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Encapsulation of Polythiophene by Glycopolymer for Water-soluble Nanowire

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A water-soluble polythiophene (PT) was prepared by the self-assembling complex with a glycopolymer. The glycopolymer of poly(*N*-*p*-vinylbenzyl-D-lactonamide) (PVLA) formed self-assembling cylindrical structure based on the amphiphilicity even after the complexation with PT. We confirmed the improved optical functionality of PT due to the longer conjugated π-orbital. It suggested that PT behaved like molecular nanowire with the self-assembled structure in the hydrophobic core of PVLA. PVLA–PT also showed specific biorecognition against corresponding lectin. These results suggested that the bioactive nanowire formation of PT with the glycopolymer was developed.

Many kinds of oligosaccharides on cell surfaces play important roles as a bioactive ligand with various interactions such as cell–cell interaction. These bioactivities of saccharide have received much attention, and have been applied to create new functional biomaterials. Poly(*N*-*p*-vinylbenzyl-D-lactonamide) (PVLA) is a well-known biomaterial for hepatocyte culture and lectin recognition.[1] PVLA has densely packed saccharide structure along the polymer back bone, and shows strong molecular recognition with sugar recognition proteins (lectins) and cells including hepatocyte.[2]

PVLA has amphiphilic structure, where hydrophilic saccharide units are attached to every repeating unit along the hydrophobic polymer backbone. The amphiphilic structure of glycopolymer induces itself to form a unique structure in aqueous solution owing to the self-assembling properties shielding a hydrophobic core inside. The structure is similar to the polysaccharide of helical structures like amylose and schizophyllan, which often form supramolecular conjugate materials with excellent functionality. It has been reported that these cylindrical polysaccharides form supramolecules with incorporating hydrophobic compounds such as fluorophores, quantum dots and carbon nanotubes.[3]

PVLA has suitable properties for supramolecular complex formation in terms of amphiphilic molecular structure, and in fact, the stable complex formation between PVLA and 8-anilino-1-naphthalenesulfonic acid (ANS) has reported previously.[4] Furthermore, PVLA and its analogs have been reported to form cylindrical conformation in water, and are suggested to have highly regulated hydrophobic space.[5] Therefore, PVLA was expected to form well-ordered complexes with hydrophobic molecules even as hydrophobic polymer with random structure.

Polythiophene (PT) derivative is known as a colored conductive polymer, having responsiveness to external stimuli such as light irradiation, temperature, solvated state, and voltage change due to the change of electron state.[6] These electrical functionalities of PT are dependent on conjugated π orbitals, but the aggregative properties of PT often interfere with π orbitals, and reduce functionality. Therefore, planar PT nanowire is desirable material in terms of electrical functionalities such as fluorescence and conductivity,[7] and stereoregulation of PT chain is necessary to obtain superior conductive materials with long conjugated π-orbitals.

In this research, the supramolecular formation of PVLA with PT in water, and its properties were investigated, where the PT used was poly{3-[2-(*N*-octadecylcarbamoyloxy)ethyl]thiophene} (thiophene polymer, ADS518PT) (American Dye Source, Inc., Quebec, Canada). As mentioned above, a cylindrical structure of PVLA is a hydrophobic nanoscale core in water, and can afford the incorporation of PT, based on the structure of PVLA. The conjugation of PT with PVLA provided the advanced optical functionality of PT due to its conformation for the PT nanowire. In addition, PVLA shows biorecognition of lectins, which can contribute to the hierarchical assembly of PVLA–PT nanowire to fabricate functional materials. PVLA–PT can be applicable as a biosensor due to the biorecognition ability of PVLA and optical properties of PT.

PVLA was synthesized via a conventional method reported by Kobayashi et al.,[1] and molecular weights were 2.14×10^5 (M_n) and 2.75×10^5 (M_w) respectively, using gel permeation chromatography with pullulan standard (Figure 1).

Initially, the amphiphilicity of PVLA was investigated by hydrophobic ANS fluorophore indicator to investigate supramolecular formation. Fluorescence intensity was largely increased and the emission wavelength of fluorophore was blue-shifted

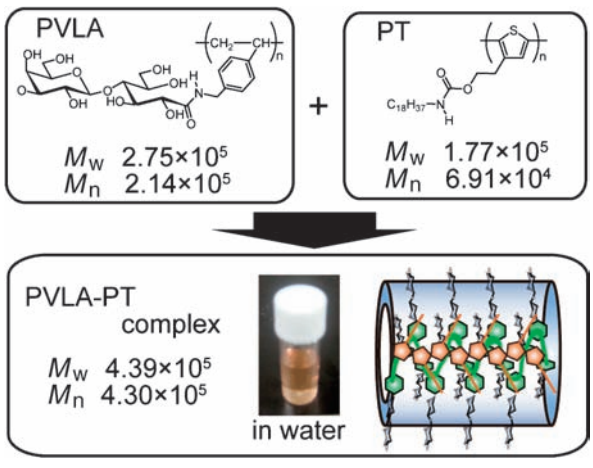

Figure 1. Molecular structure and complex formation model of polymers.

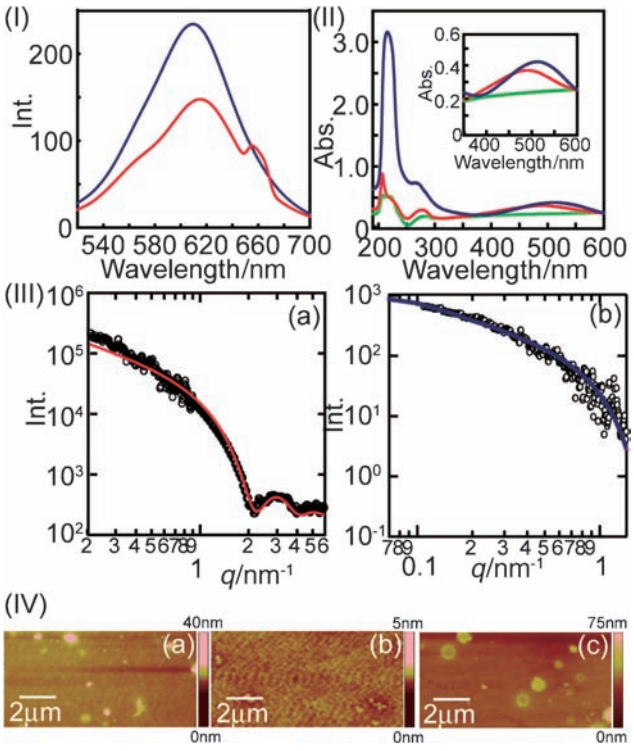

Figure 2. Characterization of PVLA–PT complex. (I) Fluorescence spectra of PT (red line) and PVLA–PT (blue line) in 10% THF aqueous solution. (II) UV–vis absorption spectra of PVLA (green line), PT (red line), and PVLA–PT (blue line) in 10% THF aqueous solution. (III) The solution X-ray scattering profile of (a) PVLA (SAXS and WAXS) and PVLA–PT (SAXS) (b). Each circle denoted experimental SAXS data, and each line indicated cylinder model fittings with parameter of $r = 1.6$ nm and $h = 40$ nm (a), $r = 2.2$ nm and $h = 40$ nm (b). (IV) Height images of PT (a), PVLA (b), and PVLA–PT (c) on mica.

from 530 to 470 nm. This indicated that PVLA held the hydrophobic space in itself and incorporated the hydrophobic fluorophore there because the fluorescence is known to be increased in hydrophobic space.[8]

Additionally, the incorporation capability was evaluated by the addition of other hydrophobic molecules such as naphthalene and anthracene with UV–vis absorption spectroscopy, and pyrene with fluorescence emission and UV–vis absorption spectroscopy.[8] The above experimental results demonstrated the possibility of PVLA as a container or carrier for molecular nanowire or drug delivery systems.

Subsequently, PVLA–PT mixtures were prepared by the addition of 1.03 mM PT in THF into 7.35 mM aqueous PVLA solution at the rate of PT solution:PVLA solution = 9:1 (v:v) under stirring (monomer unit concentration). Mixed solution was purified using a dialysis membrane (MWCO. 8000) for a day, changing the solvent of water. Then, PVLA–PT solution was freeze-dried to yield slightly red powder. This complex was readily resolubilized in distilled water regardless of existence of hydrophobic PT.

PVLA–PT complex was confirmed by NMR spectrum in deuterated water,[8] and M_n and M_w were increased to 4.30×10^5 and 4.39×10^5, respectively.

PVLA, PT, and the complex were investigated by fluorescence and UV–vis spectroscopies in detail (Figure 2(I) and 2(II)), resolubilized in 10% THF aqueous solution for the comparison of intensity increase under the same solvent surroundings. Fluorescence intensity of PVLA–PT around 610 nm was 1.6 times larger than that of precursor PT at the same concentration. Simultaneously, fluorophore emission was blue-shifted from 615 nm of PT to 609 nm. In addition, UV–vis absorption intensity of PVLA–PT was wholly larger than that of PT around 370–600 nm, and absorption wavelength of PVLA–PT was red-shifted from 490 nm of PT to 513 nm in this region. This suggested that the blue shift in fluorescence emission and the red shift with UV–vis absorption with PVLA–PT were caused by extension of the conjugated π orbital and incorporation in hydrophobic space. This assumption was accounted for by PVLA conjugated PT keeping PVLA conformation undisturbed, and then PT expanding a conjugated π orbital longer in the hydrophobic space of PVLA than precursor PT.

Subsequently, the conformations of PVLA and PVLA–PT in water was further characterized by small-angle X-ray scattering (SAXS) (Figure 2(III)). The SAXS measurement was carried out using each sample solution at room temperature for 5 min. The SAXS scattering profile was corrected for the background scattering from pure water. Based on a previous SAXS results for PVLA in water,[5] we assumed that PVLA and PVLA–PT also has a cylindrical conformation in water. The best-fit of a cylindrical form factor with a radius (r) and length (h) to the data in PVLA–PT gave us $r = 2.2 \pm 0.2$ nm and $h = 40 \pm 4$ nm, which are in good agreement with those of PVLA in water ($r = 1.6$ nm and $h = 40$ nm. Therefore, the two independent spectroscopic experimental results may lead to the important conclusion that the conformation of PVLA remains unchanged even after the PT conjugation.

A topographic image of the PVLA–PT conjugate was observed by AFM (Figure 2(IV)). PVLA–PT conjugate formed a spherical aggregate 0.71–0.82 μm diameter. On the other hand, PVLA and PT were amorphous aggregate with the width of 0.18–0.29 and 0.15–0.29 um, respectively. In addition, the height image provided the structural information of aggregate, and the spherical aggregate of PVLA–PT was thickly edged and was vacant in the center of the aggregates.

Biological functionality of PVLA–PT was investigated by recognition analyses with peanut lectin (PNA) having specificity for lactose.[9] Two-dimensional immunodiffusion in agar showed that PNA interacted with both PVLA and PVLA–PT,[10] in contrast to references because of the presentation of precipitation lines. On the other hand, Con A showed little recognition with all samples involving PVLA and PVLA–PT.[8]

Next, the interaction between PVLA–PT and PNA was quantified by fluorescence quenching assay (Figure 3). The fluorescence change based on the PNA concentration was evaluated by the Scatchard plot (Figure 3(II)), and the association constant between PVLA–PT and PNA was calculated 2.89×10^9 (M^{-1}), based on the tilt of line in the plot.[11] This value was very high, and was accounted for the multivalent interaction of lactose with PNA.

In this study, we demonstrated a self-assembled supramolecular complex of PT derivative using hydrophobic space of PVLA. PVLA–PT complex had not only water solubility but also superior optical functionality because PT formed long

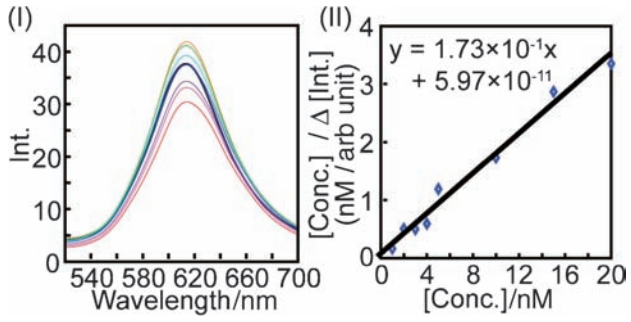

Figure 3. (I) Fluorescence intensities of PVLA–PT (black bold line) without PNA and PVLA–PT with 1 (orange), 3 (green), 5 (right blue), 10 (blue), 15 (purple), 20 (pink), and 30 nM PNA (red). (II) Scatchard plot for the calculation of the association constant K_A, based on the intensity around 614 nm with PNA addition.

conjugated π orbitals without self-quenching for enclosure by the cylinder of amphiphilic polymer. The complex also had biorecognition capability depending on the lactose ligand. We believe that the incorporation functionality of PVLA was important for the development of not only biomaterial for biosensors, tissue engineering, and drug delivery systems, but also the covering material of organic molecular nanowire for electronic devices in terms of improvement of optical functionality.

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