Parallel β-sheet as a Novel Template for Polymerization of Diacetylene

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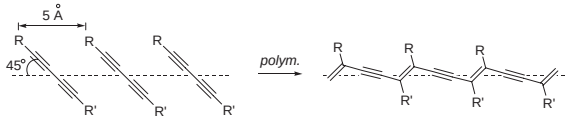
The peptide having diacetylene at its side group formed parallel β-sheet structure in crystalline solid, and solid-state polymerization of the diacetylene groups successfully occurred by UV irradiation or heating of the crystal.

Diacetylenes undergo radical polymerization in assemblies such as single-crystal, vesicle, monolayer, Langmuir–Blodgett film, and liquid crystal.[1–4] The polymerization is induced by heating or irradiation of UV, γ- and X-ray. The polydiacetylenes thus obtained are π-conjugated polymer so that they have received much attention as conductive and non-linear optical materials.[1–4] A necessary condition for the solid-state polymerization of diacetylenes is that the diacetylene groups are spaced at ca. 5 Å with a 45°-declination angle.[1a]



So far, many diacetylene derivatives have been synthesized for the crystalline-state polymerization.[1a,2] Most of them have symmetric structure, and have aromatic groups and urethane bonds at both sides so that an aromatic stacking interaction and a hydrogen bonding can space the diacetylene group with 5-Å distance.[1a,2] We assumed that the parallel and *anti*-parallel β-sheet structures which are one of the secondary structures of peptide are worth paying attention because, in these structures, hydrogen bonding arises between the amide bonds of the peptide chains to arrange them with a distance of ca. 5 Å as shown in Figure 1.[5] Because, in the parallel β-sheet structure, a distance between side groups (R) is also ca. 5 Å (Figure 1a), the structure will be applicable to template for polymerization of diacetylene when diacetylene group is introduced in the side group of peptide. On the other hand, in the *anti*-parallel β-sheet structure, two different distances appear alternatingly (Figure 1b) and both of them will not suit for the polymerization of diacetylene.

To confirm the applicability of parallel β-sheet motif to the polymerization of diacetylene, in this study, we synthesized peptides with amide groups at both N- and C-termini (Figure 2; **1** and **2**) and investigated their reactivity in solid-state. Figure 2 shows the polymerization scheme of **1** or **2** aimed in this study. The compounds **1** and **2** were synthesized from Boc-L-Tyr-OH and Boc-L-Thr-OH, respectively, by six-step reactions.[6] Although **1** and **2** were crystallized from methanol to obtain needle-like pale yellow crystals, the size of the crystals was not large enough to conduct a single-crystal X-ray crystallographic analysis. Thus, we measured FT-IR spectrum to obtain information about the arrangement of the molecules in the crystals. The amide I band (1600–1700 cm^{-1}), which represents primarily the

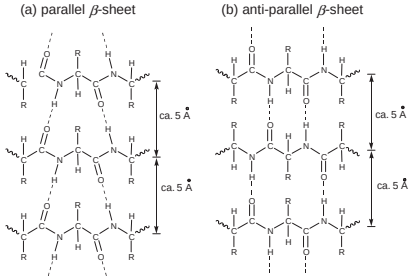


Figure 1. Structural models of parallel (a) and *anti*-parallel (b) β-sheets.

C=O stretching vibrations of the amide groups coupled to the in-plane N–H bending and C–N stretching modes,[7] is useful for an analysis of secondary structure of peptide. It is known that both the parallel and *anti*-parallel β-sheet structures show absorption at around 1630 cm^{-1} as amide I band, whereas *anti*-parallel β-sheet has an additional weak absorption at around 1690 cm^{-1}.[8] Figure 3 shows the IR spectra of the both crystals in amide I and II regions. **1** and **2** have maximum absorption at 1640 and 1637 cm^{-1}, respectively, and **2** has weak absorption at 1682 cm^{-1}. These spectral patters show that **1** and **2** form parallel and *anti*-parallel β-sheets in the crystalline solid, respectively. So far, the crystal structures of some *N*-acetyl-L-amino acid methylamides have been determined, and the both parallel and *anti*-parallel β-sheet structures have been found depending on the kind of amino acids.[9] Because *N*-acetyl-L-phenylalanine-*N*-methylamide, which has a similar structure of **1** except for having no alkyl ether substitunent at *p*-position, forms ideal parallel β-sheet structure,[9] the compound **1** would also form that structure.

Then, we applied UV light (high pressure mercury lamp) to the crystals to polymerize the diacetylene group. Figure 4a shows UV–vis reflection spectrum of crystal **1** obtained after 6-h UV irradiation. There was absorption maximum at 590 nm

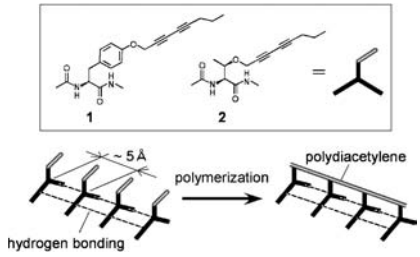


Figure 2. Chemical structures of peptides **1** and **2** (above) and schematic representation of polymerization of the peptide (below).

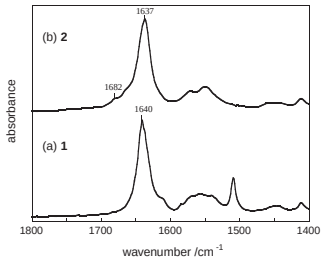


Figure 3. FT-IR spectra of amide I and II regions of crystals of **1** (a) and **2** (b).

with a shoulder at 555 nm, and this corresponds to typical absorption spectrum of the polydiacetylene crystal.[1a] On the other hand, UV–vis spectrum of **2** did not change after UV irradiation, and thus the polymerization of **2** did not proceed. The difference in polymerization reactivities of **1** and **2** should result from the difference in the crystal structures of the peptides.

The polymerization of **1** also proceeded by incubating the crystal at 150 °C in N_2 atmosphere. After 72-h incubation, absorption resulting from diacetylene group (2254 cm^{-1}) almost disappeared, indicating the almost complete conversion of diacetylene groups. The powder X-ray diffraction pattern of polymerized **1** was different from that of monomer crystal and the diffraction peaks of the polymerized **1** were as sharp as that of the monomer crystal (data not shown). These facts prove that polymerization reaction of **1** proceeds via a crystal-to-crystal process. The polymerized **1** is insoluble for common organic solvents except for trifluoroacetic acid and dichloroacetic acid (DCA). These solvents are known as good solvents for peptide because of their strong hydrogen bonding ability to peptide amide bond. Thus, the limited solubility of the polymerized **1** indicates that there is a strong hydrogen bonding interaction among the monomeric units of the polydiacetylene along the polymer strand as illustrated in Figure 2.

The polydiacetylene solution shows solvatochromism (Figure 4b). In the yellow colored DCA solution, there was an absorption maximum at 470 nm. When the non-solvent (methanol) content was 40 vol %, the absorption maximum red-shifted to 540 nm and a shoulder appeared at 500 nm, and then the color of the solution was red. The color change with increasing methanol content is probably due to revival of hydrogen bonding among the neighboring monomeric units along the polymer chain so that the effective conjugation system grows longer.

In conclusion, we succeeded in the application of the parallel β-sheet motif to the polymerization of diacetylene. The amino acid of **1** will be applicable to the stabilization of protein conformation by cross-linking reaction (polymerization) among the amino acids incorporated in a parallel β-sheet region of a protein. Moreover, if we apply the system for polypeptide, we can obtain nano-composite material which is composed of polypeptide and polydiacetylene.

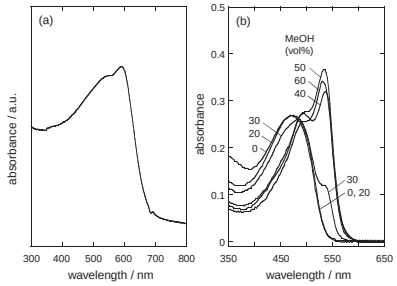


Figure 4. (a) UV–vis reflection spectrum of polymerized crystal of **1**, and (b) UV–vis absorption spectra of polymer **1** (0.03 mg/mL) in dichloroacetic acid/methanol mixed solvents.

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6 **1** was synthesized by following procedure. Boc-L-Tyr-OH was reacted with propargyl bromide to obtain Boc-*O*-2,4-octadiyn-1-yl-L-tyrosine, and the obtained compound was reacted with 1-bromo-1-pentyne to obtain Boc-*O*-2,4-octadiyn-1-yl-L-tyrosine. The resulting compound was introduced with acetyl group at N-terminus and methyl amide group at C-terminus, respectively and then **1** was obtained as a light sensitive needle-like white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (t, $J = 7.3$, 3H, CH_3CH_2), 1.54 (m, 2H, CH_3CH_2), 1.96 (s, 3H, $COCH_3$), 2.25 (t, $J = 7.1$, 2H, CH_2), 2.70 (d, $J = 4.9$, 3H, $NHCH_3$), 2.98 (m, 2H, β-CH_2), 4.59 (m, 1H, α-CH), 4.71 (s, 2H, OCH_2), 6.30 (q, $J = 4.9$, 1H, $NHCH_3$), 6.71 (d, $J = 8.0$, 1H, α-CHNH), 6.88 (d, $J = 8.5$, 2Ar-H, *m* to β-CH_2), 7.13 (d, $J = 8.5$, 2Ar-H, *o* to β-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.4, 21.2, 21.6, 23.1, 26.1, 37.8, 54.9, 56.4, 64.4, 70.1, 72.2, 81.9, 114.9, 129.7, 130.2, 156.4, 170.0, 171.6. **2** was synthesize from Boc-L-Thr-OH by the similar procedure and obtained as a light sensitive white solid. Mp 124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (t, $J = 7.3$, 3H, CH_2CH_3), 1.06 (d, $J = 6.4$, 3H, γ-CH_3), 1.50 (m, 2H, CH_2CH_3), 1.99 (s, 3H, $COCH_3$), 2.19 (t, $J = 6.8$, 2H, $\equiv CCH_2$), 2.77 (d, $J = 4.9$, 3H, $NHCH_3$), 4.04 (m, 1H, β-CH), 4.24 (m, 2H, OCH_2), 4.42 (m, 1H, α-CH), 6.48 (d, $J = 4.2$, 1H, α-CH_2NH), 6.56 (d, $J = 6.8$, 1H, $NHCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.4, 15.5, 21.2, 21.6, 23.2, 26.3, 56.2, 57.8, 64.4, 71.3, 71.5, 74.6, 81.6, 169.5, 170.2.

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