Synthesis and Hierarchical Structures of Amphiphilic Polyphenylacetylenes Carrying L-Valine Pendants

Bing Shi Li,[†,‡] Kevin K. L. Cheuk,[‡] Liansheng Ling,[†] Junwu Chen,[‡] Xudong Xiao,[§] Chunli Bai,*[,†] and Ben Zhong Tang*[,‡]

Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China; and Departments of Chemistry and Physics, Institute of Nano Science and Technology, and Open Laboratory of Chirotechnology of the Institute of Molecular Technology for Drug Discovery and Synthesis,[||] Hong Kong University of Science & Technology, Clear Water Bay, Kowloon, Hong Kong, China

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ABSTRACT: In this work, we synthesized amino acid-containing poly(phenylacetylene)s and demonstrated the possibility of tuning their hierarchical structures by internal and external perturbations. A valine-acetylene adduct, 4-ethynylbenzoyl-L-valine methyl ester (**5**), was readily polymerized by $[Rh(nbd)Cl]_2$, and the resultant "polyester" (**1**) was selectively hydrolyzed by KOH to its "polyacid" congener, poly(4-ethynylbenzoyl-L-valine) (**2**). (Supra)molecular structures of the polymers were characterized by NMR, IR, UV, CD, and AFM techniques. The macromolecules took helical chain conformations in solutions. Their Cotton effects varied to different extents, when the polymer, solvent, and pH changed respectively from **1** to **2**, from methanol to THF, and from neutral to basic. Upon evaporation of their methanol solutions, **1** and **2** self-assembled into micellar spheres and helical cables, respectively. Changing the solvent of **1** to THF changed its folding structure to helical cables, while increasing the pH of the methanol solution of **2** led to the formation of random threads. The denaturation from helical cables to random threads is probably caused by the cleavage of interstrand hydrogen bonds by base-mediated ionization of carboxyl groups of the valine pendants.

Introduction

Hierarchical organization of biopolymers forms a structural basis of life in the natural world.[1,2] While the primary structures of the biopolymers are constructed via covalent linkage of naturally occurring building blocks such as amino acids, their higher-order (secondary, tertiary, and quaternary) structures are assembled through intra- and interchain associations cooperatively aided by noncovalent forces such as hydrogen bonding, hydrophobic stacking, solvation effect, and electrostatic interaction.[3] The molecular information such as amino acid sequence and chain chirality and amphiphilicity encoded in the primary structures of the biomacromolecules plays a primary role in determining their native folding structures; for example, L-glutamic acid segments in proteins often give an α-helix structure, while L-isoleucine segments most frequently induce β-sheet formation.[4,5] The folding structures can, however, be varied or denatured by the changes in the environmental surroundings of the biopolymers, due to the noncovalent nature of the supramolecular assembling.[3,6] Loss of body fluid (an important biological medium or "solvent"), for example, can transmute organizational structures of proteins by dehydration or deprivation. Variation in pH often causes changes in the active-site structures of enzymes, which in turn alters their biocatalytic activities.[7] This is best manifested by the bell-shaped dependence of the enzymatic activity of RNase A on pH: its rate constant k_{cat} peaks in a neutral medium (pH ∼7) but sharply drops at lower (acidic) or higher (basic) pH.[8]

Amino acids are the constitutional components of proteins, which are everywhere in living systems: hair, skin, muscle, and connective tissue are proteins; almost all enzymes are proteins; ....[5] Incorporation of the naturally occurring building blocks into manmade polymers is of interest because such a meld may create new nonbiological macromolecules with biomimetic structures and properties.[9,10] For example, molecular hybridization of hydrophilic amino acids with hydrophobic synthetic polymers will give birth to amphiphilic offspring;[11] the chirality of the amino acid moieties may induce the macromolecular chains to rotate in a screw sense,[12−15] and self-assembling of the helical amphipathic polymers may generate proteomimetic organizational structures.[16,17] The amino acid moieties may be arranged, through judicious molecular design, in such a way that they wrap a conjugated polymer chain as pendant groups; in other words, a conductive molecular wire may be sheathed in a cytophilic shroud. Such biocompatible nanowires may find innovative applications in medical diagnosis as biosensors, in drug delivery devices as control elements, in tissue engineering as architectural scaffolds, and more exotically, in cytotech and nanorobotics as artificial nerves, retinas, muscles, etc.[18]

Intrigued by the captivating prospects, in this study, we chose poly(phenylacetylene) (PPA), the best-known photoconductive polyacetylene,[19,20] as a model polymer, and tried to attach L-valine, one of the 20 amino acids commonly found in proteins, to the PPA chains as pendants. By fusing the valine and acetylene units together at the monomer stage, we succeeded in synthesizing PPA derivatives with every one of their repeat units being precisely appended with one amino acid

* Corresponding authors. E-mails: tangbenz@ust.hk (B.Z.T.); clbai@infoc3.icas.ac.cn (C.B.).
[†] Center for Molecular Sciences, Chinese Academy of Sciences.
[‡] Department of Chemistry, Hong Kong University of Science & Technology.
[§] Department of Physics, Hong Kong University of Science & Technology.
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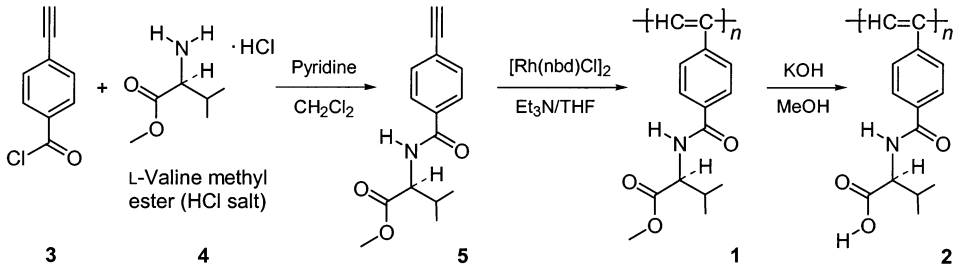
Scheme 1. Synthesis of Valine-Containing Polyphenylacetylenes: Poly(4-ethynylbenzoyl-L-valine methyl ester) (1) and Poly(4-ethynylbenzoyl-L-valine) (2)



moiety (Scheme 1). The pendant chirality induced the PPA backbone to helically spiral and the chain amphiphilicity enabled the polymer strands to self-assemble. Similar to proteins, the synthetic hybrids changed their hierarchical structures in response to the changes in their molecular structures and environmental surroundings. Changing the end-capped valine ester in **1** to "free" valine acid in **2** enhanced the chain hydrophility; this internal perturbation (structural change), as well as external stimuli (solvent and pH variations), affected the folding structures of the biomimetic polymers to various extents.

Experimental Section

Materials. Dichloromethane (DCM) was purchased from Lab-Scan and was distilled over calcium hydride. Pyridine and triethylamine (both from RdH) were dried and distilled over KOH. THF (Aldrich) was dried over 4 Å molecular sieves and distilled from sodium benzophenone ketyl immediately before use. L-Valine methyl ester hydrochloride {**4**; $[\alpha]_D^{21}$ +23.6° (*c* 2, methanol); Sigma} and phenylacetylene (PA; Aldrich) were used as received without further purification. The rhodium catalyst $[Rh(nbd)Cl]_2$ (nbd = 2,5-norbornadiene) was prepared according to published procedures.[21] 4-Ethynylbenzoic acid was prepared by palladium-catalyzed coupling of trimethylsilylacetylene with methyl 4-bromobenzoate followed by base-catalyzed desilylation, according to our previously published synthetic procedures.[22] The acid was converted to acid chloride **3**, which was used in-situ for the preparation of the valine-PA adduct or 4-ethynylbenzoyl-L-valine methyl ester **5** (see **Monomer Synthesis** below).

Instrumentation. The average molecular weights (M_w and M_n) and polydispersity indexes (PDI; M_w/M_n) of the polymers were estimated by gel permeation chromatography (GPC) using a Waters Associates liquid chromatograph equipped with a Water 510 HPLC pump, a column temperature controller, a Waters 486 wavelength-tunable UV detector, and a Waters 410 differential refractometer. Styragel columns HT3, HT4, and HT6 were used in the GPC system, which covers a molecular mass range of 10^2−10^7 Da. Polymer solutions were prepared in THF (~2 mg/mL) and filtered with 0.45 μm PTFE syringe-type filters before being injected into the GPC system. THF was used as eluent at a flow rate of 1.0 mL/min. The column temperature was maintained at 40 °C and the working wavelength of the UV detector was set at 254 nm. Monodisperse polystyrene samples (Waters) were used as the calibration standards.

The FT-IR spectra were recorded on a Perkin-Elmer 16 PC FT-IR spectrometer. The ^1H and ^{13}C NMR spectra were measured on a Bruker ARX 300 NMR spectrometer in chloroform-*d*, acetone-d_6, methanol-d_4, and/or DMSO-d_6. The deuterated solvents or tetramethylsilane (TMS) were used as the internal references for the NMR analyses. Circular dichroism (CD) measurements were performed on a Jasco J-720 spectropolarimeter in 1 mm quartz cuvettes using a step resolution of 0.2 nm, a scan speed of 50 nm/min, a sensitivity of 0.1°, and a response time of 0.5 s. Each spectrum was the average of 5−10 scans. The molar concentrations of the polymer solutions were calculated on the basis of the repeat units of the polymers.

The AFM samples were prepared by placing tiny amounts (~3−5 μL) of dilute polymer solutions (~1−5 μg/mL) on the new surfaces of freshly cleaved mica under ambient conditions. The morphological structures formed by the polymers upon natural evaporation of the solvents were imaged on a Nano IIIa atomic force microscope (Digital Instruments) operating in a tapping mode using hard silicon tips with a spring constant of ~40 N/m.

Monomer Synthesis. The valine-containing acetylene monomer **5** was prepared by amidation of 4-ethynylbenzoyl chloride (**3**) with L-valine methyl ester hydrochloride (**4**; cf., Scheme 1). Into a 100 mL round-bottom flask were added 1.02 g (6.1 mmol) of **4**, 3 mL of pyridine, and 10 mL of DCM under nitrogen. The contents were mixed by stirring and cooled with an ice bath. A solution of **3** (1.00 g, 6.1 mmol) in 10 mL of DCM was then slowly injected into the flask. The reaction mixture was gradually warmed to room temperature and stirred overnight. The mixture was then diluted with 100 mL of DCM, and the resultant solution was washed twice with dilute HCl solution and once with water. The organic layer was dried over 5 g of magnesium sulfate. After filtration of the solid and removal of the solvent, the crude product was purified on a silica gel column using a mixture of $CHCl_3$/acetone (15:1 by volume) as eluent. Evaporation of the solvents gave 1.03 g of product **5** as white solid (yield: 65.1%). IR (KBr), ν (cm^{-1}): 2105 (m, C≡C), 1738 (s, C═O), 1652 (s, C═O). ^1H NMR (300 MHz, $CDCl_3$), δ (TMS, ppm): 7.7 (m, 2H, aromatic protons *o* to C═O), 7.4 (m, 2H, aromatic protons *m* to C═O), 6.8 (d, 1H, NH), 4.7 (m, 1H, NHC*H*), 3.7 (s, 3H, CO_2CH_3), 3.2 (s, 1H, ≡CH), 2.2 (m, 1H, CH), 0.9 [m, 6H, $(CH_3)_2$]. ^{13}C NMR (75 MHz, $CDCl_3$), δ (TMS, ppm): 172.6 (CO_2), 166.4 (CONH), 134.1 (aromatic carbon attached to C═O), 132.3 (aromatic carbons *m* to C═O), 127.0 (aromatic carbons *o* to C═O), 125.6 (aromatic carbon *p* to C═O), 82.7 (Ph*C*≡), 79.6 (HC≡), 57.5 (NHCH), 52.3 (CO_2CH_3), 31.6 (CH), 19.0, 18.0 [$(CH_3)_2$]. $[\alpha]_D^{20}$ +54° (*c* 0.038, chloroform).

Polymerization. The monomer was polymerized with $[Rh(nbd)Cl]_2$ in Et_3N/THF. Into a 20 mL Schlenk tube were added 0.2 mmol of **5** and 1 mL of THF. The catalyst solution was prepared in another tube by dissolving 0.01 mmol of $[Rh(nbd)Cl]_2$ in 1 mL of THF with a catalytic amount of Et_3N, which was transferred to the monomer solution using a hypodermic syringe. The reaction mixture was stirred at room temperature for 24 h. The mixture was then diluted with 2 mL of THF, and the dilute solution was added dropwise to an acetone/ether mixture (150 mL) under stirring. The precipitate was collected by filtration and dried under vacuum at room temperature to a constant weight. The polymeric product, i.e., poly(4-ethynylbenzoyl-L-valine methyl ester) (**1**), was isolated as yellowish fibrous solid in a high yield (93.2%). M_w: 371 000. M_w/M_n: 7.93 (GPC, polystyrene calibration). IR (KBr), ν (cm^{-1}): 3032 (w, ═CH), 1740 (s, C═O), 1646 (s, C═O). ^1H NMR (300 MHz, acetone-d_6), δ (TMS, ppm): 7.7 (NH), 7.6 (aromatic protons *o* to C═O), 6.8 (aromatic protons *m* to C═O), 6.0 (cis olefin proton), 4.5 (NHC*H*), 3.6 (CO_2CH_3), 2.2 (CH), 1.0 [$(CH_3)_2$]. ^{13}C NMR (75 MHz, acetone-d_6), δ (TMS, ppm): 173.1 (CO_2), 167.7 (CONH), 146.0 (═C−), 139.7 (aromatic carbon *p* to C═O), 134.4 (aromatic carbon attached to C═O), 128.4 (H−C═, aromatic

carbons *o* and *m* to C=O), 59.3, (NHCH), 52.2 (CO_2CH_3), 31.5 (CH), 19.6, 19.3 [$(CH_3)_2$]. UV (THF, 2.20×10^{-4} mol/L), λ_{max} (nm)/ϵ_{max} (mol^{-1} L cm^{-1}): $272/6.67 \times 10^3$, $395/2.32 \times 10^3$. $[\alpha]_D^{20}$: −244.4° (*c* 0.126, chloroform).

Hydrolysis. The valine methyl ester of **1** was partially or completely hydrolyzed under controlled reaction conditions. A typical experimental procedure for fully hydrolyzing the methyl ester to the corresponding free acid is given below. To a 50 mL round-bottom flask were added 200 mg (0.77 mmol) of **1** and a methanolic solution of KOH (2 g KOH in 20 mL of methanol). After 2 h of stirring at room temperature, the mixture was poured into a dilute aqueous HCl solution. The precipitate was collected by filtration and dried under vacuum for at least 2 days to a constant weight. The "polyacid" or poly(4-ethynylbenzoyl-L-valine) (**2**) was isolated as yellowish solid in 95.3% yield. M_w: 408 000. M_w/M_n: 8.47 (GPC, polystyrene calibration). IR (KBr), ν (cm^{-1}): 3032 (w, =CH), 1732 (s, C=O), 1645 (s, C=O). ^{1}H NMR (300 MHz, DMSO-d_6), δ (TMS, ppm): 12.6 (CO_2H), 8.1 (NH), 7.6 (aromatic protons *o* to C=O), 6.7 (aromatic protons *m* to C=O), 5.9 (cis olefin proton), 4.3 (NHC*H*), 2.2 (CH), 0.9 [$(CH_3)_2$]. ^{13}C NMR (75 MHz, CD_3OD), δ (TMS, ppm): 175.1 (CO_2), 169.5 (CONH), 146.7 (=C−), 140.2 (aromatic carbon *p* to C=O), 134.0 (aromatic carbon attached to C=O), 128.7 (H−C=, aromatic carbons *o* and *m* to C=O), 59.9 (NHCH), 31.6 (CH), 19.8, 19.4 [$(CH_3)_2$]. UV (THF, 1.39×10^{-4} mol/L), λ_{max} (nm)/ϵ_{max} (mol^{-1} L cm^{-1}): $272/8.04 \times 10^3$, $395/2.70 \times 10^3$. $[\alpha]_D^{20}$: −423.3° (*c* 0.030, chloroform).

Results and Discussion

Polymer Synthesis. Two approaches have been reported for attaching amino acid pendants to a polyacetylene backbone: (1) by macromolecular complexation of amino acids with a preformed PPA acid[23] and (2) by covalent bonding of amino acid and acetylene moieties at the monomer stage.[24] The former approach enjoys simplicity in polymer preparation, whereas the latter route offers homogeneity in molecular structure. Macromolecular complexation is an addition process, which does not always proceed to completion (with every repeat unit being complexed) due to the involved steric effect.[25] Polymerization of an amino acid−PA adduct, however, affords inherent guarantee that every one of the monomer repeat unit of the resultant polymer carries one amino acid pendant. We thus in this work took the latter approach and synthesized a valine−PA monomer by an amidation reaction.

Using our previously developed synthetic method,[22] we prepared a PPA derivative, 4-ethynylbenzoyl chloride (**3**). Simple coupling of **3** with a commercially available L-valine salt, **4**, gave a valine−PA adduct, **5** (cf., Scheme 1). The ester-capped valine−PA monomer was readily converted by an acetylene polymerization catalyst $[Rh(nbd)Cl]_2$[26−28] to its polymer, **1**, in excellent yield (93%). The polymer exhibited well-defined NMR spectra in polar solvents, an example of which is given in Figure 1A. The resonance peaks well correspond to the expected molecular structure of polymer **1**, there being no any unexpected or unidentifiable signals originating from any side products or impurities. Using a base-catalyzed hydrolysis reaction, we converted "polyester" **1** to "polyacid" **2**. Through optimization of reaction conditions, we succeeded in selectively cleaving the methyl ester groups of **1** while keeping its amide bond unharmed. By changing the reaction conditions (shortening reaction time, lowering reaction temperature, reducing KOH amount, etc.), we can now control the ester hydrolysis to a desirable extent.

Two examples of the ^{1}H NMR spectra of a fully hydrolyzed product, i.e., "polyacid" **2**, are shown in panels B and C of Figure 1. The sharp peak associated with the resonance of the methyl ester protons in **1** in Figure 1A at δ 3.6 (peak *g*) completely disappeared in the spectra of **2**. As its replacement, a broad peak related to the absorption of the newly formed carboxy proton in **2** appeared at δ 12.6 (peak *h*). The resonance signal of the amide proton (peak *c*) is still clearly seen, although it has undergone a slight downfield shift due to the change in its chemical environments caused by the hydrolysis reaction. Comparison of the integrated peak areas confirmed the intactness of the amide proton or the selectivity of the hydrolysis reaction.

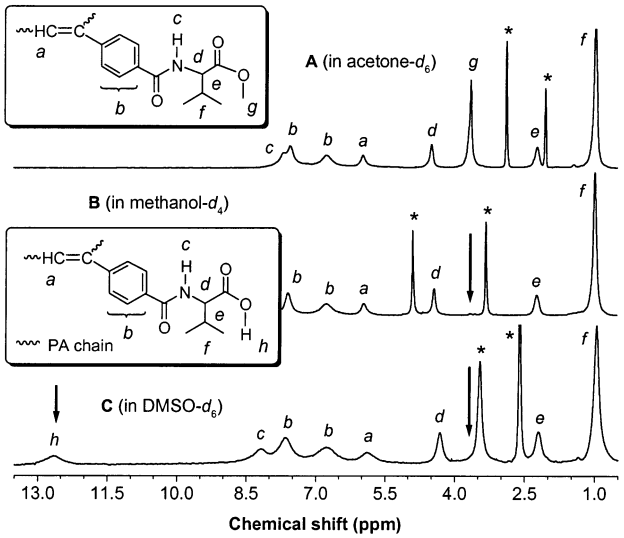


Figure 1. ^{1}H NMR spectra of (A) an acetone-d_6 solution of poly(4-ethynylbenzoyl-L-valine methyl ester) (**1**) and (B) methanol-d_4 and (C) DMSO-d_6 solutions of poly(4-ethynylbenzoyl-L-valine) (**2**). The peaks of the residual nondeuterated solvents and the water dissolved in the deuterated solvents are marked with ∗ symbols.

Chain Helicity. After confirming the molecular structures of the polymers, we investigated their chain conformations by chiroptical spectrometry. While monomer **5** showed weak CD signals in the deep UV region (<280 nm), its polymer (**1**) exhibited strong Cotton effects in the long wavelength region, where its backbone absorbed (Figure 2).[22,29] The CD and UV−vis spectral data thus clearly confirm that the polyacetylene backbone of **1** takes a helical conformation. Similarly, polymer **2** was also CD-active in the low-energy region. Its UV−vis absorption was, however, stronger than **1** over the whole spectral region. This hyperchromism is probably associated with the difference in the chain hydrophilicity caused by the difference in the pendant polarity. In the polar solvent of methanol, the chains of "polyacid" **2**, which are more polar, may be more extended, while those of the less polar "polyester" **1** may be more compactly coiled, although they both take helical conformations. The more extended chains of **2** allow its chiral pendants to be better exposed and enable its polyene backbone to be better conjugated, which in turn enhances the absorptions of its pendants in the UV region and its backbone in the visible.

The chain helicity is obviously induced by the pendant chirality.[12−14,30] The asymmetric force field generated by the chiral pendants may drive the polymer chains to spiral in a screw sense, giving helical segments with different pitches, lengths, and shapes, depending on the backbone conformation[31] (Chart 1A). Hydrogen bonding between the segments of the same and/or different

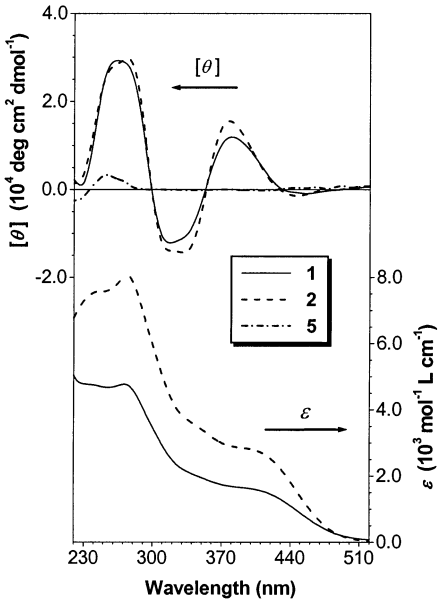


Figure 2. CD and UV−vis spectra of poly(4-ethynylbenzoyl-L-valine methyl ester) (**1**) and (B) poly(4-ethynylbenzoyl-L-valine) (**2**). The CD spectrum of ethynylbenzoyl-L-valine methyl ester monomer (**5**) is shown for comparison. Solvent: methanol. Concentration (mM): ∼1.5 (CD); ∼0.1−0.2 (UV). Temperature: ∼23 °C (room temperature).

chains may compensate the entropic cost paid for the formation of the regular helical structures, examples of such hydrogen bonds being given in panels B and C of Chart 1. The cisoid and transoid backbone conformations can be inter-changed by liable single-bond rotation (atropisomerism),[12h,32] and the entropic balance can be disturbed and shifted to another equilibrium state by breaking the noncovalent hydrogen bonds between the chain segments.[3−5,33] The susceptibility of these processes suggests the possibility of manipulating the chain helicity of the polyacetylene segments by environmental changes or external perturbations.

This proved to be the case. When the solvent of the solution of polymer **1** was changed from methanol to THF, its Cotton effects were intensified and its molar absorptivity was also enhanced (Figure 3A). Methanol is a good solvent for the amino acid pendants but a bad solvent for the PPA backbone. THF is, however, a good solvent for both the pendants and the backbone. The polymer chains thus may take a coiled conformation in methanol but an extended one in THF. The coiled chains may experience complex steric effects and bury some chain segments inside the cores during the folding process induced by the solvophobic effect.[34] The extended chains, on the other hand, may allow the formation of more regular helical structures and better exposure of the chain segments to the photoexcitation, thus leading to the observed higher $[\theta]$ and ϵ values.

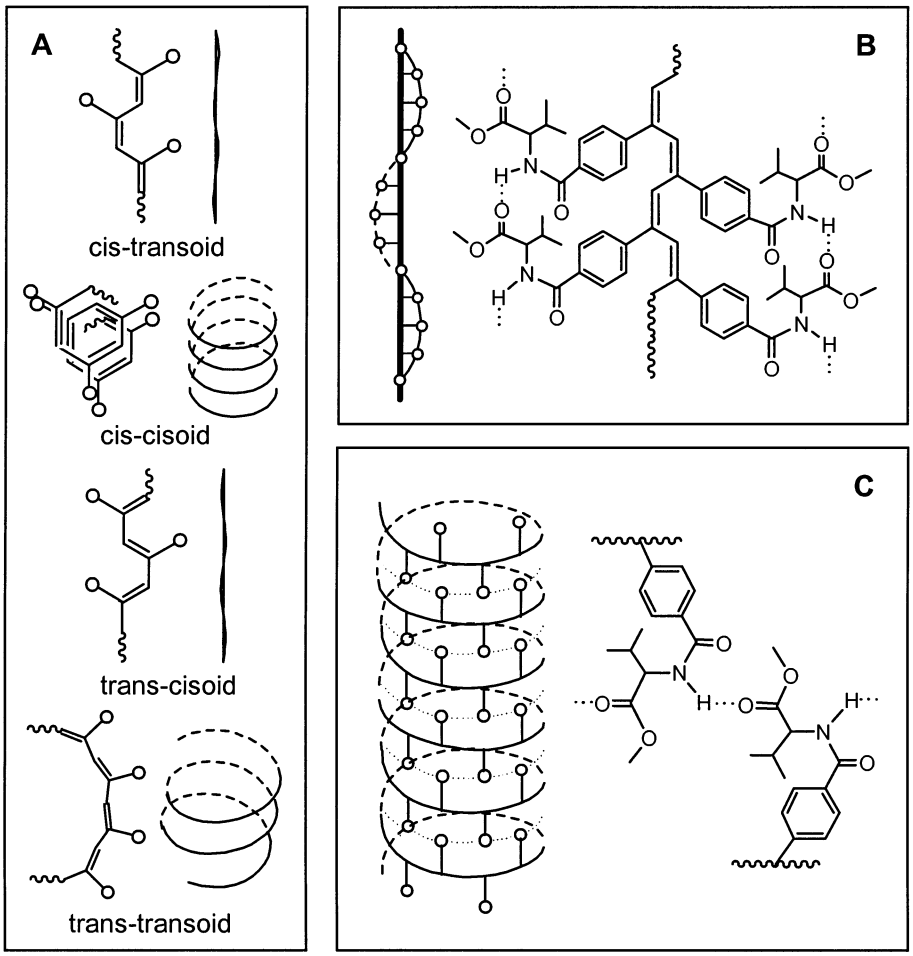
When 1 molar equiv of KOH was added into the methanol solution of polymer **2**, its CD signals were dramatically weakened, while its UV−vis absorptions were slightly enhanced (Figure 3B). When the base is admixed with the "polyacid", its carboxylic protons will be replaced by the potassium ions. The ionization of the carboxyl groups will break the hydrogen bonds (cf., Chart 2 and related discussion). Entropy-driven randomization will destroy the regular helical structures, resulting in the large decrease in the CD activity of the polymer. The ionized polymer chains might be solvated by the polar solvent molecules to a somewhat better extent, thus causing a slight increase in its UV−vis absorptivity.

Supramolecular Assembling. In the structural hierarchy of biomacromolecules, chain helicity belongs to the secondary structure, whose change often brings about variations in their higher-order structures.[1,4−6] We were able to tune the helical structures of our valine-containing PPAs by altering their chain hydrophilicity and environmental conditions, and we further investigated how these internal and external perturbations would affect their assembling behaviors and whether the manipulation could be extended to, or amplified at, the supramolecular level.

When a tiny drop of a dilute methanol solution of "polyester" **1** was placed on newly cleaved mica, pearl-shaped morphological structures were formed upon natural evaporation of the solvent (Figure 4).[35] Under similar conditions, "polyacid" **2** assembled into helical ropes, and a partially hydrolyzed polymer containing both the ester and acid repeat units (∼1:1 in molar ratio) gave an intermediate structure with spherical beads strung up by a filamentary string. In methanol, the coiled chains of "polyester" **1** may pack together to minimize the exposure of its hydrophobic backbones to the polar solvent, forming micellelike structures with their outer shells decorated by the hydrophilic amide and ester functional groups. During the solvent evaporation, the micelles may grow in size and stick together via intershell hydrogen bonding between the amide and ester groups to give the clustered pearls. On the other hand, the strong solvation power of the methanol solvent toward "polyacid" **2**[36] may force the helical chains to take an extended conformation. When methanol evaporates, the individual helical chains may aggregate in a side-by-side fashion via interchain hydrogen bonding to form spirally twisting fibrils, similar to the assembling process followed by fibrous proteins such as keratins and collagen in the formation of twisted cables.[5] The partially hydrolyzed polymer consists of both "polyester" and "polyacid" segments, and it is thus not surprising that the morphological structure formed by the polymer contains both assembling features of **1** and **2**, because the association of the coiled "polyester" segments would give the spherical knobs and spiraling of the extended "polyacid" segments would yield the helical string.

After checking the effect of the structural change (chain hydrophilicity), we examined the effects of the environmental variations (solvent and pH). While the methanol solution of polymer **1** gave pearl-shaped structures upon natural evaporation, its THF solution gave helical cables with a clear left-handed twist under similar assembling conditions (Figure 5). As discussed above (cf., Figure 3A), the polymer chains of **1** may take an extended conformation in THF because THF is a good solvent for both the backbone and the pendants. During the aggregation process accompanying the THF evaporation, the extended helical chains may twine around each other via interchain hydrogen bonding to give twisted strands, further association of which in different multiplicities (doublet, triplet, etc.) will give thicker fibrils of different diameters—this assembling mechanism is clearly suggested by the image shown in Figure 5B. It is envisioned that evaporation of a methanol/THF solution of **1** may generate transit morphologies containing both micellar and fibrillar struc-

Chart 1. Diagrammatic Illustrations of (A) Helical Polyacetylene Chains Induced by Chiral Pendants and (B, C) Hydrogen Bonds between Amino Acid Moieties in Poly(4-ethynylbenzoyl-L-valine methyl ester) (1)



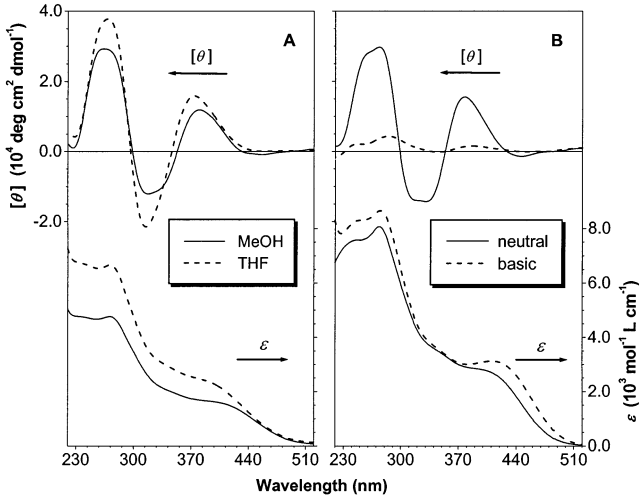


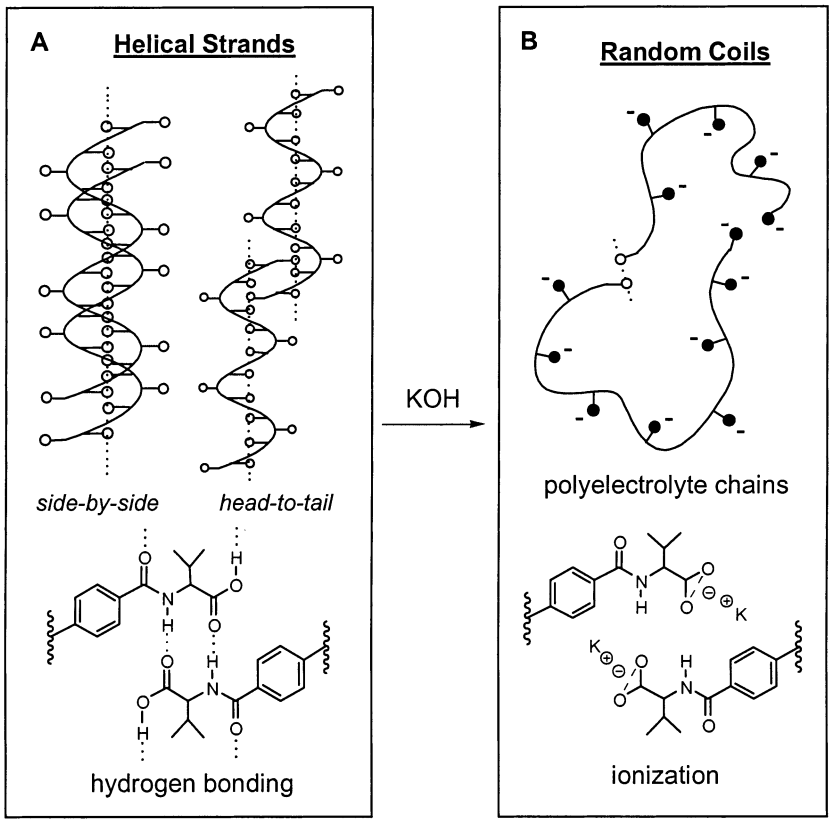
Figure 3. Effects of (A) solvent and (B) pH on the molar ellipticity ([θ]) and molar absorptivity (ϵ) of (A) poly(4-ethynylbenzoyl-L-valine methyl ester) (**1**) and (B) poly(4-ethynylbenzoyl-L-valine) (**2**). Polymer concentration (mM): ~1.5 (CD), ~0.1−0.2 (UV). Temperature: ~23 °C (room temperature). In panel B, the methanol solutions of **2** without and with KOH (1 molar equiv of **2**) are defined as "neutral" and "basic", respectively.

tures. This was indeed the case: as shown in Figure 5C, the organizational morphologies obtained from the mixture solvent system showed combined features of the assembling structures obtained from the individual solvent systems.

The pH effect on the assembling behaviors of polymer **2** is shown in Figure 6. Changing the medium pH from "neutral" to "basic" changed the organizational morphology of the polymer from continuous helical cables to discrete random threads. The helical cables were several tens of nanometers in diameter and up to several hundreds of micrometers in length. The random threads were, however, much thinner and shorter. Their "true" diameters, after subtracting the involved "tip broadening effect" in the AFM measurements,[37,38] were close to the sizes of one or two single polymer chains, but their lengths were, in many cases, still much longer than a single chain. The random threads showed almost no macroscopic screw sense. The AFM images here appeared to be visual representations of the CD spectral data given in Figure 3B. The strong Cotton effects of the methanol solution of **2** were magnified to fibrillary superhelicity via the evaporation-induced self-assembling process, while the random coils with little CD activities in the basic solution hardly organized into any regular morphological structures.

The self-weaving of the helical chains of polymer **2** into the thick, long fibrillar cables is believed to be aided by interchain hydrogen bonding, an example of which is given in Chart 2A, which sketchily illustrates how helical strands are associated through side-by-side and head-to-tail interstrand hydrogen bonding. Ionization of the carboxyl groups by the potassium ions breaks the hydrogen bonds, and entropic chaos randomize the

Chart 2. Schematic Representations of (A) Supramolecular Associations of the Helical Chains of Poly(4-ethynylbenzoyl-L-valine) (2) via Lateral and Terminal Interstrand Hydrogen Bonding and (B) Cleavage of the Hydrogen Bonds by Base-Mediated Ionization of the Carboxylic Acid Pendants and Denaturation of the Helical Strands to Random Coils by Entropy-Driven Randomization of the Polyelectrolyte Chains.



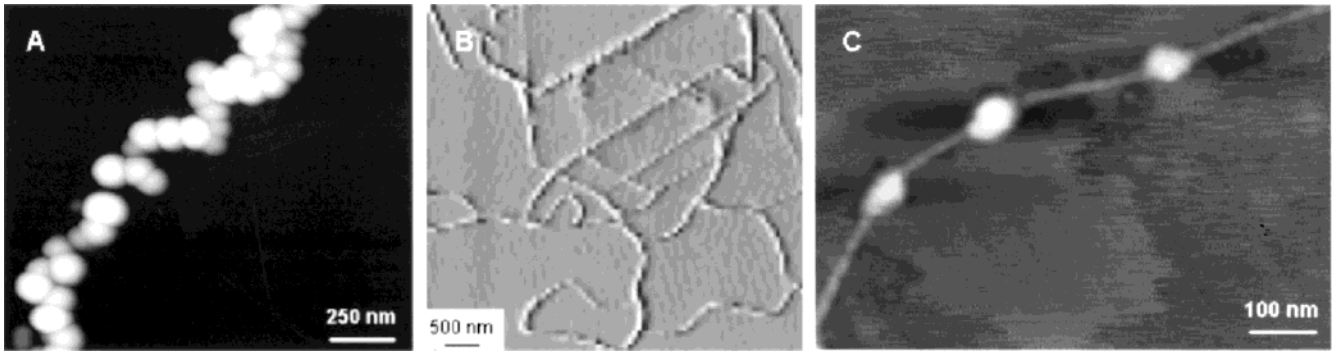


Figure 4. AFM images of (A) clustered pearls, (B) helical cables, and (C) string beads formed upon natural evaporation of methanol solutions of (A) poly(4-ethynylbenzoyl-L-valine methyl ester) (**1**), (B) poly(4-ethynylbenzoyl-L-valine) (**2**), and (C) a partially hydrolyzed **1** {or poly[(4-ethynylbenzoyl-L-valine methyl ester)-*co*-(4-ethynylbenzoyl-L-valine)]} on the surfaces of newly cleaved mica. Scale bars (nm): (A) 250; (B) 500; (C) 100. Concentration: ~1−5 μg/mL. Temperature: ~23 °C (room temperature).

macromolecular chains. The polyelectrolyte chains carrying the same negative charges repulse each other and can hardly aggregate into multistranded cables, which may account for the observed single-chain dimensionalities of the random threads. Some small fractions of the carboxylic groups may survive the ionization due to the steric hindrance and electrical repulsion involved in the polymer reaction, and the chain segments with the un-ionized carboxylic residues may still be able to fold and assemble. When the segmental residues are associated in a head-to-tail fashion, filamentary threads with lengths longer than that of a single polymer chain will be formed (Chart 2B). When the segments are associated in a side-by-side fashion, twisting filaments will be resulted. The knots of the looped threads marked by the arrows in Figure 6B might be the consequence of such segmental braiding.

Concluding Remarks

In this study, we hybridized naturally occurring building blocks with synthetic conjugated polymer chains. We generated the molecular blends by attaching the hydrophilic amino acid pendants to the hydrophobic conjugated PPA backbone. The pendant chirality, chain amphiphilicity, and hydrogen-bonding capability encoded in the primary structure conferred a rich structural hierarchy on the hybrid polymers, enabling the macromolecular chains to take helical conformations and to self-assemble into biomimetic architectural morphologies including micellar spheres and helical cables.

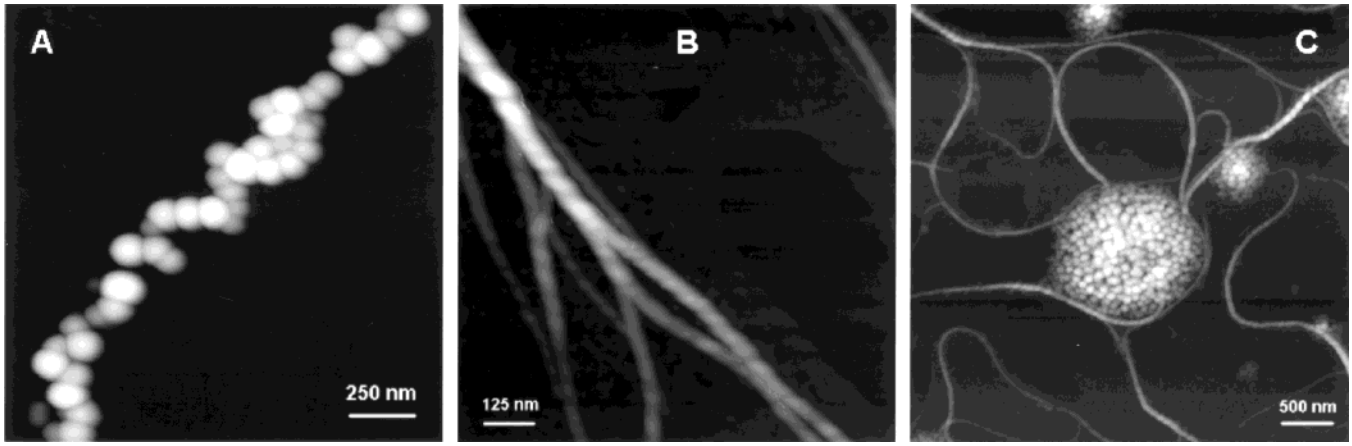


Figure 5. AFM images of (A) clustered pearls, (B) helical cables, and (C) clustered pearls plus helical cables formed upon natural evaporation of (A) methanol, (B) THF, and (C) methanol/THF (1:7 by volume) solutions of poly(4-ethynylbenzoyl-L-valine methyl ester) (**1**) on the surfaces of newly cleaved mica. Scale bars (nm): (A) 250; (B) 125; (C) 500. Concentration of polymer solutions: ~1−5 μg/mL. Temperature: ~23 °C (room temperature).

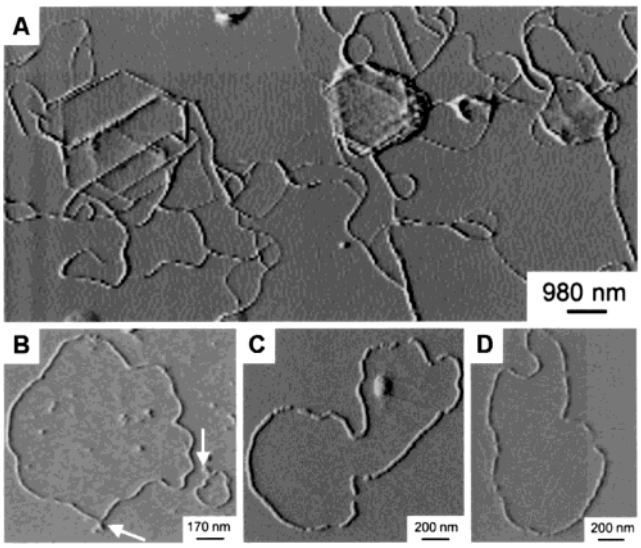


Figure 6. AFM images of (A) helical cables and (B−D) random threads formed on natural evaporation of (A) "neutral" and (B−D) "basic" methanol solutions of poly(4-ethynylbenzoyl-L-valine) (**2**) on the surfaces of newly cleaved mica. The "basic" solution was prepared by adding 1 equiv of KOH (relative to the molar amount of **2**) to the "neutral" solution of the polymer in methanol. Scale bars (nm): (A) 980; (B) 170; (C, D) 200. Polymer concentration: ~1−5 μg/mL. Temperature: ~23 °C (room temperature).

The chain helicity (secondary structure) and the organizational morphologies (higher-order structures) changed with the variations in the pendant hydrophilicity, solvent polarity, and medium pH,[39] suggesting a proteomimetic adoptability[40] and demonstrating the tunability of the hierarchical structures by internal perturbations and external stimuli.[39]

Research on self-assembling of macromolecular species has been so far focused on amphiphilic block copolymers. Preparations of such copolymers are, however, nontrivial tasks and often involve the use of synthetically demanding living polymerization techniques. The amphiphilic homopolymers used in this study were readily prepared by a simple polymerization procedure. (Indeed, the polymerization can even be carried out in open air using water as solvent.[21,41]) This synthetic advantage offers a versatile tool for the construction of amphiphilic macromolecules and has enabled us to prepare a wide variety of helical polyacetylenes containing naturally occurring species including not only different amino acids but also various saccharides and nucleosides.[41,42]

The novel structural feature of our hybrid polymers is that a conjugated polyacetylene backbone is wrapped up with a coat of naturally occurring pendants. Cytotoxicity assays reveal that all the polymers are cytocompatible.[41] Some monosaccharide-containing polyacetylenes can even stimulate the growth of living HeLa cells when the polymer solutions are added into the culture media or when the polymer films are precoated on the microtiter plates.[41,43] We are currently exploring the exciting possibility of utilizing our biomimetic polymers as biocompatible nanowires for cytotech, especially bioelectronics, applications.

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