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Probing Strain in Thermoplastic Elastomers Using Fluorescence Resonance Energy Transfer

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ABSTRACT: Strain in polyetherurea thermoplastic elastomers with poly(tetramethylene oxide) soft blocks and well-defined bisurea hard blocks was probed through changes in fluorescence resonance energy transfer between both covalently linked and randomly dispersed donor and acceptor probe molecules. The covalently linked fluorophores are shown to have superior strain sensitivity, while the dispersed probes give information on the affinity of deformation at the nanometer length scale. The method is proposed to be generally applicable to study deformation of elastomers at the molecular scale.

Introduction

Strain−induced spectral changes are useful probes for measuring deformation at the molecular level in elastomeric materials. While many devices are known to gauge macroscopic strain,[1] information on strain at the nanometer length scale is much harder to obtain. In polymers, this information would be highly useful in order to understand deformation mechanisms in cross-linked networks with different topologies, and at different length scales.[2]

Several spectroscopic techniques have been used to measure strain in polymeric materials. The position of bands in the Raman spectra of oriented fibers of polydiacetylene single crystals,[3] aromatic polyamides,[4] poly(*p*-phenylenebenzobisthiazole),[5] carbon fibers,[6] etc. shift substantially when the fibers are subjected to stress. Although band shifts are high in polydiacetylene single crystals, the use of Raman spectroscopy to probe strain in isotropic polymeric matrices of polypropylenes, polyamides etc. has been less successful due to low sensitivity, but incorporation of diacetylenes in polyurethane thermoplastic elastomers was shown to lead to strongly enhanced sensitivity.[3b] Shifts in Raman bands of single walled carbon nanotubes (SWCN's) added to the matrix of polyurethane acrylate have also been used to study molecular deformation in polymers such as polyurethanes.[7] Rubner and co-workers have designed and synthesized various mechanochromic (color change induced by strain) polydiacetylene networks by irradiation of various polyurethane-diacetylene segmented copolymers.[8] Recently, fluorescent methods based on excimer, exciplex and energy transfer have been widely used to study properties such as orientation, diffusion, relaxation and residual strain of elastomeric polymers and gels.[9−12] For example, fluorescence of poly(*N*-vinylcarbazole) has been used to study the residual strain in commercial styrene−isoprene−styrene (SIS) triblock copolymers.[13] Changes in the relative intensity at two wavelengths (I_{350}/I_{370}) were observed upon elongations up to 400% with the changes being strongest for strain between 0 and 200%. "Self assessing" photoluminescent polyurethane based on excimers formed from cyano-substituted oligo(*p*-phenylene vinylene)[14] and polymeric material exhibiting optically detectable response to changes in pressure have also been reported in the literature.[15] Quite recently, a fluorescence energy transfer-based mechanical stress sensor for proteins composing of a green fluorescence protein pair (cerulean as donor and venus as acceptor) linked to each other with a stable α helix have been used.[16]

Fluorescence resonance energy transfer (FRET) between a donor and an acceptor chromophore within 2 to 6 nm has seldom been used to study deformation of polymer networks and gels. A single patent of its use in pressure sensitive paints exists—however, without giving details about the relation between strain and optical response.[15] The strong distance dependence of the efficiency of energy transfer, which varies with the inverse of sixth power of the separation between donor and the acceptor, would make this technique highly advantageous for optically probing deformation of polymeric materials as compared to fluorescent probe molecules which are based upon excimers and exciplexes.[17] Here we use FRET in a thermoplastic elastomer with bisurea hard blocks to probe strain at the nanometer length scale. In the experiments we compare changes in FRET ratio for two kinds of systems. In one of the systems the donors are randomly anchored to the fibrous hard blocks of the TPE and the acceptor molecules are randomly dispersed in the matrix. In the second system, a polymeric linker connects two anchored donor moieties to an acceptor to give a donor−linker−acceptor−linker−donor molecule (Figure 1).

Experimental Section

Materials and Methods. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker 400 spectrometer in deuterated solvent. MALDI-TOF was performed on a Perseptive DE PRO Voyager MALDI-TOF mass spectrometer using cyano-4-hydroxycinnamic acid as the calibration matrix. Infrared spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer with a Universal ATR sampling accessory. UV/vis spectra were recorded on a Perkin-Elmer Lambda 900. Fluorescence spectra were recorded on an Edinburgh Instrument FS920 double-monochromator spectrometer with a Peltier cooled red-sensitive photomultiplier. Size exclusion chromatography was performed on a Shimadzu LC10-AT, using Polymer Laboratories PL Gel 5 μm mixed-D column (linear range of MW: 200−40000 g/mol), a Shimadzu

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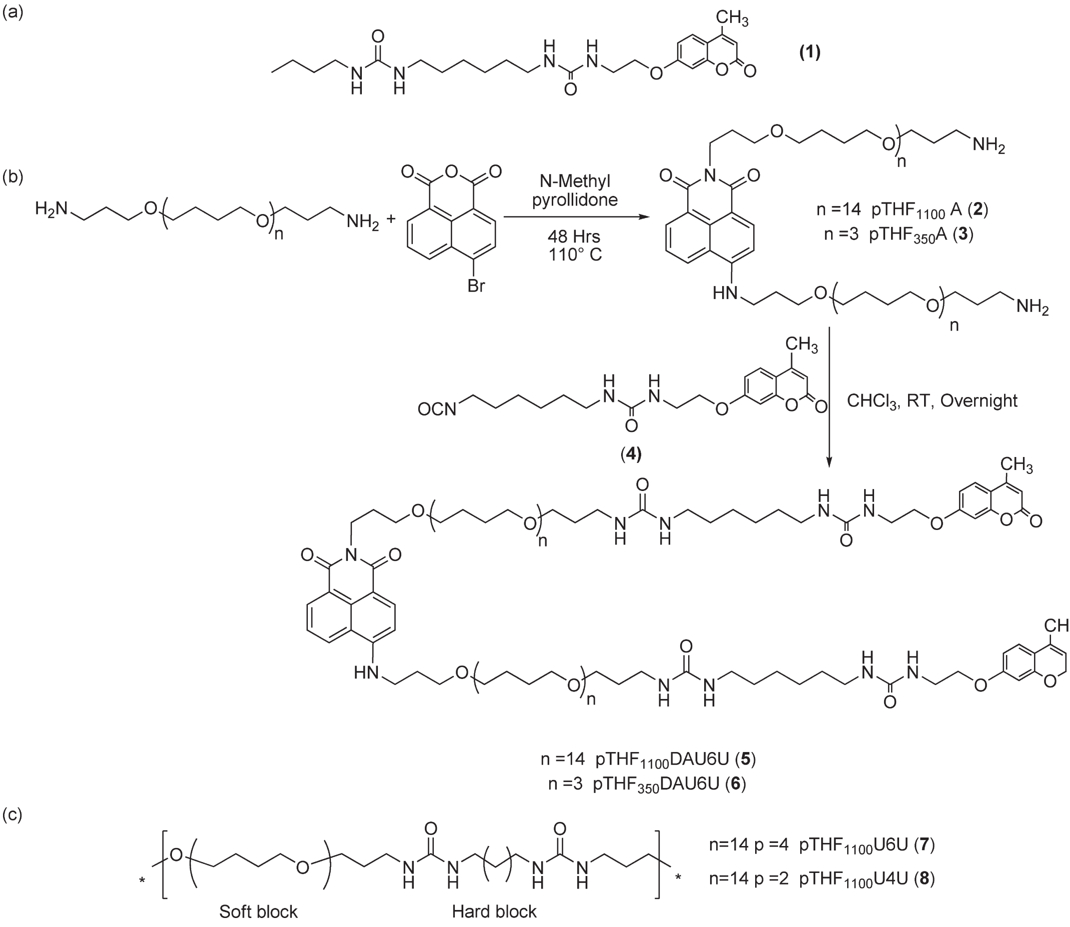
Scheme 1. (a) Noncovalent-Linked Donor Molecule (1), (b) Synthesis of Probe Molecules (5 and 6) and (c) Polymers Having Bisurea Unit as a Hard Block and pTHF Unit as a Soft Block (7 and 8)



SPD-10AV UV−vis detector at 254 nm and chloroform as elunet at a flow rate of 1 mL/min (20 °C). Polystyrene standards were used for calibration. Chemical shifts are reported in ppm and referenced to tetramethylsilane and chloroform/dimethyl sulfoxide (proton and carbon). Solvents used in the synthesis were reagent grade. The reagents and poly(tetrahydrofuran) (M_n = 1100 and 350) used in this study were purchased from Aldrich, Fluka, or Acros and were used without additional purification.

Synthesis. A detailed description of the synthesis and characterization of probe molecules (**1**−**5**) and their synthetic intermediates is available in the Supporting Information.

Preparation of Polymer Films and Fluorescence Measurements. To obtain films containing randomly dispersed **1** and **2**, probe molecules **1** (0.0049 mg, 3.0×10^{-6} M) and **2** (0.0128 mg, 1.5×10^{-6} M) were taken in a solution of polymer **7** (80 mg) in chloroform (2.5 mL) with a few drops of methanol. For films containing **5** and **6**, probe molecules **5** (0.0198 mg, 2.4×10^{-6}M) or **6** (0.0102 mg, 2.4×10^{-6}M) were dissolved along with polymer matrices **7** or **8** (80 mg) in chloroform (2.5 mL) with few drops of methanol. The samples were stirred to dissolve the polymer and the probe molecules in order to obtain a clear solution. The solution was then poured into a Teflon mold and covered with a watch glass for slow evaporation. After the slow evaporation, the films obtained were peeled off and dried in vacuum for 5 h. Films of length 1.1 cm and width 1 cm were cut and clamped onto a quartz plate using a Scotch-brand tape. Two marks were made on the films using a marker to determine strain after the samples were stretched. For fluorescence experiments, dwell time was set to 0.2 s, number of scans to 3 and step size to 1 nm. The emission and excitation slits were set to 4 nm. The samples were excited at 325 nm and the emission spectra was recorded from 345 to 640 nm. For excitation spectra, the emission were monitored at 375 and 516 nm respectively.

Results and Discussion

Segmented poly(THF)−bisurea polymers have been shown to be thermoplastic elastomers with excellent mechanical properties due to the high degree of phase separation between the soft p (THF) and the hard bisurea segments.[18] Polymers **7** and **8** (Scheme 1), which we use here as matrix for the FRET probes, show Hookean behavior upon elongation up to 100%, with plastic deformation at higher strains, and a strain at break of 1000%.[18] Previously, we have shown that probe molecules provided with “matching” bisurea moieties, which have the same the number of methylene units between urea groups as in the host are specifically bound to the fibrous hard blocks of these polymers, while incorporation of probe molecules with “nonmatching” bisurea units are not incorporated in the hard blocks of the host.[19]

As FRET pairs, disubstituted napthalimides such as 9-butyl-4-butylamino-1,8-naphthalimide (acceptor) and 7-*N*,*N*-diethylamino-4-methylcoumarin derivatives (donor) were used.[20] The structure of the probe molecules and their synthesis is outlined in Scheme 1. Donor molecules **1** were provided with bisurea groups that match the bisurea groups constituting the hard blocks of the matrix, and hence these chromophores are incorporated in the hard blocks as indicated in Figure 1a. Acceptor molecules **2** and **3** do not have bisurea groups and are provided with pTHF substituents to favor dissolution in the pTHF soft phase.

Films suitable for strain measurements were prepared by solution casting polymer **7** in the presence of a small amount of

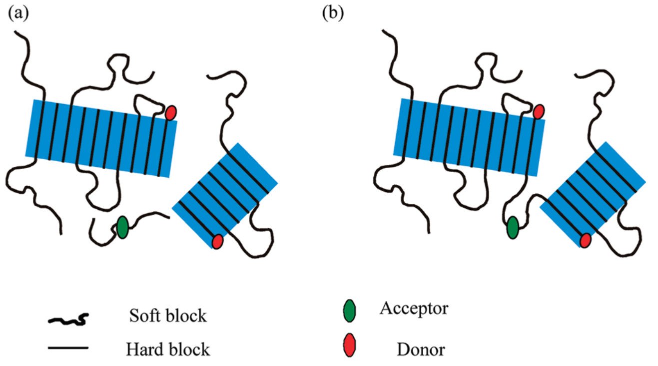


Figure 1. Two kinds of systems studied to probe strain in thermoplastic elastomers (TPE): (a) the donors are randomly anchored to the hard blocks of the TPE and acceptor molecules are randomly dispersed in the matrix; (b) the two anchored donor molecules are connected to an acceptor through a polymeric linker.

donor (**1**) and acceptor (**2**) in a 2:1 ratio to give a final concentration of 3.0×10^{-6} M and 1.5×10^{-6} M of chromophores **1** and **2** in the film. These films were elongated to strains between 0 and 500% and the FRET ratio $(I_{507})/(I_{376})$ was determined. Upon elongation, the FRET ratio decreased only slightly from 0.57 (0% elongation) to 0.55 at 100% elongation (Figure 2). However, when the material containing the FRET pair was elongated to 500% strain, the FRET ratio decreased significantly to a value of 0.33. After relaxation of the sample the ratio had increased to 0.43, but it did not recover to its original value, in line with a tensile set (elongation remaining after deformation) of approximately 220% observed for this material after straining to 500%.[18]

The change in FRET ratio at low strains using randomly dispersed probe molecules (**1** and **2**) is very limited. Therefore, we synthesized covalently linked D–A–D probes (**5** and **6**) which combine donor and acceptor moieties in a single molecule by linking them with poly(THF) spacers. The donor moieties were connected to the polymer linkers via bisurea units that are incorporated in the hard blocks of the matrix. In this way, the molecules probe deformation of the network of fibers instead of the overall deformation of the matrix (Figure 1b). Furthermore, use of these probe molecules at sufficiently low concentration will predominantly give intramolecular energy transfer. Under these conditions, the FRET ratio of unstrained films is no longer dependent on probe concentration, but only on the length of the poly(tetrahydrofuran) linker used (M_n = 350 and 1100 in **5** and **6**, respectively).

The structure of the probe molecules and their synthesis is outlined in Scheme 1. The disubstituted naphthalimides (**2** and **3**) were obtained by reacting 2 equiv of bisaminopropylpoly(tetrahydrofuran) (M_n = 1100 or 350) with 4-bromonapthalene anhydride.[21] In the next step, the product (disubstituted-poly(tetrahydrofuran)naphthalimide) was reacted with the isocyanate obtained from 4-methyl-7-ethyloxycoumarinureidohexylamine (**4**) to yield the probe molecules **5** and **6** (see Supporting Information for detailed synthetic procedure, purification and characterization of the products of each synthetic step).

A low concentration (2.4×10^{-6} M) of the probe molecules (**5** and **6**; Scheme 1) was mixed into the host matrix (**7**) and FRET ratios were determined analogous to the films with randomly dispersed donor and acceptor chromophores. At this concentration of the DAD probe **5** with linkers of number average molecular weight 1100 the FRET ratio of unstrained samples was 0.59. The ratio is plotted as a function of strain in Figures 3 and 4d. In contrast to the elastomer containing randomly dispersed donors and acceptors, the FRET ratio (I_{507}/I_{376}) decreased strongly to 0.38 upon straining to 100%. The FRET ratio reached a value of 0.17 at 460% strain. Annealing the films at 60 °C for 5 h before applying strain did not significantly influence the change in FRET ratios, annealed film with $(I_{507})/(I_{376})$ = 0.64 gave a FRET ratio of 0.22 after straining to 475%. Similar experiments were performed with the probe molecule having donor–acceptor attached to shorter poly(tetrahydrofuran) chain (M_n = 350) (**6**, Scheme 1). The FRET ratio at 0% is higher (1.49) than for probe **5**, in line with the shorter p(THF) linker of probe **6**, which keep the chromophores of the FRET pair closer together. Upon applying 500% strain, a FRET ratio of 0.69 was reached, corresponding to a decrease of 53% in the energy transfer efficiency (Figure 4a).

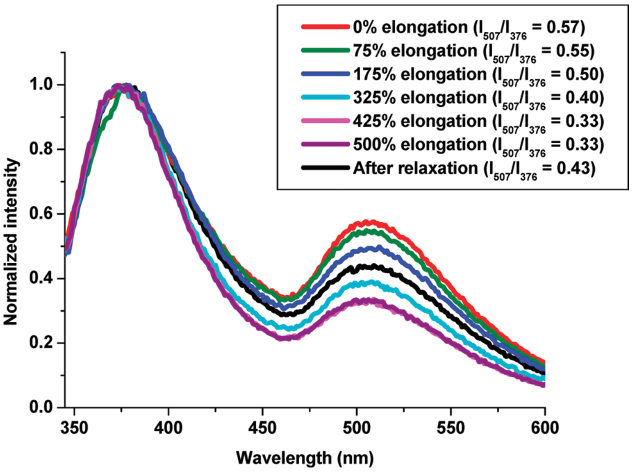


Figure 2. Normalized fluorescence spectra showing the variation of the intensity of donor's emission (I_{376}) and acceptor's emission (I_{507}) with respect to tensile elongation for 2:1 ratio of **1** (3.0×10^{-6} M) and **2** (1.5×10^{-6} M) in **7**.

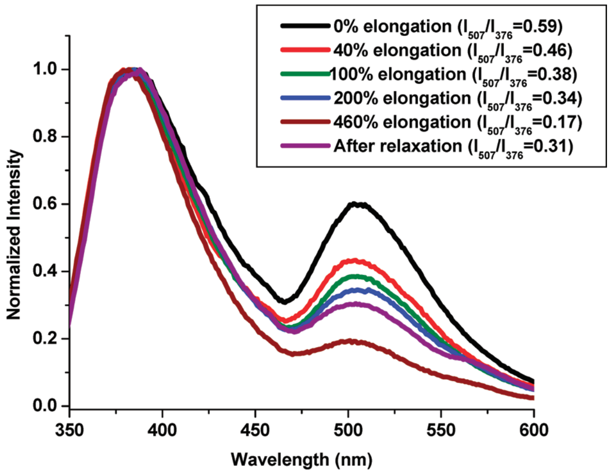


Figure 3. Normalized fluorescence spectra showing the variation of the intensity of donor's emission (I_{376}) and acceptor's emission (I_{507}) with respect to tensile elongation for 2.4×10^{-6} M of **5** in **7**.

Previously, we have demonstrated using AFM and fluorescent studies with bispyrene-based guest molecule in matching and non matching polymer matrices that bisurea guest molecules are phase separated from the hard blocks of the matrix when the spacing between urea groups of matrix and probe do not match.[22] In order to understand the importance of matching bisurea groups of matrix and D–A–D strain probes, fluorescence studies were performed with polymer **8** as the matrix. This polymer has tetramethylene units between its urea groups. The films prepared by mixing 2.4×10^{-6} M of **5** in nonmatching matrix **8** were subjected to tensile elongation and fluorescence measurement. The initial FRET ratio was 0.78, a value higher than observed with the matching polymer **7** (I_{507}/I_{376} = 0.59)

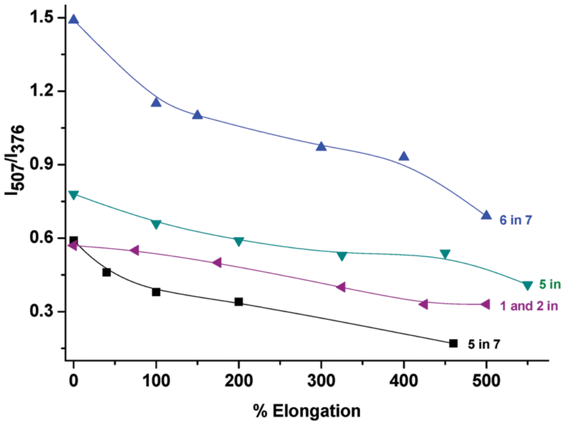


Figure 4. FRET ratio (I_{507}/I_{376}) as a function of strain for (a) 2.4×10^{-6} M of **6** in **7**, (b) 2.4×10^{-6} M of **5** in **8**, (c) 3.0×10^{-6} M and 1.5×10^{-6} M of **1** and **2** in **7**, and (d) 2.4×10^{-6} M of **5** in **7**.

(Figure 4b). The change in FRET ratio after 100% elongation ($I_{507}/I_{376} = 0.66$) was 15%, significantly less than the decrease of 35% observed in a matching polymer matrix (**7**), indicating the importance of matching of the bisurea groups of matrix and probe for maximum strain sensitivity of the system.

The experiments described above show that fluorescence energy transfer may be used as probe for strain. It is evident from comparison of the randomly dispersed probes and the combined D−A−D probe that the latter is more sensitive in detecting small strains. The superiority of the combined probe molecule originates in its topology. When the acceptor is covalently linked to both donors, as in probes **5** and **6**, the distance between donor and acceptor strongly increases with elongation, because it can be assumed that the initial distance between donor and acceptor in **5** and **6** is on the order of the expectation value of the end-to-end distance of an unperturbed coil with the corresponding molecular weight[23] (2.0 and 3.2 nm respectively), while it is equal to the contour length of the p(THF) segment between donor and acceptor (3.0 and 9.5 nm) when the polymer chain is completely stretched. These considerations predict that the initial FRET ratio is higher for probe **6** than for probe **5**, as is indeed the case. They also correctly predict a stronger change in FRET ratio for **5**, although the higher fraction of molecules of **6** that form a loop within a single fiber will also contribute to this difference.

Although the change in FRET ratio of the randomly dispersed probes is less than for the combined probes, they may give valuable information about the *affinity* of deformation at the molecular scale. In *affine deformation* the macroscopic deformation applied on a body is translated uniformly to the microscopic level. Affinity of deformation usually breaks down at length scales below the size of characteristic inhomogeneities.[2] Thus, calculation of changes in FRET upon deformation may give detailed insight in the effect of morphology of polymer **7**, which consists of rigid fibers in a soft matrix, on the nonaffinity of deformation. For affine deformation of randomly dispersed acceptors, the distance from an acceptor molecule to the nearest donor is decreasing, since elongation of the sample results in contraction perpendicular to the axis of elongation directions. Since FRET is dominated by nearest neighbors, the FRET ratio is therefore also expected to increase. However, it is not straightforward to predict changes in FRET ratio because overall FRET efficiency is a complex function of ensemble average distance when non-nearest neighbors are also taken into account. In order to use changes in FRET to study the affinity or nonaffinity of deformation in this and other polymers, future efforts will be directed toward developing a quantitative description of the relationship between affine deformation and change in FRET ratio.

Conclusion

In conclusion, we have designed and synthesized fluorescent strain probes based on FRET to study molecular deformation in poly(tetrahydrofuran) based thermoplastic elastomers. While the linked probes are found to be superior in terms of strain sensitivity, probably the most valuable insights from the experiments is that the randomly dispersed probes allow investigation of the nonaffinity of deformation of elastomers. Since the changes in FRET are not determined by the chemical nature of the host material, but only on geometrical changes at the nanometer length scale we expect the technique to be applicable to the study of affinity of deformation of any transparent elastomeric material. Future work will be directed at quantitatively relating the changes in FRET ratio to the details of the morphology in a range of elastomeric materials with different architectures.

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Supporting Information Available: A detailed description of the synthesis and characterization of probe molecules including schems showing the reactions and structures of the compounds formed (**1**−**5**) and their synthetic intermediates and figures showing UV-vis spectra, GPC traces, fluorescence spectra, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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