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Single molecule of a π-conjugated polymer slowly twinkles in solution at room temperature

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Abstract

A phenylacetylene monomer, 4-(decyloxycarbonyl)phenylacetylene (DecCPA), was synthesized and polymerized using the combined catalyst system, i.e. $[Rh(norbornadiene)Cl]_2$ and triethylamine, to give a yellow solid π-conjugated polymer [poly(DecCPA)] of high molecular weight and *cis*-transoidal stereo-regular main chain. Poly(DecCPA) was measured using the total internal reflection fluorescence microscopy, which is a single molecule detection technique that is carried out in a tetrahydrofuran solution at room temperature with the light-twinkling of a single molecule being observed. This twinkling is a dynamic photon emission phenomenon of single poly(DecCPA) molecule that we mention specifically as a resultant from the time scale — changing very slowly in the time scale of milliseconds and seconds — with the changed fluorescent intensity being associated with the changing single polymer molecule conformation caused by the micro-Brownian motion. Although molecular dynamics was of the picosecond time scale, generally, this poly(DecCPA) molecule had the mode of the molecular motion of π-conjugated main chain. This was very slow in the second time scale as was shown. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Single molecule detection; π-Conjugated polymer; Near-field

1. Introduction

What function does a synthetic polymer have in the single-molecular-level? Does it have such a dynamic multi-function as protein, which is a kind of biopolymer? Mankind has not yet understood the fundamental properties of synthetic polymers and hence cannot still bring out the maximum of capabilities of them.

Polymer is a highly capable, useful and indispensable material. Conjugated polymers, investigated as advanced materials for photonic and electronic applications, have made progress, and have caused an ever-increasing interest from the view point of both academic research and industrial development [1,2]. The conjugated polymers are used as laser dyes [3], organic light-emitting diodes [4], and so on. In addition, they are also used as an optical resolution membrane material [5–8].

Although conjugated polymers are very interesting and important materials, the mechanism in single-molecular-level of these photonic and electronic functions are still not clear owing to the varied and complicated structure of the conjugated system in the polymer molecules. Thus, the polymers are a complex system of molecules. Therefore, if direct observation of a single molecule of conjugated polymer were achieved, the polymer function at the single molecule unit, i.e. the single-molecule device would be clear in the molecular level.

On the other hand, imaging of single protein molecules using fluorescence microscopy has made remarkable progress after the development of single fluorophore imaging methods, which were used for specimen on an air-dried surface [9]. Single fluorophores in aqueous solution have been imaged using a total internal reflection fluorescence microscope (TIRFM), in which the background fluorescence is greatly reduced [10,11], when a laser beam is totally reflected in the interface between the glass surface and solution, illumination is limited to only the vicinity near the glass surface. In our previous study, this single molecule imaging method was extended to the organic polymer solution system for the direct observation of single molecules of photofunctional rigid-rod conjugated polymer that is a poly(aryleneethynylene) having anthracene units in the main chain [12,13].

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In this study, we report the single molecule imaging of a stereo-regular π-conjugated polymer and the detection of a single molecule slow-twinkling of the photoluminescence in tetrahydrofuran solution at room temperature for the first time.

2. Experimental

2.1. Measurements

^{1}H NMR spectra were measured on a Bruker DPX-400 (400 MHz). GPC was analyzed using Toso TSKgel GMH_{HR}-H(S) columns and a Shimadzu SPD-10AVP UV detector (254 nm). IR spectra were measured on a Shimadzu FTIR-4000 spectrophotometer and UV–Vis spectra on a Shimadzu UV-260 spectrophotometer. Emission spectra were obtained with a Hitachi F-2500 spectrofluorophotometer.

2.2. Materials

Dichloromethane (Kanto Chemical) was distilled over phosphorus pentachloride (Wako Chemicals), chloroform (Kishida Chemicals) over calcium chloride (Wako Chemicals), triethylamine (Kanto Chemicals) over calcium hydride and tetrahydrofuran (THF, Kishida Chemicals) was distilled over sodium. 4-Bromobenzoic acid (Tokyo Kasei), 1-decanol (Kanto Chemicals), 2-methyl-3-butyne-2-ol (Lancaster), and the catalyst system were obtained commercially.

2.3. Synthesis

Synthesis of poly(DecCPA) was shown in Scheme 1. All the following reaction procedures were conducted under dry nitrogen.

2.3.1. 4-Decyloxycarbonyl-1-bromobenzene (1)

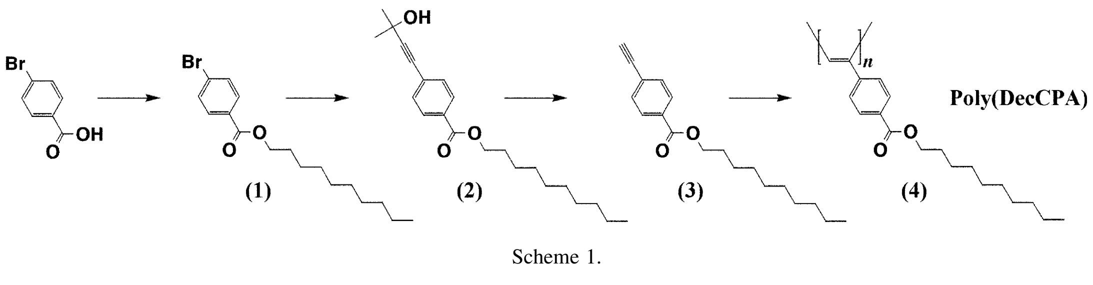
To a solution of 4-bromobenzoic acid (4.27 g, 21.2 mmol) in dry dichloromethane (90 ml) was added 1-decanol (3.51 g, 22.0 mmol), followed by 4-(dimethylamino)pyridine (DMAP) (248 mg, 2.03 mmol). Dicyclohexylcarbodiimide (DCC) (4.78 g, 21.7 mmol) was added and stirred at room temperature under nitrogen until the reaction was complete. The reaction mixture was filtered to remove the precipitated dicyclohexylurea and was evaporated to dryness under reduced pressure. The crude product was purified by silica gel chromatography eluted with hexane/ethyl acetate (80/20 v/v) to give **1** (TLC $R_f = 0.78$). The product was a pale-yellow liquid. Yield: 63.2%; ^{1}H NMR ($CDCl_3$, TMS) δ 7.90 (d, 2H, 2C*H* in phenyl), 7.58 (d, 2H, 2C*H* in phenyl), 4.30 (t, 2H, OCH_2), 1.76 (m, 2H, $-OCH_2CH_2-$), 1.43 (m, 2H, CH_2CH_3), 1.27 (m, 12H, $-(CH_2)_6-CH_2CH_3$), 0.88 (t, 3H, $-CH_3$); IR (cm^{-1}, NaCl) 2930 (C–H), 1720 (C=O), 1275 (C–O).

2.3.2. Decyl 4-(3-hydroxy-3-methylbut-1-ynyl)benzoate (2)

In triethylamine (100 ml) were dissolved **1** (11.0 g, 32.3 mmol), 2-methyl-3-butyn-2-ol (5.09 g, 60.5 mmol), dichlorobis(triphenylphosphine)palladium (25.3 mg, 3.60×10^{-2} mmol), cuprous acetate (29.1 mg, 1.60×10^{-1} mmol), and triphenylphosphine (53.6 mg, 2.04×10^{-1} mmol). The solution was stirred for 24 h at the reflux temperature. The resulting salt was removed by filtration and the solvent of the filtrate was evaporated to yield a crude product, which was purified by silica-gel chromatography using hexane/ethylacetate (80/20 v/v) as an eluent (TLC $R_f = 0.49$). The product was a yellow viscous liquid. Yield: 98.9%; ^{1}H NMR ($CDCl_3$, TMS) δ 7.97 (d, 2H, 2C*H* in phenyl), 7.46 (d, 2H, 2C*H* in phenyl), 4.31 (t, 2H, $-OCH_2-$), 2.04 (s, 1H, $-OH$), 1.77 (m, 2H, $-OCH_2CH_2-$), 1.63 (s, 6H, $(CH_3)_2C-$), 1.44 (m, 2H, $-CH_2CH_3$), 1.27 (m, 12H, $-(CH_2)_6-CH_2CH_3$), 0.89 (t, 3H, $-CH_3$ in decyl); IR (cm^{-1}, NaCl) 3400 (O–H), 2850 (C–H), 1720 (C=O), 1260 (C–O).

2.3.3. 4-(Decyloxycarbonyl)phenylacetylene (3)

Sodium hydride (60 wt%, 690 mg, 17.3 mmol) was added to a toluene solution (120 ml) of **2** (11.0 g, 32.0 mmol), and the mixture was heated to 105°C with stirring for 2 h. After the usual work up, the product was purified with silica-gel chromatography using toluene/hexane (50/50 v/v) as an eluent (TLC $R_f = 0.50$). Yellow liquid was obtained in 53.4% yield. ^{1}H NMR ($CDCl_3$, TMS) δ 7.99 (d, 2H, 2C*H* in phenyl), 7.55 (d, 2H, C*H* in phenyl), 4.31 (t, 2H, OCH_2), 3.22 (s, 1H, $C\equiv CH$) 1.76 (m, 2H, $-OCH_2CH_2-$), 1.43 (m, 2H, $-CH_2CH_3$), 1.27 (m, 12H, $-(CH_2)_6-CH_2CH_3$), 0.88 (t, 3H, $-CH_3$); IR (cm^{-1}, NaCl) 3250 (H–C), 2960



Scheme 1.

(C–H), 2100 (C≡C), 1700 (C=O), 1275 (C–O). Anal. calcd for –($C_{19}H_{26}O_2$)–: C, 79.68; H, 9.15. Found: C, 79.95; H, 9.61.

2.3.4. Poly[4-(decyloxycarbonyl)phenylacetylene] [poly(DecCPA)] (4)

To the monomer **3** (172 mg, 6.01×10^{-1} mmol) in chloroform (2.50 ml) was added the (bicyclo[2.2.1]hepta-2,5-diene)chlororhodium (I) dimer {[Rh(norbornadiene)Cl]$_2$} (0.80 mg, 1.7×10^{-3} mmol) in dry triethylamine (0.50 ml), and the solution was stirred for 4 h at room temperature. The polymerization mixture was poured into methanol, and the polymer was purified by reprecipitation from chloroform solution into methanol and then dried in vacuo for 24 h. Yellow solid was obtained in 88.4% yield. ^{1}H NMR ($CDCl_3$, TMS) δ 7.62 (br, 2H, C*H* in phenyl), 6.66 (br, 2H, C*H* in phenyl), 5.75 (br, 1H, *H*C=C– in main chain), 4.19 (br, 2H, –OCH_2–), 1.69 (br, 2H, –OCH_2CH_2–), 1.35–1.25 (br, 14H, –$(CH_2)_7$–), 0.85 (br, 3H, –CH_3); IR (cm^{-1}, KBr) 3000 (C–H), 1720 (C=O), 1277 (C–O); GPC (based on polystyrene standard): $M_w = 4.23 \times 10^5$, $M_w/M_n = 2.80$; *cis*%: >98 (from ^{1}H NMR measurement; see Section 2.4); UV–Vis (7.05×10^{-5} mol/l THF solution) $\lambda_{max} = 395$ nm ($\varepsilon = 3790$), $\lambda_{\varepsilon>50} = 520$ nm. Anal. calcd for –($C_{19}H_{26}O_2$)–: C, 79.68; H, 9.15. Found: C, 78.92; H, 9.33.

2.4. Determination of stereo-regularity in the main chain (cis%) [14–16]

The *cis* percent of the configuration of the double bonds in the main chain was determined by the ratio of the integral (i.e. area) of the peak at about δ 5.7, 6.6 and 7.6 ppm in ^{1}H NMR spectra [14]. In addition, polyacetylenes involve four types of backbone configurations: *cis*-transoidal, *cis*-cisoidal, *trans*-transoidal, and *trans*-cisoidal [15]. It has been shown that the poly(phenylacetylene)s prepared with [Rh(norbornadiene)Cl]$_2$ possess about 100% *cis*-transoidal main chain structure [16]. It was found by ^{1}H NMR measurement that a *cis*-content of the poly(DecCPA) also had over 98% stereo-regular main chain.

2.5. Instrumentation of a TIRFM

A spectroscopic device was incorporated into a TIRFM (Fig. 1) based on an optical microscope (IX-70-22FL/PH, OLYMPUS, Japan) [10,11]. The linearly polarized 441.6 nm-line 70 mW output of a helium–cadmium continuous wave (CW) laser (M.4.70, Liconix, USA) was used. After passing through a neutral density filter, a focusing lens, and a cubic prism, the laser beam was totally reflected. The incident angle at the quartz slide-to-solution was 78° to the normal, and the critical angle was 74.5°. The fluorescence emission from the specimen was collected with an oil-immersion microscope objective (1.40 NA, 60×, Plan Apo, Nikon, Japan). For imaging, the fluorescence was filtered by a barrier filter (BA475, OLYMPUS, Japan) and then focused by a relay lens onto an ICCD camera (ICCD 325, Photek, USA). The images were recorded on a videocassette recorder through an image processor (ARUGUS C5510, Hamamatsu Photonics, Japan).

3. Results and discussion

3.1. Synthesis and characterization of poly(DecCPA)

By using the combined catalyst system, i.e. [Rh(norbornadiene)Cl]$_2$ and triethylamine, the polymerization of DecCPA monomer proceeded to give a yellow solid poly(DecCPA) of high molecular weight and controlled *cis*-transoidal main chain. Poly(DecCPA) also had an expanded conjugated high stereo-regular main chain that absorbed the wavelength of 441.6 nm as the wavelength of the laser used in this system, and emitted fluorescence of peak wavelength 510 nm (Fig. 2). The ability of application with a TIRFM is discussed in Section 3.2.

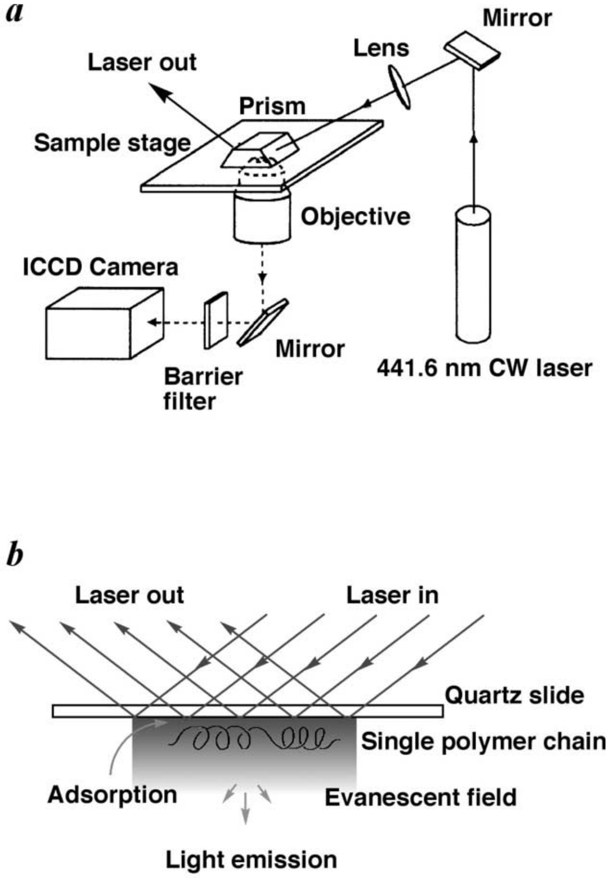


Fig. 1. Schematic drawing of the optical system for single fluorophore imaging (see instrumentation of TIRFM for details). Schematic of the principle of measurement in visualization of individual single conjugated polymer.

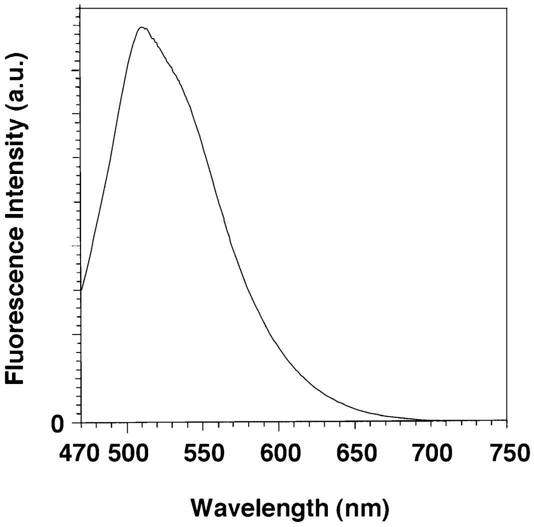


Fig. 2. Fluorescence spectrum of poly(DecCPA) THF solution at room temperature. Concentration is 1.04×10^{-5} mol/l. Wavelength of excitation (λ_{ex}) is 442 nm.

3.2. Fluorescence images for individual single poly(DecCPA) molecules

After putting poly(DecCPA) THF solution (1×10^{-9} mol/l, 2.0 μl) onto the surface of a quartz slide glass, the fluorescence images were measured with the TIRFM (Fig. 1). Fluorescence of a single molecule was very weak in order to decrease the background luminescence, we reduced the optical excitation volume, using evanescent-field illumination of TIRFM [10,11]. When a laser is incident on a quartz slide-to-medium interface at above the critical angle, the light is totally and internally reflected, and an evanescent field is produced just beyond the interface (Fig. 1). This evanescent field is localized near the interface with the 1/*e* penetration depth of 144 nm, depending on the incident angle of the laser. Combining the low background optics with the local excitation, the background was reduced to 1–3 photons/s per diffraction limit area, i.e. >2000-fold lower than that for a conventional epifluorescence microscope [10,11]. Thus, single fluorophores, i.e. single poly(DecCPA) molecules could be clearly visualized at a full-video rate (1/30 s/frame) (Fig. 3). A typical time course of the fluorescence intensity of an individual bright spot, which is indicated by the white arrow, is shown in Fig. 3. Fluorescence intensity of a single poly(DecCPA) greatly fluctuated during the first 113 frames (0–3.77 s). After the irradiation of the laser beam for a certain period of time, i.e. at the 113th video frame, the fluorescence intensity dropped to baseline in a single-step manner due to desorption, confirming that each single spot arises from a single fluorophore. The observed process was the desorption of a single poly(DecCPA) molecule. Evidence that a single fluorescent spot was produced from a single poly(DecCPA) molecule with TIRFM in THF solution was obtained as follows. The poly(DecCPA) was easily dissolved in THF, and this solution was a dilute one (1×10^{-9} mol/l). Therefore, poly(DecCPA) molecules were very difficult to associate under the conditions of the TIRFM measurement. Besides, the fluorescent spot was desorbed in a single step (at the 113th frame in Fig. 3). If the single fluorescent spot was associated with multiple molecules, the desorption must occur in multi-steps. Furthermore, as the concentration of poly(DecCPA) increased, the number of fluorescent spots increased whereas the fluorescence intensity distribution of the individual spots remained a constant, consistent with the fluorescence spots arising from single poly(DecCPA) molecules.

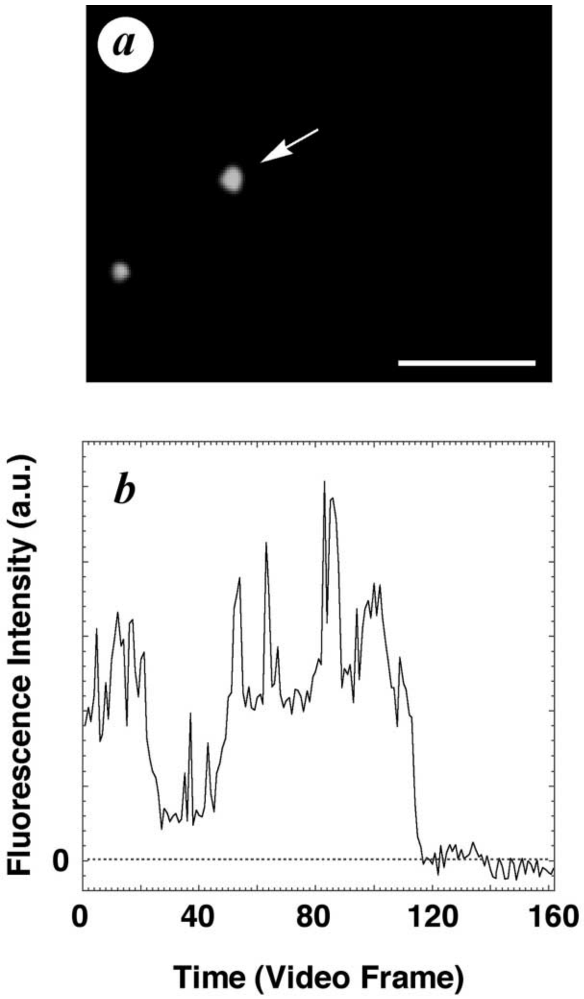


Fig. 3. TIRFM imaging. A micrograph of poly(DecCPA) molecules. Arrow indicates typical fluorescence spot due to a single molecule of poly(DecCPA). Dynamic behavior of a fluorescence image intensity of a single molecule observed at the video rate (30 frames = 1.00 s).

In the period of the first 113 frames (0–3.77 s in Fig. 3), this twinkling is the dynamic photon emission phenomenon of single poly(DecCPA) molecule that we mentioned specifically as a resultant from the time scale — changing very slowly in the time scale of milliseconds and seconds — with the changed fluorescent intensity being associated with the single changing polymer molecule conformation. This molecular dynamic photonic phenomenon, i.e. changing of π-conjugated system by the conformational change in the single polymer chain was caused by the micro-Brownian motion in the THF solution at room temperature having a thermal energy of k_BT, where k_B is the Boltzmann constant and T is the temperature. Although the molecular dynamics of the picosecond time scale, generally, this poly(DecCPA) molecule had the mode of the molecular motion of π-conjugated main chain. This was very slow in the second time scale as was shown. In addition, this behavior would be unobservable in bulk studies using the technique that makes the average of large number of molecules a measuring sample.

This is the first report that twinkling of a single molecule of a conjugated polymer by slow conformational changing of the conjugated polymer chain was directly detected in solution at room temperature. Novel single-molecule device having dynamic photonic function, was reported by this paper using the Brownian motion, will be developed in the near future.

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References

[1] Shinohara K, Yasuda S, Kato G, Fujita M, Shigekawa H. J Am Chem Soc 2001;123:3619.
[2] Martin RE, Diederich F. Angew Chem Int Ed Engl 1999;38:1350.
[3] Meier H. Angew Chem Int Ed Engl 1992;31:1399.
[4] Bleier H. Organic materials for photonics. Amsterdam: Elsevier, 1993. p. 77.
[5] Shinohara K, Aoki T, Kaneko T, Oikawa E. Polymer 1995;36:2403.
[6] Aoki T, Shinohara K, Kaneko T, Oikawa E. Macromolecules 1996;29:4192.
[7] Aoki T, Shinohara K, Oikawa E. Makromol Chem Rapid Commun 1997;13:565.
[8] Shinohara K, Aoki T, Kaneko T, Oikawa E. Chem Lett 1997:361.
[9] Trautman JK, Macklin JJ, Brus LE, Betzig E. Nature 1994;369:40.
[10] Funatsu T, Harada Y, Tokunaga M, Saito K, Yanagida T. Nature 1995;374:555.
[11] Funatsu T, Harada Y, Higuchi H, Tokunaga M, Saito K, Ishii Y, Vale RD, Yanagida T. Biophys Chem 1997;68:63.
[12] Shinohara K, Yamaguchi S, Higuchi H. Polym J 2000;32:977.
[13] Shinohara K, Yamaguchi S, Wazawa T. Polymer 2001;42:7915.
[14] Lee D, Lee D, Soga K. Makromol Chem Rapid Commun 1990;11:559.
[15] Simionescu CI, Percec V, Dumitrescu S. J Polym Sci, Part A: Polym Chem 1977;15:2497.
[16] Furlani A, Napoletano C, Russo MV. J Polym Sci, Part A: Polym Chem 1989;27:75.