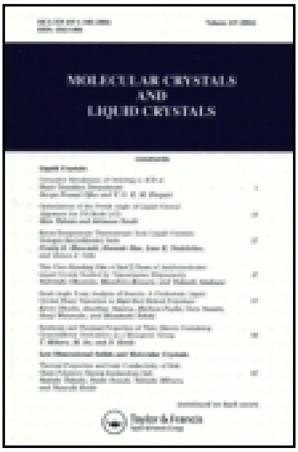

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INVESTIGATIONS OF PHOTOCHROMIC POLYPEPTIDES

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Abstract Bacteriorhodopsin provides researchers with a vision of the possibilities for an engineered optical material. Photochromic polypeptides are synthetic materials that have similar potential applications as bacteriorhodopsin. The light-induced coil-to-helix conformation change can be used to develop materials having light-induced changes in birefringence. Recent investigations of light-sensitive polypeptides in solution and as thin films give increased knowledge of structure-property relationships. The behavior of bacteriorhodopsin-based optical materials provides a list of materials requirements needed to explore the feasibility of photochromic polypeptide thin film development. Major challenges include decreasing photofatigue, designing films with thermal back reaction half-life in seconds and large light-induced changes in refractive index.

BACTERIORHODOPSIN-BASED OPTICAL MATERIALS

Bacteriorhodopsin(*bR*) is a photochromic protein isolated from a halophilic bacterium.[1-3] Because the protein posseses a net charge and permanent dipole moment, preparation of oriented films is straightforward. The essential features of this optical material result from the photoconversion between two states: bR_{570} and M_{412}, having absorption maxima at 570 and 412 nm, respectively(Figure 1).

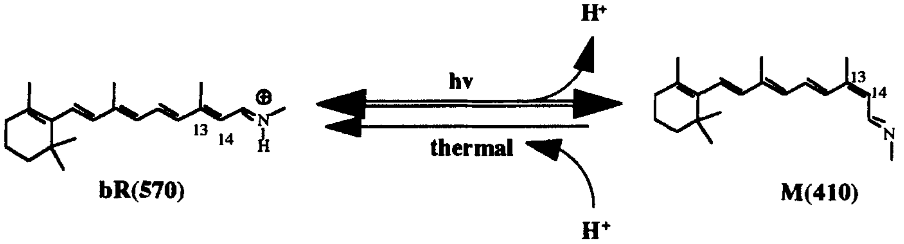

FIGURE 1 Simplified Bacteriorhodopsin Photocycle

Upon illumination, the bacteriorhodopsin(bR_{570}) thin film undergoes a complex photocycle accompanied by a photovoltage. When in the bR_{570} state, the chromophore is an *all-trans* retinal protonated Schiff base covalently-linked to the protein. Conversion to the M_{412} state results from deprotonation of the Schiff base linkage and isomerizarion to *13-cis* retinal. Investigation of applications have focused on bacteriorhodopsin and the M_{412} photointermediate. The two states can be interconverted by illumination with green (*i.e.* $\lambda_1 = 514.5$ nm) followed by blue light (*i.e.* $\lambda_2 = 400$ nm), or by illuminating with green light and allowing thermal decay of the M_{412} state(Figure 1). The lifetime of the M_{412} state can be varied from milliseconds to hours by changing solvent, etc.

Bacteriorhodopsin films have been used in numerous proof-of-concept experiments demonstrating optical devices, including an artificial retina[4], real-time holography[5], schlieren optics[6], optical data storage[7], optical image processing[8] and spatial light modulation.[9] The advantages of bacteriorhodopsin over synthetic photochromic polymers include a well-understood photocycle, ease of thin film processing, high environmental stability and no evidence of photofatigue. Its disadvantages include high production cost, limits on the ability to engineer changes in the photointermediate absorption spectra and small photoinduced changes($\Delta n = 0.001$) in the real refractive index component.

PHOTOCHROMIC POLYPEPTIDE STRUCTURE-PROPERTY RELATIONSHIPS

Photochromic polypeptides are materials with the potential for use in similar applications to bacteriorhodopsin films. Structure-property relationships investigated include solvent control of photoinduced conformation changes, development of an accelerated thermal helix-to-coil conversion, and light-induced aggregation changes. In order to find the outer limits of photoinduced coupling between chromophore and polypeptide, succinylated poly(L-lysine) has been modified with the spiropyran 6-nitro-BIPS (BIPS = spiro(2H-1-benzopyran-2,2'-indoline) and light-induced conformation changes measured as a function of solvent composition.[10] The spacer group length between polypeptide α-carbon and the dye is 12 atoms, assuring minimum polypeptide-dye interaction. The polypeptide is an α-helix when dissolved in trifluoroethanol and a coil when in hexafluoroisopropanol. Photoinduced conformation changes occur in a narrow solvent composition window near the midpoint of the solvent-induced helix-to-coil transition. Zimm-Bragg theory can be used to calculate light-induced free energy changes per monomer.[11] The experiment requires measurement of helix content in a mixture of helix- and coil-forming solvents both under dark- and light- adaptation. The data can be used to measure initiation(σ) and propagation(s) parameters. Furthermore, the light-induced free energy change per monomer can be obtained,

$$\Delta G_{LA} - \Delta G_{DA} = -RT\ln\left(\frac{x_{LA}}{x_{DA}}\right) \quad (1)$$

where LA and DA stand for light and dark-adapted, respectively and x is the volume fraction of the coil-formimg solvent giving 50% helix content when the mixture is either light- or dark-adapted. The light-induced free energy change per monomer was measured in both spiropyran-modified succinylated poly(L-lysine) investigated in our laboratory[10] and calculated from literature data(Figure 2).[12-16] In all systems the light-induced free energy change per monomer was < *RT*, where *RT* is the Maxwell-Boltzmann thermal energy. The results suggest a mechanism whereby collective interactions between monomer units and solvent make possible the translation of a small light-induced energy difference (< *RT* per monomer) into large macromolecular conformation changes (>*RT* per macromolecule). These two systems are examples of polypeptides having a 'gated photoresponse'.[15,16]

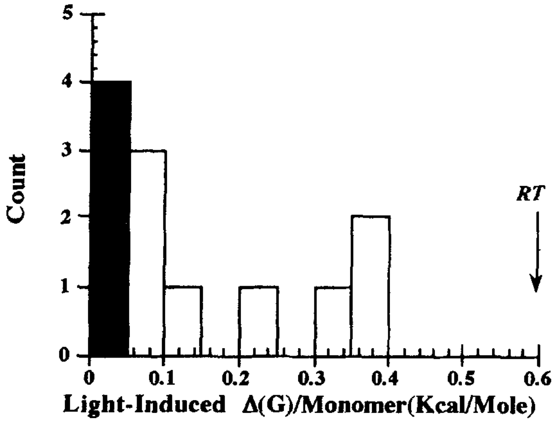

FIGURE 2 Histogram of light-induced free energy changes per mole monomer. Grey area emphasizes free energy changes less than 50 cal/mole/monomer

When the polypeptide is in gel or film form, a half life of ~10 sec is required to mimic a bacteriorhodopsin film. Control of the thermal back reaction half life is essential in the use of a photochromic polypeptide in optical applications. In order to accelerate the thermal back reaction of a photochromic polypeptide, poly(L-glutamic acid) has been modified with 6,8-dibromo-BIPS[17]. The polypeptide modified with 6,8-dibromo-BIPS has a thermal back reaction half-life of 80 sec, approximately 100 times smaller than poly(L-glutamic acid) modified with 6-nitro-BIPS. The rate constant of the dye thermal back reaction reaction equals that of the helix-to-coil interconversion, demonstrating coupling between the dye and the polypeptide state.

Two recently-described photochromic polypeptides show light-induced turbidity changes. An elastin polypentapeptide modified with phenylazoaniline undergoes an inverse temperature transition with a turbidity increase upon heating.[18] The phase transition temperature of the modified polypeptide increases from 32 °C to 42 °C after irradiation of the dye to the *cis* form. Irradiation of the polypeptide to the *cis* form causes a turbidity decrease, while subsequent conversion to the *trans* form increases the turbidity. An amphiphilic photochromic polypeptide has been synthesized by using *p-p'*-diaminoazobenzene as the initiator site for polymerization of the *N*-carboxyanhydride of L-glutamic acid γ-methyl ester.[19] Following polymerization, the authors prepare a rigid monolayer film of the polypeptide on an air-water interface. By introduction of sodium hydroxide into the aqueous phase beneath the solid condensed monolayer, they were able to selectively hydrolyze side chains oriented into the aqueous phase, thereby creating an amphiphilic, photochromic polypeptide. The dark-adapted polypeptide forms a micelle which deaggregates when light-adapted.

PHOTOCHROMIC POLYPEPTIDE THIN FILMS

Photochromic polypeptide thin films, including cholesteric[20], electrostatically self-assembled[21], Langmuir-Blodgett[22] and spin-cast[23] films, have been prepared. Induced circular dichroism appears in thermotropic cholesteric films of poly(γ-benzyl L-glutamate-*co*-γ-dodecyl L-glutamate) doped with an alkoxyazobenzene-anthraquinone bichromophore.[20] The authors investigate a series of these photochromic dichroic dyes in order to determine the relation between structure and induced circular dichroism. The induced circular dichroism intensity is influenced by cholesteric pitch, alkoxy tail length and the nature of the spacer group. The dichroism undergoes a reversible change in intensity upon interconversion between the *cis* and *trans* forms. These materials have applications as circular optical devices, photorecording media and optical filters. Optically clear, dichroic multilayer thin films of charged polypeptides with oppositely charged dyes are obtained by an electrostatic self-assembly technique.[21] The authors prepare films by alternately dipping a silanized glass slide into a poly(L-lysine) solution and then a dye solution. The films are composed of alternating monolayers of poly(L-lysine) and either the azo dye congo red or the light-sensitive dye copper phthalocyanine tetrasulfonate. The dyes exhibit intense induced circular dichroism, suggesting a highly chiral environment. Upon adhesion to the thin film surface, poly(L-lysine) undergoes a coil-to-helix conversion. The dyes tend to orient with respect to the dipping axis.

Detailed investigations of photochromic 'hairy rod' poly(L-glutamate) monolayers[22] have demonstrated the factors influencing the photomechanical effect, including temperature, surface pressure and the nature of the polymer. At constant surface pressure the monolayers exhibit an expansion upon irradiation with ultraviolet light resulting from *trans*-to-*cis* azobenzene photoconversion. Visible light irradiation causes the monolayer to shrink. The author proposes a two-step mechanism where photoisomerization occurs, followed by a rearrangement of the polar *cis* isomers towards the water surface.

The nonlinear optical properties of poled, spin-cast thin films of poly(L-glutamate) covalently modified with nonlinear chromophores, including spiropyran, have been investigated.[23] The measured second order nonlinear *d* coefficients compare favorably to those obtained in conventional polymeric systems, allowing for comparable efficiency of second harmonic, sum frequency and difference-frequency-generating processes.

HIGH-PERFORMANCE POLYPEPTIDE OPTICAL MATERIALS

Table 1 lists materials requirements for development of a thin film mimicing bacteriorhodopsin.

TABLE I. Requirements for high-performance polypeptide optical materials

- Diffraction-limited performance
- High photofatigue resistance
- High environmental stability
- Engineered chromophores to meet wavelength range, quantum yield requirements
- Processable into high optical quality oriented thin films
- Oriented thin film has the ability to produce measurable photovoltage
- Large shift in optical absorption maximum and extinction coefficient during photocycle
- Ability to vary thermal relaxation time from milliseconds to years
- Photochemical interconversion of intermediates

Currently, no single synthetic material has all these properties. However, literature data suggests that the elements for a biomimetic thin film exist, but need to be brought together into a single system. Numerous examples of high optical quality, oriented thin films have been described.[20-23] Photovoltage induced by spiropyran photochromism has been demonstrated.[24] The authors describe a protein sensor based on the potentiometric response of plasticized polymer doped with an antigenic spirobenzopyran derivative. The electrode potential increased 100 mV after 5 minutes of UV illumination. The original potential was recovered by visible-light irradiation or thermal decay with a half-life of 4 min. Large illumination-dependent changes in the absorption spectra can be seen in azobenzene[22] and spiropyran[24] systems. Diffraction-limited performance requires a high optical density ($OD > 3$) film. Thin films(1μ) are straightforward to prepare by spin coating, the Langmuir-Blodgett method or electrostatic self-assembly. Thick bacteriorhodopsin films ($200\ \mu$) are prepared by casting in a polymer matrix[25], a procedure that could be used in synthetic systems. Polypeptide systems allow for the possibility of tailor-made chromophores with user-defined absorption spectra, thermal decay rate and quantum yield. A current disadvantage to synthetic photochromic dyes is their tendency to bleach and undergo side reactions. Understanding the mechanism of bacteriorhodopsin photostability will assist in designing photostable synthetic systems. A novel system that may shed light on photostability are recently-described novel bacteriorhodopsin analogues based on azo chromophores .[26]

Photochromic polypeptides have the ability to undergo light-induced conformation and turbidity changes resulting from refractive index mismatch between the macromolecule and solvent. The large conformation changes only occur in solution, while the polypeptide retains the α-helix conformation when in thin film form.[21] It is possible that holograms resulting from a large modulation ($\Delta n = 0.1$) of the real refractive index component could be written in gels containing these polymers. The photostability of bacteriorhodopsin demonstrates that a suitable molecular environment will inhibit photofatigue. Invention of fatigue-resistant photochromic polypeptide films will make possible a new class of optical materials going beyond bacteriorhodopsin's capabilities.

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