Photomodulation of the Inverse Temperature Transition of a Modified Elastin Poly(pentapeptide)

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Received July 29, 1993
Revised Manuscript Received December 6, 1993

The inverse temperature transition exhibited by elastin-like polypeptides has been shown to provide a basis for an intriguing array of energy transduction processes.[1] For example, the elastin poly(pentapeptide) **1** can be fabricated into cross-linked gels that remain swollen in water at temperatures below 25 °C but then deswell, or contract, upon a rise in temperature. Modification of sequence **1** has produced a series of related polypeptides that undergo phase mixing or swelling transitions in response to changes in pH,[2] ionic strength,[3] pressure,[4] and oxidation/reduction[5] or upon enzymatic phosphorylation.[6] We report herein the photomodulation of the inverse temperature transition of the modified elastin polypeptide **2b**. This development provides a route to protein-based polymeric materials and gels capable of photomechanical transduction.[7]

$$\left[\text{ValProGlyValGly}\right]_n \quad \text{or} \quad \text{poly(VPGVG)} \qquad \mathbf{1}$$

$$\text{poly}[f_v(\text{VPGVG}), f_x(\text{VPGXG})] \qquad \mathbf{2}$$

a: X = —HNCHCO—
|
$CH_2CH_2CO_2H$

b: X = 0.5 —HNCHCO— / 0.5 —HNCHCO—
|
$CH_2CH_2CO_2H$ / $CH_2CH_2CO_2NH$—C$_6$H$_4$—N=N—C$_6$H$_5$

where $n \geq 120$ and f_v and f_x are mole fractions with $f_v + f_x = 1$.

Structure **2a** was synthesized as previously described[8] and verified by nuclear magnetic resonance. The mole fractions of pentamers were determined by amino acid analyses to be f_v = 0.68 and f_x = 0.32, i.e., **2a** may be represented as poly[0.68-(VPGVG), 0.32(VPGEG)].

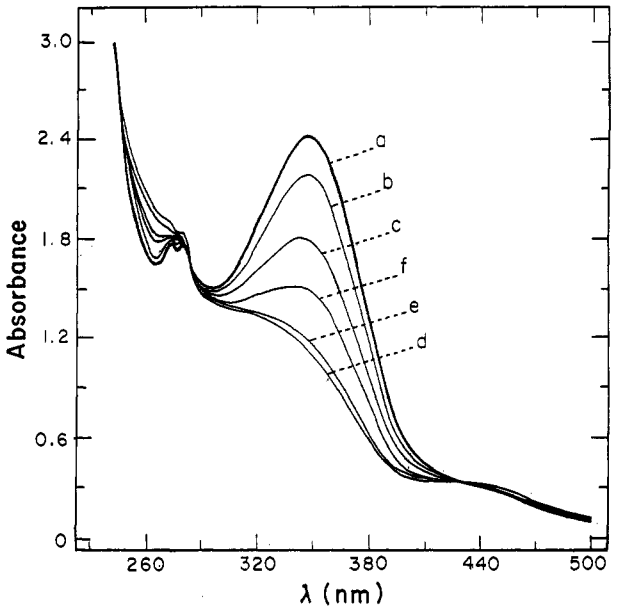

Figure 1. Electronic absorption spectra of **2b** after irradiation at 350 nm or from the electronic flash unit (room temperature): (a) dark-adapted 24 h; (b) 5 s at 350 nm; (c) 20 s at 350 nm; (d) 45 s at 350 nm; (e) 15 flashes at 1 flash/s; (f) 45 flashes at 1 flash/s.

The photosensitive copolypeptide **2b** was prepared in the following manner. Copolypeptide **2a** (31.6 mg, 0.019 mmol of -CO_2H) was dissolved in 2 mL of *N*,*N*-dimethylformamide (DMF). To this solution were added 8.2 mg (0.042 mmol) of phenylazoaniline, 6.6 mg (0.049 mmol) of hydroxybenzotriazole, and 8.2 mg (0.040 mmol) of dicyclohexylcarbodiimide. The solution was stirred for 3 days at room temperature, and 1 drop of 1 M acetic acid was added to facilitate precipitation of the dicyclohexylurea byproduct. The precipitate was removed by centrifugation, and the polymer was recovered by precipitation into excess diethyl ether, washed repeatedly with ether, and then dried overnight at 40 °C. The yield was 28.2 mg (89%). Thin-layer chromatography revealed no contamination by unconjugated phenylazoaniline, and the ultraviolet absorption spectrum indicated amidation of 55% of the glutamic acid side chains of **2a**.[9,10]

Figure 1 shows the changes in the electronic absorption spectrum of **2b** that occur upon irradiation of a 0.5 wt % solution of the copolymer in phosphate-buffered saline (0.15 N NaCl/0.01 M sodium phosphate, pH 3.5). The dark-adapted copolymer exhibits the expected absorption spectrum of the *trans*-azobenzene chromophore, with absorption maxima at 348 and 428 nm (curve a). Irradiation at 350 nm (Rayonet minireactor, four 350-nm lamps) results in reduction in the intensity of the 348-nm absorption band, with the photostationary state being reached in ca. 45 s under the conditions of this experiment (Figure 1, curves b–d).[11] Further irradiation from a longer wavelength source (a Sunpak Thyristor Auto 522 electronic flash unit with the window removed) restores the 348-nm absorption via partial photoreversion to the *trans* form of the chromophore (curves e and f). The state represented by curve f does not change upon further irradiation and is estimated to consist of ca. 50% *trans* and ca. 50% *cis* chromophore. The tight isosbestic points at 280 and 425

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(7) Photomodulation of the lower critical solution temperature of poly(*N*-isopropylacrylamide) has been described (Kungwatchakun, D.; Irie, M. *Makromol. Chem., Rapid Commun.* **1988**, *9*, 243–246), and photomechanical transduction (the conversion of light energy into mechanical work) has been reported in modified poly(*N*-isopropylacrylamide) gels (Irie, M. *Pure Appl. Chem.* **1990**, *62*, 1495–1502).
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(9) The molar extinction coefficient reported by Fissi and Pieroni (Fissi, A.; Pieroni, O. *Macromolecules* **1989**, *22*, 1115–1120) for poly(L-glutamic acid) containing 85 mol % azobenzene units in the side chains was used to estimate the degree of amidation.
(10) The absence of observable phenylazoaniline from the thin-layer chromatogram limits the amount of the unconjugated chromophore to less than 0.5% of the amount bound to the polypeptide.
(11) The photostationary state consists of ca. 30% of the *trans* and 70% of the *cis* forms of the chromophore under these conditions of irradiation (Ferritto, M. S.; Tirrell, D. A. *Biomaterials* **1990**, *11*, 645–651).

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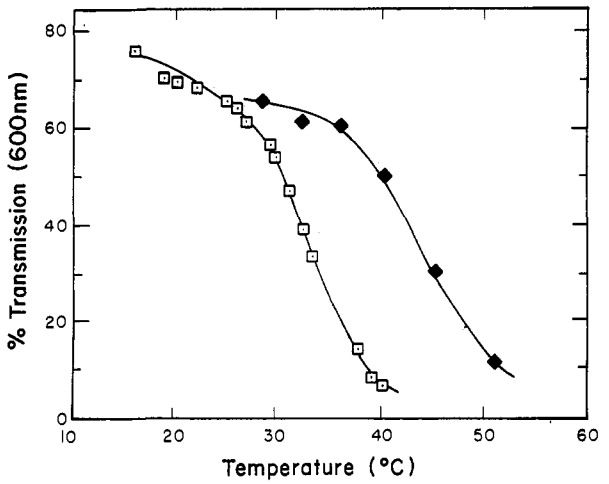

Figure 2. Temperature-dependent turbidity of aqueous samples of **2b** (c = 5 mg/mL in pH 4.1 phosphate-buffered saline solution). Transmission values obtained on a Beckman DU-7 spectrophotometer with a Lauda K-4/RD circulating bath: (□) dark-adapted 24 h; (◆) flashed 45 times.

nm indicate that the *cis–trans* interconversion occurs without significant degradation of the chromophore.

Figure 2 shows that the inverse temperature transition of **2b** is sensitive to the configuration of the azobenzene chromophore. Phase separation of the polymer, as reported by an abrupt increase in the turbidity of the sample, occurs at ca. 32 °C for the *trans* form and at ca. 42 °C for the *cis* form of **2b** when buffered at pH 4.1. Elevation of the transition temperature upon *trans–cis* photoisomerization is consistent with the increased dipole moment of the *cis*-azobenzene isomer[12] and with the established correlation between the polarity of the side chain and the temperature at which phase separation is observed.[13,14]

The shift in phase transition temperature from 32 °C to 42 °C upon *trans–cis* isomerization opens a window, near 40 °C, for photomodulation of the transition at a constant pH of 4.1. Figure 3 illustrates this phenomenon. At 40 °C, the relatively hydrophobic *trans* form of the polymer affords turbid biphasic suspensions. Irradiation at 350 nm results in conversion to the 70% *cis* form, with corresponding dissolution of **2b** and decreasing

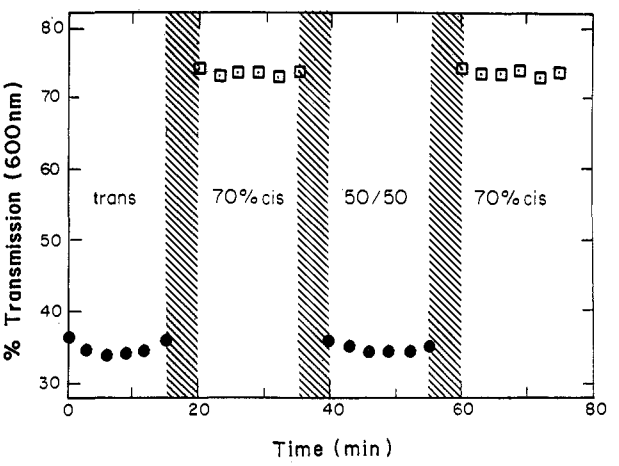

Figure 3. Photomodulation of phase separation of aqueous samples of **2b** (c = 5 mg/mL in pH 4.1 phosphate-buffered saline solution, 40 °C). 70% *cis* samples were prepared by irradiation for 5 min at 350 nm; 50/50 sample by irradiation with 50 flashes from the electronic flash unit. Hatched intervals represent periods of irradiation.

sample turbidity. Further irradiation from the longer wavelength source reforms ca. 50% of the hydrophobic *trans* isomer and drives a second cycle of phase separation. Thermal reversion of the *cis* isomer under these conditions is negligible, and the process appears to be fully reversible under photocontrol.

These results illustrate that attachment of one azobenzene chromophore in ca. 30 amino acid residues is sufficient to render photosensitive the inverse temperature transition of elastin-like polypeptides. This phenomenon can be exploited to achieve reversible photomodulation of the transition at 40 °C. Extension to cross-linked gels and photomechanical transduction processes in protein-based polymers is being pursued.

Acknowledgment. This work was supported in part by the NSF Materials Research Laboratory at the University of Massachusetts and by Contracts Nos. N00014-90-C-0265 and N00014-89-J-1970 from the Department of the Navy, Office of Naval Research. Luke A. Strzegowski held a fellowship provided by the Research Experiences for Undergraduates program of the MRL. Manuel Bueno Martinez was a Visiting Scientist from the University of Seville, Spain, and was supported by the DGICYT.

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