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Polymer 42 (2001) 7915–7918

polymer

www.elsevier.nl/locate/polymer

Polymer Communication

First observation of spectral fluctuation in a single molecule of a rigid-rod π-conjugated polymer

Ken-ichi Shinohara[a,b,*], Shingo Yamaguchi[b], Tetsuichi Wazawa[c]

[a]Center for Interdisciplinary Research (CIR), Tohoku University, Aramaki-aza-aoba, Sendai 980-8578, Japan
[b]Department of Metallurgy, Graduate School of Engineering, Tohoku University, Aoba-yama 02, Sendai 980-8579, Japan
[c]Single Molecule Processes Project, International Cooperative Research Project (ICORP), Japan Science and Technology Corporation (JST), 2-4-14 Senba-higashi, Mino, Osaka 562-0035, Japan

Received 8 December 2000; received in revised form 28 February 2001; accepted 1 March 2001

Abstract

The photonic function of the light-emission from a rigid-rod conjugated polymer, poly(9,10-anthracenediyl-ethynylene-1,4-phenylene-ethynylene) [poly(AEPE)], was measured using total internal reflection fluorescence microscopy (TIRFM), which is a single molecule detection technique that is carried out in methanol at room temperature. The light-emission from a single polymer molecule as it slowly and dynamically changes over a cycle lasting a few seconds has been successfully detected using TIRFM with a built-in spectroscope. Special mention must be made of the fact that we were able to observe for the first time fluctuations in the wavelength of the emission spectra resulting from structural changes in a single polymer molecule. Although poly(AEPE) had a rigid-rod main chain, these changes occurred very slowly over a time-scale of several seconds at room temperature. Thus, single molecule spectroscopy appears to be a powerful tool for studying the dynamic behavior of single macromolecules. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Conjugated polymer; Single molecule spectroscopy; Near-field

1. Introduction

Conjugated polymers are useful substances that exhibit many excellent properties, which already make them indispensable as advanced materials for mankind [1,2]. For example, conjugated polymers have been used as laser dyes [3], photoconductors [4], organic light-emitting diodes (OLED) [5], piezoelectric materials [6], and have possible applications in optical data storage [7] and signal processing [8]. There are also some uses as an optical resolution membrane material [9–12].

However, the polymer considered in this study is an extremely complicated example due to its generally varied and dynamic structure, so we have had difficulty in clearly defining the correlation between structure and function at the molecular level. In other words, questions such as ‘What kind of polymer structures indicate the molecular functions?’ have been very difficult to clearly answer at the molecular level. This introduces uncertainty as to whether a proposed molecular design is beneficial or not when we are trying to synthesize novel conjugated polymers with improved functions at the molecular level. Therefore, we thought that if we could directly observe an individual molecular structure and its functionality for a single conjugated polymer, it might then become possible to keep this speculation to a minimum, thus enabling a very clear discussion of the relationship between the molecular structure and function at the molecular level. Bout et al. [13] reported their observation of a single molecular spectrum for a conjugated substituted poly(phenylenevinylene) in a polystyrene matrix, but this was not a *in solvo* system. If the direct measurement of single molecules of a conjugated polymer in solution can be achieved, the observation of unique phenomena can be expected, e.g. the host–guest interaction of single molecules, leading towards the development of novel systems consisting of molecular devices in solution. We have already reported the results of fluorescence imaging experiments conducted on single molecules of the conjugated poly(9,10-anthracenediyl-ethynylene-1,4-phenylene-ethynylene) [poly(AEPE), Fig. 1], which has a rigid-rod main chain, in a tetrahydrofuran (THF) solution at room temperature using a total internal reflection microscope (TIRFM) [14]. In the present study, we conducted single molecule fluorescence imaging and single molecule spectroscopy for conjugated poly(AEPE) having a rigid-rod

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* Corresponding author. Tel.: +81-22-217-7313; fax: +81-22-217-7313.
E-mail address: shinoken@material.tohoku.ac.jp (K. Shinohara).

0032-3861/01/$ - see front matter
PII: S0032-3861(01)00220-8

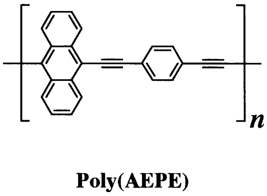

Fig. 1. Chemical structure of poly(9,10-anthracenediyl-ethynylene-1,4-phenylene-ethynylene) [poly(AEPE)].

main chain in methanol at room temperature, and we could successfully detect a unique light-emitting phenomenon which fluctuated dynamically with in the time-scale of several seconds.

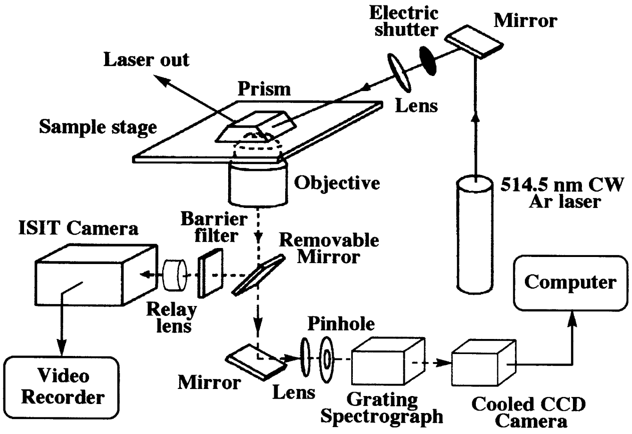

Fig. 2. Schematic drawing of a TIRFM with a built-in spectroscope.

2. Experimental section

Poly(AEPE) [$M_w = 6.1 \times 10^3$, $M_w/M_n = 3.5$ (GPC, based on polystyrene standard) [14] THF solution (1×10^{-9} mol/1, 3.0 μl) was first cast uniformly on a quartz glass slide and then the surface of this glass was immediately washed with a THF solvent (500 μl × 2). This is a good solvent for poly(AEPE) in this instance, because it has been proven that light-emission from a single molecule can be observed if the poly(AEPE) is in a dilute THF solution [14]. After the surface of the glass was dried on a clean bench, it was again cast with methanol (3.0 μl) and sealed in a glass cover. It was then examined under the TIRFM at room temperature, using both the single polymer molecule fluorescence imaging and single molecule spectroscopy methods. Poly(AEPE) molecules do not dissolve in methanol. If a thin THF solution of poly(AEPE) is cast on the surface of a glass slide and is immediately washed away using THF, a small number of single molecules of poly(AEPE) that are physically adsorbed onto the surface of the glass slide may remain that are not washed off. If methanol is then cast again at this particular moment, it will become possible to clearly detect poly(AEPE) in the methanol by using the single molecule fluorescence imaging- and single molecule spectroscopy methods. Single molecule imaging and single molecule spectroscopy was conducted using a TIRFM with a built-in spectroscope (Fig. 2), as described in previous work [15]. The linearly-polarized 514.5-nm line output of a continuous wave (CW) argon laser (Model 2017-06S; Spectra Physics, Mountain View, CA, USA) was modulated to circularly polarized light by a quarter-wave plate and attenuated by a neutral density filter. After passing through a focusing lens and a cubic prism, the laser beam was totally reflected at the silica glass slide–liquid interface. The fluorescence emission from the specimen was collected with an oil-immersion microscope objective (1.40 NA, 60×, PlanApo; Nikon, Tokyo, Japan) and switched for either imaging or spectroscopy using a mirror. For imaging, the fluorescence was filtered by a barrier filter (570DF30; Omega Optical, Brattleboro, VT, USA) and then focused by a relay lens onto the faceplate of an image intensifier (Model VS4-1845; Video Scope International, Sterling, VA, USA) coupled to an SIT camera (C2400-08; Hamamatsu Photonics, Shizuoka, Japan). The images were recorded on a video cassette recorder. For the spectroscopy measurements the fluorescence was passed through a notch filter (HNPF-514.5-1.0; Kaiser Optical Systems, Ann Arbor, MI, USA) and focused onto a pinhole by an achromatic lens. Only that fluorescence which passed through the pinhole was brought into the spectrograph and camera system. The diameter of the pinhole was 150 μm, which corresponded to 1.5 μm at the specimen, since the images at the pinhole were magnified by 100×. The fluorescence was wavelength-dispersed by using a spectrograph (reciprocal linear dispersion, 13.0 nm/mm; Model M10-TP; Spectral Instruments, Tokyo, Japan) and the spectral image was projected onto the focal plane of a back-illuminated cooled CCD camera (CCD; 1024 × 256 pixels, each 27-μm square; CCD15-11-0-232; Wright Instruments, London, UK). The dispersion-axis bin-size of the cooled CCD camera was kept to 6 (pixels) so that the apparent dispersion per bin was 13.0 (nm/mm) × 27 (μm/pixels) × 6 (pixels/bin) = 2.11 (nm/bin). The spectrum detected with the cooled CCD camera was stored and analyzed using a personal computer. The laser beam was blocked by a mechanical shutter synchronized to an electronic shutter in the cooled CCD camera.

3. Results and discussion

3.1. Imaging and spectral fluctuation of a single molecule

Multiple bright spots formed by the fluorescence of single molecules in the poly(AEPE) having a rigid-rod main chain in methanol were observed during a single molecule imaging experiment conducted using the TIRFM, as shown in Fig. 3. To obtain the fluorescence emission spectra of a single poly(AEPE) molecule, the fluorescence within a circle of 1.5-μm diameter at the specimen plane was passed

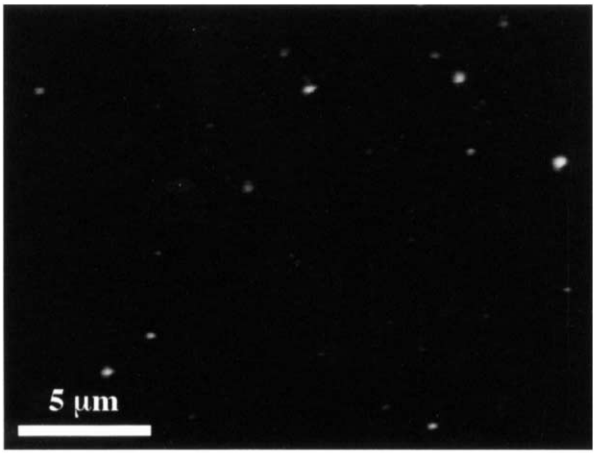

Fig. 3. TIRFM image shows one frame image of single poly(AEPE) molecules taken at the video rate (exposure time, 1/30 s) in methanol at room temperature. Wavelength of excitation was 514.5 nm. The laser power was 6.4 mW. The observation wavelength was from 550 to 585 nm.

through a pinhole (see Section 2). The fluorescence was then wavelength dispersed by wavelength with a spectrograph, and an image of the spectrum was taken with a cooled CCD camera. Fig. 4 shows how the single molecule spectra of the fluorescence emission from one of the bright spots of single poly(AEPE) molecules changed with time. Six different spectra can be obtained by totaling the data for a duration of 2 s. These six spectra can be associated with the three different conditions listed below.

> [*State* I] Spectra (1) and (2): The fluorescence intensity gradually decreases from 525 nm to 650 nm.
> [*State* II] Spectra (3) and (4): The wavelength of the emission maximum is observed around 570 nm.
> [*State* III] Spectra (5) and (6): The fluorescence intensity decreases completely to the level of the background light.

The examination of spectra (1) and (2) revealed that a single molecule of poly(AEPE) was strongly adsorbed onto the surface of a quartz-glass slide substrate and the single polymer chain became bent, therefore, the π-conjugated system became shorter [*State* I]. Spectra (3) and (4) show that the interaction with the substrate weakened and the single polymer chain turned itself into a linear structure through molecular motion, and therefore, the π-conjugated system became relatively long [*State* II]. Spectra (5) and (6) can be explained by the single polymer chain becoming detached from the substrate [*State* III]. Special mention must be made of the fact that the fluctuations in the light-emission spectra resulting from a structural change in a single polymer molecule having a rigid-rod main chain occurred very slowly over a time-scale of seconds at room temperature.

This is the first report in which changing spectral behavior in light-emission due to slow conformational changes of a conjugated single polymer molecule have been directly detected at room temperature. This is a molecular dynamic photonic phenomenon, which is due to changes in a π-conjugated system caused by conformational changes in a single polymer chain, which were caused in turn by micro-Brownian motion in methanol at room temperature. The dynamic photo-function was caused by the thermal energy of k_BT, where k_B is the Boltzmann constant and T is the temperature. Although molecular dynamics generally works on a picosecond time scale, the poly(AEPE) molecule having a rigid-rod main chain exhibited a mode of molecular motion in which the π-conjugated main chain reacted very slowly over a time scale of several seconds, as shown here. In addition, this behavior would be unobservable in bulk studies using techniques that take the

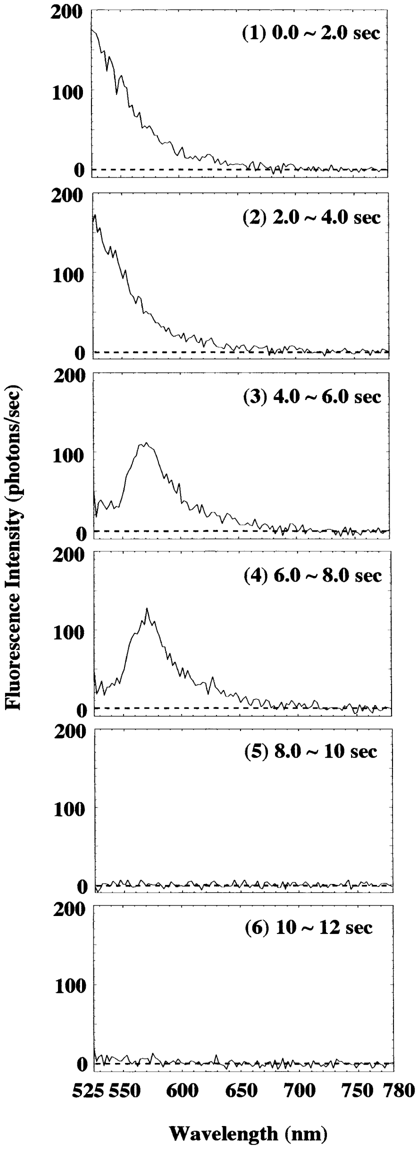

Fig. 4. Slow-dynamic single molecule spectra of poly(AEPE) on glass surface in methanol at room temperature. Spectra were taken with the collection time of 2.0 s for each spectrum at a laser power of 6.4 mW, wavelength of excitation was 514.5 nm.

average of a large number of molecules for the measurement sample. A novel single-molecule device that incorporates a dynamic photonic function using Brownian motion, as shown in this study, will be developed in the near future.

Acknowledgements

This study was partially supported by a grant-in-aid for Scientific Research on Priority Areas (A) No. 286 of 'Near-field Nano-optics' from the Ministry of Education, Science and Culture, Japan (No.11122204).

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