Photodynamic protein incorporated in conducting polymer and sol-gel matrices: toward smart materials for information storage and processing

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ABSTRACT

We report our development of biomaterials for information processing and signal transduction by incorporation of photodynamic proteins into conducting polymer and sol-gel matrices. Our aim is to develop biomaterials with high optical quality, good thermal and mechanical stability, and superior opto-electronic characteristics for applications in biosensor, signal transduction, and information processing. A novel three-dimensional optical memory system based on a light transducing protein, bacteriorhodopsin, is designed and demonstrated.

1. INTRODUCTION

Biological macromolecules as materials possess superior intelligent properties because they are evolved through nature's selection process to function efficiently in response to their environment. During the past few years, several groups including our own have studied biomaterials based on biological macromolecules for potential applications in information processing and signal transduction[1-6]. Examples to be explored here are the photodynamic proteins, bacteriorhodopsin(bR), and phycobiliproteins. Bacteriorhodopsin is a light transducing protein that converts photon energy into chemical energy. Phycobiliproteins are molecular assemblies located on the outer membranes of algal cells that function as light receptors to funnel ambient light into the photoreactive center to drive photosynthesis. These proteins possess unique photochromic and photoelectric characteristics that have potential for applications in information processing and signal transduction. The rapid and reversible photochromic properties of bR has been used in optical memory and spatial light modulators. The large index change induced by photochromic transition has been applied for real-time holography and pattern recognition. The unique opto-electronic property has been used for neural network and artificial retina. Recently, we also developed a novel three dimensional optical memory system based on bacteriorhodopsin. All these applications require films of photodynamic proteins with high optical quality, good thermal and mechanical stability, and superior opto-electronic characteristics. A number of approaches has been used to develop these biomaterials with the desired properties. These include application of the Langmuir-Blodgett (LB) technique and incorporation of protein in water soluble polymer such as poly(vinyl alcohol)[6, 7].

Our aim is to develop smart materials for information processing and signal transduction by integrating the evolved specific signal transduction mechanisms of the biological macromolecules within monolayers or thin films of the conducting polymers or sol-gel matrices. In our previous studies, we have designed a generic "cassette" system that can incorporate biological molecules into conducting polymer in a LB film by the streptavidin-biotin interaction[4]. This system can be applied to any biomolecules that can be biotinylated. However, many applications require thick films with good mechanical stability. In this paper, we report our progress in developing biomaterials for information processing and signal transduction by incorporating photodynamic proteins in a conducting polymer and a sol-gel matrix. We also report our investigation on a novel three-dimensional optical data storage system based on bacteriorhodopsin to demonstrate the potential applications of these materials.
2. INCORPORATION OF PHOTODYNAMIC PROTEINS IN CONDUCTING POLYMER AND SOL-GEL MATRICES

2.1 Incorporation of bacteriorhodopsin in conducting polymer

Electrically conducting polymers have attracted interest due to their improved processability and possible applications in electro-optics[8]. Among these systems, polyheterocycles such as polythiophenes and polypyrroles are an important class of materials. The former can be tailored to achieve the desired bulk property and may be melt or solution processed[9]. Conjugated polymers not only act as a ‘rugged’ support for the attachment of biomaterials, but also may be used in signal transduction by coupling the properties of the biological macromolecule with the opto-electronic properties of the conducting polymer. These properties result from the extensive π–conjugation of the latter. The polythiophenes are also found to be good photoconductors[10]. The charge transport in these types of materials is facilitated through the π-conjugation. To demonstrate the opto-electronic signal transduction between a photodynamic protein such as bacteriorhodopsin and the conjugated polymer, we investigated the absorption and photoconducting characteristics of bR/conducting polymer composite films. Because bR is stable in aqueous buffer, a water soluble conjugated polymer, poly(3-thienylaceticacid) (PTAA), is synthesized for the preparation of the composite films.

Bacteriorhodopsin is the light transducing protein in the purple membrane of the microorganism Halobacterium halobium. The bR molecule consists of a single chain of 248 amino acids and a retinylidene that is covalently linked to a specific lysine residue of the protein moiety via a Schiff base linkage. In the living cell, bR acts as a light driven proton pump to convert light energy into chemical energy. Upon absorption of a photon, bR undergoes a photocycle characterized by a series of intermediates with different absorption spectra. Oriented bR film possess very interesting opto-electronic characteristics that has the potential to be used in image processing, optical neural networks, and artificial retina. Incorporating the photodynamic protein in the conducting polymer has the potential to provide a efficient means to transport the signal, generated in the biomolecules.

2.1.1 Method

PTAA is synthesized by polymerizing ethyl 3-thienyl acetate (Aldrich) using ferric chloride as a catalyst, followed by the alkaline hydrolysis of tetrahydrofuran (THF) soluble poly(ethyl-3-thienylacetate)[11]. The number and weighted average molecular weights of the precursor polymer poly(ethyl-3-thienyl acetate) are found to be 4,200 and 8,350 g/mole (using Gel Permeation Chromatography) respectively. The resulting polymer is soluble in dilute sodium hydroxide solution as well as in dimethylformamide. The structure of the conducting polymer is shown in Figure 2.1.

![Fig. 2.1 Structure of the poly(3-thienylaceticacid).](http://proceedings.spiedigitallibrary.org/)

Bacteriorhodopsin is isolated from Halobacterium halobium according to the method of Stoeckenius[12]. It is mixed with the conjugated polymer solution in dilute NaOH (58 mM). The resultant solution is cast on quartz windows and dried overnight under vacuum for the photocycle measurements. The UV-Visible absorption studies are performed with the solutions of different molar ratios of bacteriorhodopsin/conducting polymer. The concentration of the PTAA is kept constant in all the measurements, while changing the concentration of bR. The samples for photoconductivity measurements are prepared by casting a solution of mixtures (ratio of 0.05 to 1 of conducting polymer to bR by weight) on interdigitated electrodes followed by drying of the samples under vacuum. The samples are excited at 514 nm using a laser power of about 25 mW to study the photocurrent behavior of the composite films. For comparison, the photoconductivity of bR films without photoconducting polymer is also measured.
2.1.2 Results and discussion

Absorption spectra of mixtures of polymer and protein in different ratios are shown in Figure 2.2. The conjugated polymer PTAA solution in dilute NaOH shows an absorption maximum at 440 nm, with the tail extending up to 600 nm. The absorption maximum of bacteriorhodopsin is at 570 nm. When bR solution is added to the conducting polymer, the absorption peak at 440 nm shows a slight increase in intensity, with a small blue shift. This is probably due to the presence of protein in the M-state which has an absorption spectrum peak at 412 nm. This result suggests that M-state lifetime increases significantly in the conducting polymer/protein mixture which is partly due to the high pH of the solution.

![Absorption spectra of bR/PTAA mixtures with different bR/PTAA ratio.](image)

Fig. 2.2 Absorption spectra of bR/PTAA mixtures with different bR/PTAA ratio.

The protein activity of bR incorporated in conducting polymer is tested by its photocycle. It is known that when bR is excited, it undergoes a photocycle that has several different intermediates with distinct absorption spectra. The M state, one of the intermediates that can be stabilized at -40 °C, has a large blue shifted absorption spectrum. Figure 2.3A shows the absorption spectra of bR/PTAA film before and after illumination at 570 nm. The difference spectrum obtained suggests that the photochromic transitions involved are bR and M states, respectively (Figure 3.2B). These results clearly indicate that bR is active in the bR/PTAA film and one can switch back and forth between these two photochromic states with light of appropriate wavelength.

The result of the photoconductivity measurement from a bR/PTAA film is shown in Figure 2.4. Photocurrent as a function of applied voltage is measured. For comparison, we also show results from bR film without the photoconducting polymer. The Figure shows that the photoconductivity increases upon the addition of the conducting polymer. This result indicates that the conjugated backbone of the photoconducting polymer can facilitate the charge transportation of the signal generated by bR upon photoexcitation. Although the increase in the photoconductivity is small, this is probably due to the small amount of the conducting polymer (5% by weight) in the film. The optimization of the amount of conducting polymer in the composite to achieve high photocurrents is under investigation.
Fig. 2.3  Absorption spectra of photochromic states bR and M. The two curves in Figure A correspond to the spectra from bR/PTAA film before and after illumination at 570 nm, respectively. The curve in Figure B is the difference spectrum.

Fig. 2.4  Photocurrent as a function of applied voltages for bR and bR/PTAA film.
2.2. Incorporation of photodynamic protein into sol-gel matrices

The sol-gel process is a technique to produce transparent glass without high temperature processing. This transparent glass has superior optical, mechanical, and thermal properties. Sol gel process involves hydrolysis and polycondensation of alkoxides. The following schematic shows a typical sol-gel process to produce silica glass (R is an alkyl group):

\[
\begin{align*}
\text{OR} & \quad \underset{\text{RO} - \text{Si} - \text{OR}}{\text{OH}} + 4 \text{H}_2\text{O} \quad \longrightarrow \quad \underset{\text{OR}}{\text{HO} - \text{Si} - \text{OH}} \\
\text{OH} & \quad \underset{\text{OH}}{\text{HO} - \text{Si} - \text{OH}} + n \underset{\text{OH}}{\text{HO} - \text{Si} - \text{OH}} - 4(n+1) \text{H}_2\text{O} \quad \longrightarrow \quad \underset{\text{O}}{\text{O}} - \text{Si} - \text{O} - \text{Si} - \text{O} \\
\text{OH} & \quad \underset{\text{OH}}{\text{HO} - \text{Si} - \text{OH}} \quad \underset{\text{OH}}{\text{HO} - \text{Si} - \text{OH}} \quad \underset{\text{OH}}{\text{HO} - \text{Si} - \text{OH}} \quad \underset{\text{OH}}{\text{HO} - \text{Si} - \text{OH}} \quad \underset{\text{OH}}{\text{HO} - \text{Si} - \text{OH}}
\end{align*}
\]

The appropriate alkoxide is first hydrolyzed to form silanol groups (Si-OH) and alcohol. Subsequent condensation reactions involving the silanol groups produce siloxane bonds (Si-O-Si). The process of condensation to form siloxane groups continues to occur during aging. Finally, the gel is dried to produce a transparent glass with good optical quality. All the reactions can take place at room temperature. The low temperature process makes it possible to incorporate biological material into glass matrices. However, conventional synthetic conditions for sol-gel glass formation are too harsh for most proteins because of the sensitivity of the proteins to alcohol and extreme pH environments[13, 14]. Encapsulation of enzyme and other proteins using a modified sol-gel protocol has been reported[13, 14]. We report here encapsulation of phycobiliproteins in a sol-gel matrix.

Phycobiliproteins are molecular assemblies located on the outer membranes of marine algae cells that function as light receptors to funnel ambient light into the photoreactive center to drive photosynthesis[15]. The light receptor portions of these proteins are open chain tetrapyrroles coupled to the protein residues through thioether linkages. The phycobiliproteins are arrayed in vivo in an antennae-like structure called the phycobilisome, each with a region of maximum and relatively narrow wavelength of absorption in the visible spectrum. Light energy is transferred by a Forster type mechanism with over 90% efficiency. Phycoerythrin (PE), one of the outermost phycobiliprotein shows very strong fluorescence (more than 20 times larger than fluorescein) with high quantum yields and a large Stokes shift (a factor of 2.7 larger than fluorescein). These proteins are widely used as biochemical and biotechnological probes because of their interesting optical properties[16]. Sol-gel immobilized phycobiliprotein with appropriate pore size has the potential for application in biosensors and signal transduction.

2.2.1 Methods

We use the sol-gel system based on tetramethoxy silane (TMOS) for protein encapsulation. The TMOS silica sol is prepared by mixing 0.1 mole of TMOS with 3.6 ml of 2.5 mM HCl. After sonicator for 30 minutes, the silica sol (1 ml) is mixed with 2 ml of phycoerythrin (50µg/ml) in 0.01 M sodium phosphate buffer (pH 7). The PE doped sol is then transferred to a cuvette. The sample gels in about 2 minutes and is then aged for about 2 weeks. During the age process, the PE doped sol-gel sample is washed with deionized water once a day to remove alcohol. The washing process is essential since the alcohol accumulated could denature the protein. The aged gel is then dried at room temperature.
2.2.2 Results and discussion

The activity of the sol-gel immobilized protein is tested by its absorption and fluorescence spectrum. Figure 2.5 shows absorption spectra of PE doped TMOS gel sample. For comparison, the absorption spectrum of PE in solution is also shown. These two spectra have almost identical profiles. The similarity between these spectra indicates that the sol-gel matrix seems to have no effect on the absorption spectrum of the protein.

![Absorption spectra](image)

**Fig. 2.5** Absorption spectrum of PE in sol-gel matrix (A); and in solution (B).

Figure 2.6 shows the fluorescence spectra of PE in sol-gel and solution. The fluorescence is measured (SLM instruments) with an excitation wavelength of 490 nm. The fluorescence of PE in the sol-gel matrix is found to be very similar to that in solution except for a slight red shift. It is known that the denatured PE loses its fluorescence capability. The strong fluorescence signal observed clearly indicates that immobilized PE is active in the sol-gel matrix. We are currently working on a biosensor based on the sol-gel immobilized enzyme and photodynamic proteins. One objective is to design and develop a sol-gel system with large pore size that will allow the diffusion and detection of the targeted molecules.

![Fluorescence spectra](image)

**Fig. 2.6** Fluorescence spectrum of PE in sol-gel matrix (A); and in solution (B).
3.

PROTEIN BASED TWO-PHOTON THREE-DIMENSIONAL OPTICAL MEMORY

High capacity and high speed memories are becoming increasingly important for the rapid development of information technology. The computational power, once limited by the CPU, is now determined by the interconnection and data access bandwidth. Present memory technologies, such as magnetic disk, optical disk, and semiconductor memory, store information in a planar surface. The two-dimensional nature of the present storage devices limits the storage density to \(-1/\lambda^2\), where \(\lambda\) is the wavelength. If a visible or infrared laser is used, this yields a storage density of approximate \(10^8\) bits/cm\(^2\). In contrast, if information is stored in a three-dimensional format, maximum storage density is limited by \(1/\lambda^3\) with a data storage density of \(10^{12}\) bits/cm\(^3\) can be achieved. In addition, three-dimensional (3-D) optical storage offers the potential of providing parallel access. Two-photon three-dimensional optical memory using organic, semiconductor, and biomolecular materials has been investigated [17-19]. However, lack of materials and efficient data access methods have impeded its development. We present in this section a bacteriorhodopsin based 3-D erasable optical storage system that uses the nonlinear optical properties of bacteriorhodopsin to carry out nondestructive reading. Specifically, two photon induced photochromic transition is used for writing and the second harmonic (SH) generation is used for reading. The 3-D memory system based on this reading method has the advantage of fast speed, high contrast, and potential for parallel access. Bacteriorhodopsin is a good material for this 3-D memory because it possesses long term thermal stability and a high threshold to photochemical degradation, exhibits large quantum efficiencies for photochromic transition, and has a large two-photon absorption cross section and second order optical nonlinearity. In addition, synthetic and genetic engineering technology can be used to modify the protein to optimize its properties for specific applications.

3.1 Design

Bacteriorhodopsin exhibits an unusually large optical nonlinearity due to the large light induced change in dipole moment. The observed two photon absorptivity (\(\delta\)) is 290 GM, which is almost a factor of 10 larger than that observed for the isolated retinal chromophore[20]. This large two-photon absorptivity coupled with the high quantum yields for the transition provides an ideal photochromic material for 3-D memory. The two photon induced photochromic behavior can be summarized in the following schematic diagram:

\[
\begin{align*}
\lambda &= 1140 \text{ nm}; \ 2\omega; \ \Phi_1 \sim 0.65 \\
\text{bR [State 0]} &\rightarrow \text{M [State 1]} \\
\lambda &= 820 \text{ nm}; \ 2\omega; \ \Phi_2 > 0.65
\end{align*}
\]

Fig. 3.1 Two-photon photochromic states of bR and the corresponding absorption spectra.

Where \(\Phi_1\) and \(\Phi_2\) are the quantum yields of the forward and reverse phototransition respectively. Information can be stored in these photochromic states as binary states which are arbitrarily assigned 0 and 1 in the figure.

The 3-D optical memories we investigated use the nonlinear optical properties of bacteriorhodopsin for the data access. Figure 3.2 shows a schematic diagram of an erasable 3-D optical storage system based on bacteriorhodopsin. The storage medium is a disk made from oriented bacteriorhodopsin in a polymer matrix. The system is adaptable to a rotating disc format similar to a compact disc or optical disc system. The difference
between this system and the conventional 2-D optical disk is that multiple layers of 2-D arrays of data can be stored and accessed. Therefore, a bacteriorhodopsin disk can be considered as a stack of 2-D optical disks. The data storage capacity equals the data density of the 2-D disk multiplied by the number of the disks in a 3-D stack. However, this approach is different from the current method of increasing capacity by using multi-disk readers or jukeboxes. The multi-disk drives require multiple optical heads, and the jukebox approach suffers from protracted seek time due to the requirements of mechanical mounting and initial spin up. The design shown here is much more compact and has a fast data access time. For example, a bacteriorhodopsin disk with thickness of 1 mm can hold 100 layers of data assuming that the separation of each data stack is less than 10 μm. Writing in this memory is performed by two-photon absorption and reading is accomplished by SH generation. Because both two-photon excitation and SH generation depend quadratically on intensity, reading and writing with 3-D resolution can be accomplished by this nonlinear process with either a focused beam or the overlap of two beams. The response of materials to the nonlinear reading and writing beam is confined to the focal plane or the overlap region. The results of two-photon studies on bacteriorhodopsin indicate that this protein has a very large two-photon absorption cross-section that can be used to accomplish the writing operation. We will focus on data access method of using second harmonic generation.

Fig. 3.2 Schematic diagram of two-photon three-dimensional optical memory based on bacteriorhodopsin. The storage medium is a disk made from oriented bacteriorhodopsin in a polymer matrix.

3.2 Experiment

Optical SH generation is the lowest second-order nonlinear optical process, in which the second-order polarizability of a material is responsible for the generation of light at the second harmonic frequency. To use this process to reliably assign state, the storage medium must possess large second order susceptibility in one of the photochromic states and a large difference in SH generation efficiency between different photochromic states. Previous studies by Chen et al. have indicated that the bR state has a very large second order nonlinearity[6, 21]. When a 1.06 μm laser is used as a fundamental beam, the SH efficiency of purple membrane in bR state is at least an order of magnitude larger than that in M state. However, if a 1.06 μm laser performs 3-D reading, the SH signal generated will be absorbed by a neighboring molecule, which results in
destruction of the data. To achieve nondestructive reading in a 3-D memory, one must choose a reading laser beam with the wavelength of the fundamental and SH photon outside the absorption band of the molecule.

The 1.54 μm laser beam is obtained from a Raman shifting cell pumped by a Q-switched Nd:YAG laser with a 10-Hz repetition rate and 10-ns pulse-width. The infrared beam at 1.064 μm from the Nd:YAG laser is focused and passed through a Raman shifting cell with pressurized methane gas to obtain multi-Stokes and anti-Stokes lines. The first Stokes line at 1.54 μm is selected through a dispersion prism. This IR beam with an average power of less than 2 mJ is used as the fundamental beam. The energy of the reading beam and its SH beam are not in resonance with bR or M states. A Glan laser polarizer and a half-wave plate are used to change the polarization state of the fundamental beam. The transmitted SH signal is measured by a photomultiplier tube and the signal is averaged with a boxcar integrator. A separate tungsten lamp with appropriate filters is used to induce the photochromic switch between the bR and M state.

3.3 Results and discussion

Figure 3.3 shows the measured second harmonic signal generated from molecules in the photochromic states bR and M. L1 is the light to switch molecules in the bR state to the M state. L2 is the light to convert molecule in the M state back to the bR state. The molecule is initially in the bR state. The first peak intensity corresponds to the SH signal from molecules in the bR state. When L1 is turned on, molecules in the bR state are photochemically pumped to M state. We observe a decrease in the SH intensity. If we then turn off L1 and turn on L2, molecules in the M state are switched back to the bR state and the SH intensity returns to the high value. There is a large change in the SH signal when one of the switching lights is turned on to initiate photochromic switching. This change indicates that there is a large difference in second order nonlinearity between molecules in the bR and M states even for a beam with wavelength away from resonance. The contrast ratio between signals from bR and M states is about 6, which is large enough to assign state. Because both the fundamental and SH photon of a 1.54 μm beam is not absorbed by either photochromic state, this result demonstrates that SH generation can be used to perform nondestructive reading in a 3-D optical memory.
Besides the nondestructive nature of using SH generation to perform 3-D reading, this method has the advantage of fast speed and potential for parallel access. Because the SH generation does not involve molecular transition, the SH photon is generated instantaneously as the fundamental beam passes through the nonlinear media. The response time is more likely limited by the photon detection device.

The bacteriorhodopsin based 3-D optical storage system discussed above is only one of many possible architectures. Other forms of bacteriorhodopsin polymer matrices such as an oriented bacteriorhodopsin cube can also be used in a conventional 3-D optical storage system. Since the SH beam has a well-defined beam direction, reading with the SH signal can take advantage of the parallelism inherent in the optical system. For example, an array of laser diodes can be used to access an array of data simultaneously. In addition, the linear and nonlinear optical properties of bacteriorhodopsin are affected by the chromophore and its interaction with the protein environment[22]. Therefore, these properties can be optimized through synthetic chemistry and biotechnology.

4. SUMMARY

We have developed biomaterials for information processing and signal transduction by integrating photodynamic proteins with conducting polymers and sol-gel matrices. This integration provides a versatile means to improve optical, mechanical, and electrical properties of materials based on biological macromolecules. A novel three-dimensional optical memory system based on bacteriorhodopsin that uses two-photon absorption and second harmonic generation to carry out nondestructive reading and writing operations is designed and demonstrated.

5. ACKNOWLEDGMENTS

The authors wish to thank Dr. Ravindran for his assistance in conductivity measurement. Financial support from ARO under grant DAAL 03-9-0004 is gratefully acknowledged.

6. REFERENCES


22. Z. Chen, M. Sheves, A. Lewis, *Submitted to Biophys. J.*