mica at room temperature (imaged on a Digital Instruments Nanoscope E atomic force microscope).

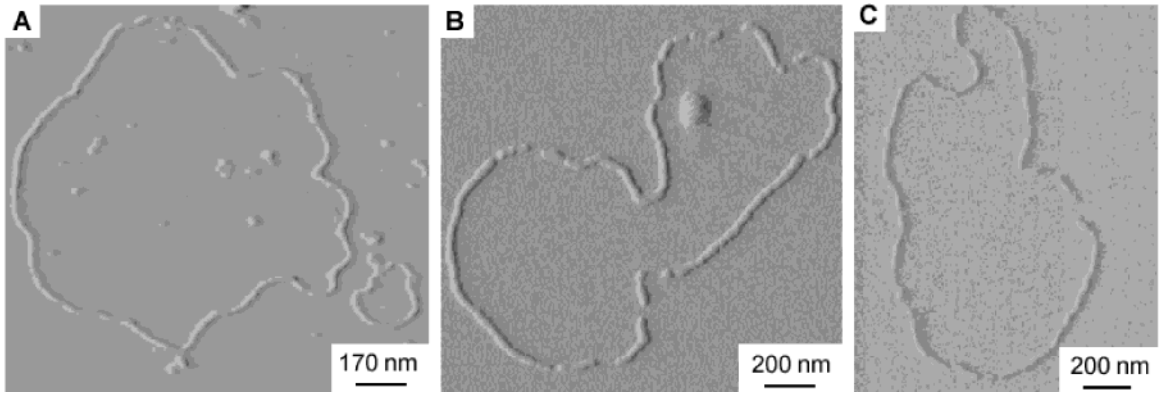


Figure 3. Unraveled nanofibers formed by **1** upon natural evaporation of its methanol solution (concentration: 4 μg/mL) containing 1 molar equiv of KOH (i.e., KOH/**1** = 1:1) on mica at room temperature (imaged on a Digital Instruments Nanoscope E atomic force microscope).

C of Figure 3 seem to have been completely unwound, with the randomly coiled ringlike structures comprising many short and discrete filaments. As discussed above, the polymer chains are linked and bundled mainly by the interstrand hydrogen bonds but the ionization of the carboxy groups of the amino acid moieties by KOH hampers the hydrogen bond formation (cf., Chart 1). When the terminal interstrand hydrogen bonding becomes difficult, the chain ends will understandably become less "sticky". In other words, the carboxylate ions at the ends of one polymer chain will be less likely to link with the ends of another chain carrying the same charges, and the filaments thus become disconnected and broken. When an overwhelming majority of the carboxy groups is dissociated by the potassium ion, the polymer becomes a polyelectrolyte. The chains of a polyelectrolyte may not be easily associated and the filaments thus become unknotted and thin.

To have a quantitative picture, we counted the nanofibers and evaluated their statistic average sizes. The widths of the nanofibers obtained from the methanol solution are big (~47−180 nm) yet polydisperse (Figure 4). It is well-known that the vertical resolution of an AFM image is much better (on the order of a few angstroms) than the lateral resolution (on the order of a few tens of nanometers) due to the tip-broadening effect.[15,19] Subtracting the broadening effect from the apparent average width of the nanofibers (~92 nm), it is estimated that their "true" average width is ~59 nm,[20] which is much bigger than their average height (~3 nm). This suggests that the nanofibers are mainly formed via the

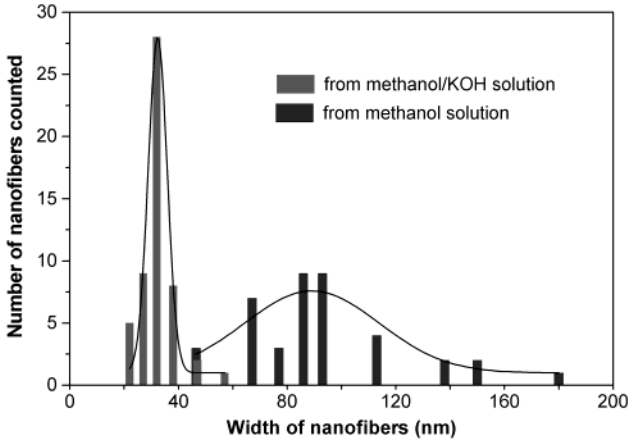


Figure 4. Histograms of measured widths for the nanofibers formed by **1** upon natural evaporation of its methanol and KOH/methanol solutions on mica at room temperature, with the solid lines showing the width distributions.

side-by-side packing of the polymer chains. On the other hand, the apparent widths of the nanofibers obtained from the methanol/KOH solution are confined in a narrower range (22−57 nm with a sharp peak at 32 nm). Their "true" average width corrected for the broadening effect is ∼3 nm, close to their measured average height (2.36 ± 0.69 nm). These values are in accordance with the theoretical diameter[16] of a single polymer chain (2.22 nm). This implies that the nanofibers shown in Figure 3 are basically the single polyacetylene chains, substantiating our argument that the electrically charged macromolecules can hardly be bundled by the lateral force of hydrogen bonding due to the electrical repulsion between the polyelectrolyte chains.

In summary, we successfully manipulated the structures of a nonbiological "test tube" polymer at "all" levels of organizational hierarchy using a simple external stimulus. By varying the pH of the methanol solutions of **1**, we continuously tuned the helicity of the polymer chains and demonstrated the full reversibility of the "denatured" macromolecular chains refolding back to their "natural" conformations. We modulated the aggregative packing of the polymer chains and the organizational structures of the nanofibers. We also correlated the chain helicity, hydrogen bonding, and organizational assembly involved in the construction of the hierarchical architectures in a visually clear fashion. The nanofibers of single polyacetylene chains swathed in a polyelectrolyte sheath of negatively charged ions are structurally unique and functionally intriguing. Such nanofibers may, for example, be both electronically and ionically conductive and may find potential applications as artificial nerves in nanostructured biomimetic systems.[22,23] Cytotoxicity of the biomimetic polymers is now under active investigation in collaboration with our colleagues in the Department of Biology of our University. Encouraging preliminary results have been obtained,[24,25] a detailed account of which will be given upon completion of the bioactivity evaluation.

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