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Phycobiliproteins Encapsulated in Sol-Gel Glass

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Abstract. Light transducing phycobiliproteins are encapsulated in optically transparent sol-gel matrices. Absorption and fluorescence spectroscopies are used to characterize the effect of the sol-gel encapsulation on the conformation and aggregation states of the three major phycobiliproteins present in phycobilisomes: phycoerythrin, phycocyanin, and allophycocyanin. It is found that the effects of sol-gel entrapment on the spectroscopic properties are significantly different for the three phycobiliproteins. The results indicate that phycoerythrin undergoes only minor change in its native structure when entrapped in sol-gel. However, significant changes in conformation and aggregation state occur when phycocyanin and allophycocyanin are entrapped in sol-gel matrices. A thin film of sol-gel encapsulated phycoerythrin is also coated on an optical fiber surface and strong fluorescence from the evanescent wave excitation is detected. The potential applications of sol-gel encapsulated phycobiliproteins in biosensors are discussed.

Keywords: sol-gel glass, phycobiliproteins, biomaterial, biosensor

Introduction

Materials based on biological macromolecules have received considerable attention for potential application in optical information processing and biosensor devices [1–5]. Examples include the light harvesting and light transducing proteins, such as bacteriorhodopsin (bR) and phycobiliproteins. Although photoactive biological proteins have been extensively investigated for their light transducing properties, only recently has the possibility of using such proteins as active components of photonic devices been explored. Bacteriorhodopsin has already demonstrated great promise as a potential optical material in dynamic holograms for pattern recognition [3], artificial photoreceptors for image processing [2, 5, 6], bipolar artificial neurons for neural networks [7], and storage medium for 3-D optical memory [1, 8]. Phycobiliproteins represent another important class of light transducing proteins that may support applications similar to those already demonstrated with bacteriorhodopsin [9].

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Phycobiliproteins are biomolecular assemblies located on the outer thylakoid membranes within marine algae. They are light transducing proteins that harvest and funnel ambient light into the photoreactive center to drive photosynthesis [10]. The phycobiliproteins (phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC)) are organized as stacked discs in the phycobilisome, each with a region of maximum and relatively narrow wavelength of absorption in the visible spectrum. In this unique arrangement phycobiliproteins are able to efficiently absorb and transfer light energy at low intensity levels via a Forster mechanism, to chlorophyll for photosynthesis with greater than 90% efficiency [11].

Phycobiliproteins are bulky, water soluble proteins that display very intense fluorescence with high quantum yields and a large Stokes shift [12]. The individual phycobiliproteins possess very interesting optical properties and are widely used today as fluorescent markers in biochemical and biomedical research [12]. In dried thin film form, phycoerythrin has demonstrated the ability to retain these optical properties as well as exhibit some interesting photovoltaic and photoconducting behavior [9]. These interesting optical and opto-electronic properties make phycobiliproteins promising candidates for signal transduction and biosensor applications.

However, to demonstrate the feasibility of phycobiliproteins for optical devices, phycobiliprotein based biomaterials with high optical quality and good thermal and mechanical stability first have to be developed. A number of approaches have been attempted to manipulate these proteins into useful architectures while still maintaining the desired optoelectronic properties. These include both the application of the Langmuir-Blodgett (LB) technique and the incorporation of protein into water soluble polymers such as polyvinyl alcohol [4, 13, 14]. In our previous studies, we have designed a generic "cassette" system that can incorporate biotinylated phycobiliprotein into a conducting polymer LB film matrix using the streptavidin-biotin interaction [4]. This system may be applied to any biomolecule that can be biotinylated. However, many applications require thick films with high optical quality and good thermal and mechanical stability. Recently, several groups have reported the incorporation of biological molecules into inorganic transparent glass using the sol-gel technique [15–17]. The sol-gel technique involves the hydrolysis and polycondensation of alkoxides to produce transparent glass without high temperature processing. The resulting transparent glass has superior optical, mechanical, and thermal properties. The encapsulation of bR in a transparent sol-gel glass has been reported by Weetall et al. [18] and Wu et al. [19]. The effect of sol-gel entrapment on the structure of albumin and myoglobin has been studied by Edmiston et al. [20] using absorption and fluorescence spectroscopies.

In this paper, we describe the encapsulation of phycobiliproteins into an optically transparent sol-gel matrix. Absorption and fluorescence measurements are used to characterize the effects of sol-gel encapsulation on the structure and function of phycobiliproteins. Although a number of biomolecules have been immobilized in sol-gel matrix, little information is available about the physical environment of the immobilized protein and the interactions involved. Phycobiliproteins are a family of homologous proteins built up from α-type and β-type subunits, each subunit has covalently attached open chain tetrapyrrole prosthetic groups. The spectroscopic properties of phycobiliproteins are very sensitive to the protein environment and aggregation states [21]. A comparison of the absorption and fluorescence spectroscopic properties of different phycobiliproteins entrapped in sol-gel matrices will provide information about the physical environment of the protein in the sol-gel glass and the effect of sol-gel entrapment on the protein conformation and aggregation states. In addition to the study of spectroscopic properties of phycobiliprotein doped sol-gel monolith, immobilization of light transducing phycobiliprotein on an optical fiber surface using sol-gel techniques is investigated. The fluorescence from the evanescent wave excitation from a PE coated optical fiber is measured and the potential applications of phycobiliproteins for biosensors are discussed.

Methods

Phycobiliproteins (B-phycoerythrin, C-phycocyanin, and allophycocyanin) were purchased from Cyanotech Co. (Hawaii) and used without further purification. A tetramethoxy silane (TMOS, Aldrich) based sol-gel system was used for the protein encapsulation. Sol-gels were prepared using procedures similar to those previously described [16]. Briefly, the TMOS silica sol was prepared by mixing 15.25 g of TMOS with 3.6 ml of 2.5 mM HCl. After sonicating for 25 minutes at 4°C, the silica sol (1 ml) was mixed with 2 ml of phycobiliprotein (50 μg/ml) in 0.01 M sodium phosphate buffer (pH

7). The phycobiliprotein doped sols were then transferred to cuvettes. The samples gelled in about 2 minutes and were then sealed with parafilm. Fresh gels were characterized with absorption and fluorescence spectra one hour after gelation. The samples were then stored at room temperature for aging and drying. During the two week aging process, the phycobiliprotein doped sol-gel samples were washed with deionized water three times to prevent the denaturation of protein due to the accumulation of alcohol. The absorption and fluorescence spectra of the aged gels were taken two weeks after gelation. The aged gels were exposed to ambient air for drying. Because complete drying results in partial bleaching of the samples, the drying process was terminated when about 2/3 of the solvent (by weight) had evaporated, which took about two to three weeks. The dried gel was then sealed with parafilm and the absorption and fluorescence spectra of dried gels were measured. The final protein concentration in sol-gel matrix is approximately 100 $\mu g/cm^3$.

The absorption spectra were taken using a uv/vis spectrophotometer (Perkin-Elmer Lambda 9). The fluorescence spectra were taken with a fluorescence spectrophotometer (SLM 8000 C, SLM Inc., New Jersey). The excitation wavelengths were set at 490 nm for PE, and 550 nm for PC and APC.

A multimode step-index optical fiber (SpecTran Co., Sturbridge, MA) was used for the thin film coating of PE by the sol-gel technique. The fiber consisted of a pure silica core with diameter of 200 μm. About 10 cm of the fiber jacket was removed by dipping the fiber in chloroform solution for approximately 15 min. The cladding of the tip was then etched away by hydrofluoric acid (HF, 48% by volume). The fiber tip was then tapered by controlling the vertical dipping and lifting speed of the fiber tip in the etching solution. A thin film coating of the PE doped sol-gel was then deposited on the fiber tip by dipping into the sol-gel solution for approximately 1 minute and then aging and drying at room temperature. To measure fluorescence from evanescent wave excitation, a 514 nm laser beam from an argon ion laser was used as the light source. The laser beam was coupled to the fiber through a beam splitter. The fluorescence signal transmitted back through the same fiber was then collected and focused to the input slit of a spectrograph with a CCD array. The signal detected by the CCD array was finally processed by a personal computer.

Results

Phycobiliprotein doped sol-gel glass exhibits good optical quality and no large scale aggregation of protein is observed. The protein is dispersed in the sol-gel matrix homogeneously and is optically transparent as shown in the photograph in Fig. 1. The phycobiliproteins PE

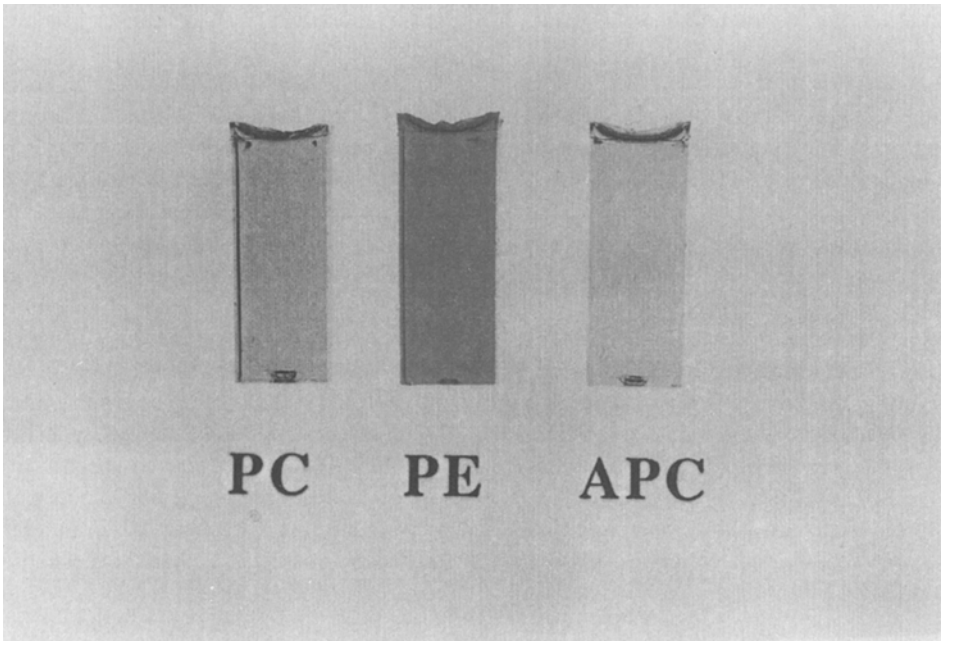

Figure 1. Photograph of sol-gel doped phycobiliproteins.

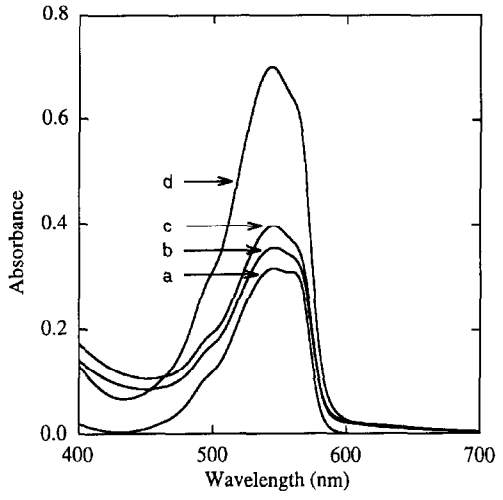

Figure 2. Absorption spectra of the PE in aqueous suspension and at different stages of the sol-gel process. (a) PE in buffer, (b) fresh gel, (c) aged gel, (d) dried gel

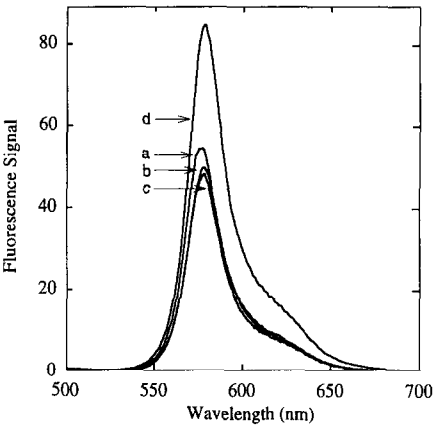

Figure 3. Fluorescence spectra of the PE in aqueous suspension and at different stages of the sol-gel process. (a) PE in buffer, (b) fresh gel, (c) aged gel, (d) dried gel.

and PC retain their characteristic colors while the color of APC changes slightly from blue to gray. When these samples are illuminated with a green beam (514 nm) from an argon ion laser, strong fluorescence from the phycobiliproteins is observed.

Phycobiliproteins have characteristic absorption and fluorescence bands. These spectroscopic properties are very sensitive to the protein environment and the conformational state [21]. Absorption and fluorescence spectroscopies are therefore used to monitor the effect of the encapsulation on the structure changes of phycobiliproteins throughout the sol-gel process.

Figure 2 shows absorption spectra of PE in buffer (a), and at different stages of the sol-gel process (b–d). The PE in solution has three characteristic absorption peaks at 500 nm, 543 nm, and 565 nm. PE in fresh gel retains the peak positions of these absorption bands. However, there is a small change in the relative absorbance of the band at 565 nm and 543 nm between PE in solution and in sol-gels. The absorbance at 565 nm diminished in sol-gels compared to the band at 543 nm. As the PE doped sol-gel ages and dries, the absorption band at 565 is further diminished in comparison to the band at 543 nm. Although part of this change can be attributed to the increased light scattering in the sol-gel samples, the PE in sol-gels show a small change (<5%) in the relative intensity of the absorption bands at 565 and 543 nm even after correction for scattering (scattering is corrected by subtracting a λ^{-4} scattering curve from the sol-gel absorption spectra). The optical density (OD) of the absorption band increases during the aging and drying process because of the shrinkage of the sol-gel that results in an increase in the density of the protein. Because 2/3 of the solvent (by weight) evaporates during the aging and drying process and the dried gel has approximately 1/3 of the volume of the fresh gel, the protein concentration increases by a factor of 3. If the gel shrinks isotropically and no change in extinction coefficient of the protein occurs in the sol-gel process, the predicted OD of dried gel is about a factor of $3^{2/3}(\approx 2.1)$ larger than that of the fresh gel. The measured OD of dried gel (Fig. 2d) is about a factor of 2 larger than that of the fresh gel (Fig. 2b), which is very close to the value predicted assuming no change in extinction coefficients due to sol-gel entrapment.

Figure 3 shows the fluorescence spectra of PE in buffer (a), and at different stages of the sol-gel process (b–d). The fluorescence emission spectrum of PE in solution peaks at 575 nm, while the spectra of PE in sol-gels are 2 nm redshifted as compared to that in solution. The profiles of the fluorescence emission spectra for PE in buffer and in sol-gel are nearly identical apart from this redshift. The dried sol-gel shows an increase in fluorescence intensity because of the increase in PE density due to shrinkage. The peak fluorescence intensity of dried sol-gel (Fig. 3d) is approximately a factor of 1.7 larger than that of the fresh gel (Fig. 3b).

Figure 4 shows absorption spectra of PC in buffer (a), and at different stages of the sol-gel process (b–d). Absorption spectra of PC in buffer and sol-gels are nearly identical except the peak position of the absorption spectra of PC in sol-gel is approximately 3 nm blueshifted. This small blueshift of the absorption band of PC entrapped in sol-gel glass is highly

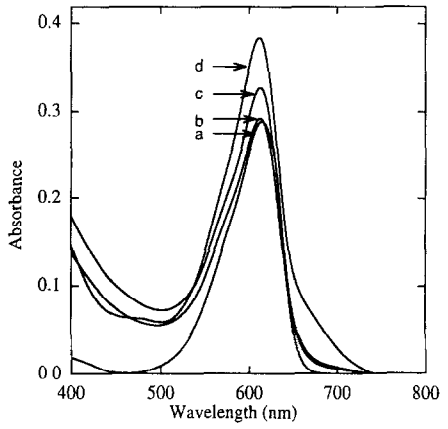

Figure 4. Absorption spectra of the PC in aqueous suspension and at different stages of the sol-gel process. (a) PC in buffer, (b) fresh gel, (c) aged gel, (d) dried gel.

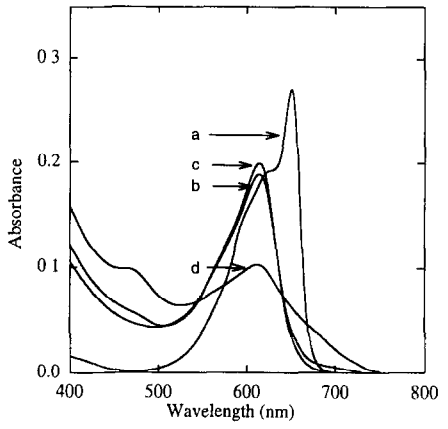

Figure 6. Absorption spectra of the APC in aqueous suspension and at different stages of the sol-gel process. (a) APC in buffer, (b) fresh gel, (c) aged gel, (d) dried gel

reproducible. In addition, dried sol-gel shows a small shoulder around 670 nm and the absorption band is also broadened. This shoulder appears at a similar wavelength as the absorption band of denatured PC [22]. This suggests that while PC is not denatured in fresh and aged sol-gel, a small fraction of PC denatures in the drying process. The OD of the aged and dried sol-gel samples increases as a result of shrinkage. However, the final OD of dried PC doped sol-gel is only a factor of 1.3 larger than that of the fresh gel, which is much smaller than the factor of 2 observed from PE doped sol-gel. Some of this difference in OD may be accounted for by the denatured PC fraction.

Figure 5 shows fluorescence spectra of PC in buffer (a), and at different stages of the sol-gel process (b–d).

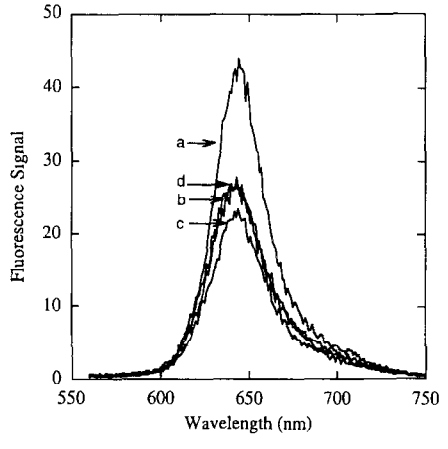

Figure 5. Fluorescence spectra of the PC in aqueous suspension and at different stages of the sol-gel process. (a) PC in buffer, (b) fresh gel, (c) aged gel, (d) dried gel.

The fluorescence emission spectra of PC in buffers and sol-gel are similar except that the fluorescence emission peak of PC in sol-gel is 3 nm blueshifted. However, the intensity of the fluorescence of PC in sol-gel decreases dramatically in comparison to PC in buffer. For PC in fresh gel, the fluorescence intensity is only 61% of the signal from PC in buffer, even though these two samples have similar absorbance. The fluorescence intensity from aged and dried PC doped sol-gel does not change significantly as compared to the fresh gel.

The largest effect of sol-gel on the phycobiliproteins is observed for APC. Figure 6 shows absorption spectra of APC in buffer (a), and at different stages of the sol-gel process (b–d). The APC in buffer has a characteristic absorption peak at 650 nm. A dramatic change in absorption spectra is observed when APC is entrapped in sol-gel; the prominent 650 nm band disappears. The 650 nm band is associated with the formation of the trimer $(\alpha\beta)_3$ [22]. The disappearance of this band at 650 nm indicates the loss of the trimer aggregation structure. There are a number of factors that could affect the aggregation state, including pH, ionic strength, and protein concentration [22]. Figure 7 shows the absorption spectra of APC titrated from pH 7 to pH 3.5. The band at 650 nm starts to diminish when the pH is below 5, and completely disappears at pH 3.5. The absorption spectrum of APC at low pH (Fig. 7g), which corresponds to the $\alpha\beta$ monomer, resembles the spectrum in sol-gel (Fig. 6b).

We have tried two modified protocols to determine whether the band at 650 nm can be preserved in the sol-gel process. One protocol is to mix 1 ml of 100 mM sodium phosphate buffer (pH 7.0) with the silica sol

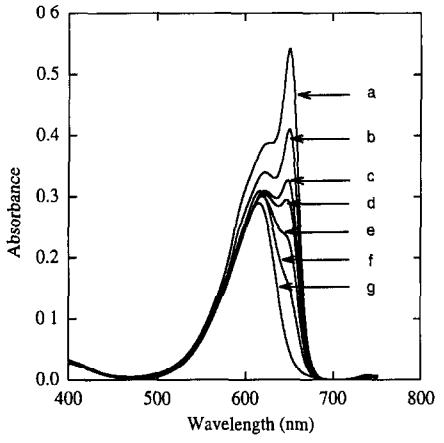

Figure 7. Absorption spectra of the APC in aqueous suspensions of different pH (a–d: pH 7.0, 5.9, 4.9, 4.6, 4 3, 4.0, 3.5).

before APC is added. This ensures that pH of the sol is near neutral pH before the protein is added. The second protocol is to immerse the fresh sol-gel in 100 mM sodium phosphate buffer (pH 7.0) immediately after gelation occurs. However, neither of these methods yields samples with an absorption band at 650 nm.

The dried sol-gel containing APC shows a broadened absorption spectrum in comparison of APC in buffer. The OD of the sol-gel sample also decreases significantly during the drying process. The absorbance of the dried sol-gel is only about 50% of fresh gel. The decrease in absorbance, together with the broadening of the spectrum, indicates that a large fraction of APC denatures during the drying process.

Figure 8 shows fluorescence spectra of APC in buffer (a), and at different stages of the sol-gel process (b–d).

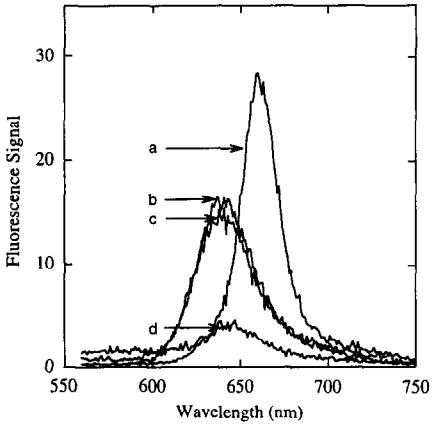

Figure 8. Fluorescence spectra of the APC in aqueous suspension and at different stages of the sol-gel process. (a) APC in buffer, (b) fresh gel, (c) aged gel, (d) dried gel.

A large shift in the emission spectra of APC in buffer and sol-gels is observed. While the fluorescence spectrum of APC in buffer peaks at 660 nm, the peak fluorescence spectra of APC in sol-gels are blueshifted to 640 nm. This is another indication that the native state of APC is changed by the sol-gel encapsulation. The fluorescence intensity of APC in fresh and aged gels is about 57% of APC in buffer, and during drying, the fluorescence intensity is further decreased. The fluorescence intensity of the dried gel is only 30% of the fresh gel. This supports the evidence from the absorption spectra that a large fraction of APC denatures during the drying process.

We also determined the leaching of phycobiliproteins from the sol-gel matrices. Phycobiliprotein doped sol-gel was equilibrated in aqueous solution for two days and the sample was then removed and placed in a clean cuvette. The absorption spectrum of the samples after equilibrating in aqueous solution shows no decrease in absorbance. In addition, the aqueous solution used for equilibrating the phycobiliprotein doped sol-gel was checked and no absorption band was observed. These results indicate that phycobiliproteins entrapped in sol-gel matrices do not leach out under the condition studied.

To demonstrate the potential application of the phycobiliprotein-doped sol-gel for fiber optic biosensors, a thin film of PE was immobilized on the surface of a tapered optic fiber by the sol-gel technique. The resulting fluorescence spectrum of PE on the fiber surface from evanescent wave excitation is shown in Fig. 9. The emission peak at 577 nm is a direct signature of the native PE protein and clearly indicates the presence

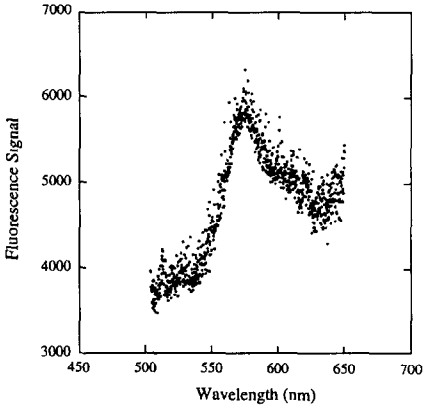

Figure 9. Fluorescence spectrum from a thin film of PE coated on the optical fiber by the sol-gel technique.

and optical properties of sol-gel encapsulated PE on the optical fiber surface.

Discussion

Phycobiliproteins are light transducing proteins that harvest and funnel ambient light into the photoreactive center to drive photosynthesis [10, 11]. They are a family of homologous proteins built up from α-type and β-type subunits of 17 to 22 kilodaltons. Each α and β subunit has covalently attached open chain tetrapyrrole prosthetic groups. The bilin chromophores associated with phycobiliproteins are rotational isomers of each other. However, their absorption and fluorescence bands differ. All of the major phycobiliproteins exist as oligomers of an $\alpha\beta$ monomer. The majority of purified biliproteins form disc-shaped trimeric or hexameric aggregates, with a dimension of between 100–120 Å in diameter and 30–60 Å in thickness [21].

In phycobilisomes of diverse biliprotein composition, phycobiliproteins are always organized in the order of their absorption maxima (PE, PC, and APC). The spectroscopic properties of phycobiliproteins are very sensitive to the protein environment and aggregation states [21]. Formation of higher aggregates is associated with an increase in the intensity of the long wavelength absorption band as well as increases in fluorescence lifetime and quantum yield [11]. It has been observed that absorption spectroscopic features of phycobiliproteins do not change on formation of the $\alpha\beta$ monomer from the constituent α and β subunits [21]. The absorption spectrum of the $\alpha\beta$ monomer is almost identical to the sum of the spectra of the constituent α and β subunits. However, the fluorescence quantum yield of the monomer is substantially higher than that of the individual subunits. Only the formation of larger aggregates results in large changes in both absorption and fluorescence spectra of phycobiliproteins [21]. When these proteins denature, due to heat or other environmental changes, their absorption characteristics change and their fluorescence responses diminish [21]. Absorption and fluorescence spectroscopy are therefore very sensitive probes to monitor the effect of the sol-gel encapsulation on the protein conformation.

PE is the outermost phycobiliprotein in the phycobilisome. It has a molecular weight of 240 kilodaltons. In addition to the α and β chains that carry two and three phycoerythrobilin chromophores respectively, PE contains a third polypeptide chain, the γ chain. PE is isolated as $(\alpha\beta)_6\gamma$ complexes with the γ chain as a linker polypeptide. The presence of the γ chain makes the hexamer aggregates $(\alpha\beta)_6\gamma$ of PE extremely stable [11].

The measured absorption of PE in sol-gel matrices is almost identical to that of the PE in buffer except for a small (5%) decrease in the 565 nm band. The increase in OD of dried sol-gel is similar to the predicted increase due to the shrinkages of sol-gel. This indicates that the extinction coefficient of PE does not change when it is entrapped in sol-gel matrices. The fluorescence spectra of PE in sol-gel are also very similar to that of PE in solution except for a 3 nm redshift. The change in the relative intensity of the two bands at 565 nm and 543 nm can be attributed to a small conformational change due to the rigid sol-gel matrix. The fact that changes in absorption and fluorescence are small indicates that the native conformation of the protein is not significantly altered by entrapment in sol-gel glasses. In addition, it has been known that spectroscopic properties of PE are very sensitive to the aggregation state. Dissociation of hexamer to monomer or subunits is accompanied by a blue shift in absorption and fluorescence spectrum and a reduction in extinction coefficient [21]. The fact that the fluorescence spectrum of PE in sol-gel is redshifted, together with the constant extinction coefficient observed in the sol-gel, indicates that the aggregation state of PE in sol-gel is also preserved. The high stability of PE in sol-gel is probably due the γ linker polypeptide that makes the protein aggregate a more stable structure.

PC in the rods of phycobilisomes is a hexamer complex, $(\alpha\beta)_6$. The α and β subunits each carry one and two phycocyanobilin chromophores, respectively. Isolated PC at physiological pH has a molecular weight of about 200 kilodaltons. It exists in various states of aggregation as a result of changes in pH, ionic strength, temperature, and protein concentration [23]. At low concentration (for example 1 μM), PC dissociates to $\alpha\beta$ monomer. The PC hexamer has an absorption maximum at 621 nm, dissociation of the hexamer to monomer shifts the absorption to 615 nm with a decreased extinction. Further dissociation of monomer into subunits does not change the absorption spectrum. However, the fluorescence yield is reduced significantly when monomer is dissociated into subunits [23]. Denatured PC shows a large redshift and broadening absorption band with more than a factor of 2 decrease in absorption extinction coefficient [22].

The measured absorption spectrum of PC in buffer peaks at 615 nm. This indicates that most of the PC

is in the $\alpha\beta$ monomer state which agrees with the low protein concentration used in this experiment. The absorption spectrum of PC in fresh gel is almost identical to that of the PC in buffer except for a small blueshift. However, the fluorescence intensity of the fresh gel is significantly reduced as compared to that of PC in buffer. This strongly suggests that a large fraction of PC monomer dissociates into α and β subunits when PC is entrapped in sol-gel. The spectroscopic feature of PC in fresh and aged sol-gel is very similar to that of PC in buffer, indicating that protein is not denatured upon entrapment in sol-gel. However, dried sol-gel shows a small shoulder around 670 nm, which is at a wavelength similar to the absorption band of the denatured PC [22]. This suggests that while PC is not denatured in fresh and aged sol-gel, a small fraction of PC denatures in the drying process. This is probably due to the shrinkage of the pore size during the drying process or to the loss of water's stabilization of the tertiary structure of this protein. However, rehydration of the dried sol-gel did not restore the spectroscopic properties of PC. This suggests that the shrinkage of the sol-gel denatures the PC and the denaturation is irreversible. The final OD of dried PC doped sol-gel is only a factor of 1.3 larger than that of the fresh gel, which is much smaller than the factor of 2.1 predicted if no change in absorption extinction coefficient occurs because of the sol-gel matrix. This is another indication that a small fraction of PC loses its native structure during the drying process.

APC is found in the core complex of phycobilisomes. It is isolated as a trimer complex, $(\alpha\beta)_3$, with a molecular weight of 100 kilodaltons. Each α and β subunit carries one phycocyanobilin chromophore. The aggregation states of APC depend on pH, ionic strength, temperature, and protein concentration. The absorption spectrum of the $\alpha\beta$ monomer peaks at 616 nm. However, the spectrum of the trimer shows a sharp absorption band at 650 nm [22]. Similar to PC, formation of $\alpha\beta$ monomer from subunits α and β reduces the fluorescence quantum yield but does not change the absorption spectrum. Denatured APC protein has an absorption band similar to the denatured PC [22].

The measured absorption spectrum of APC in buffer peaks at 650 nm, indicating that APC is in a trimer complex. However, upon entrapment in the sol-gel, the prominent peak at 650 nm completely disappears. The fluorescence peak is also shifted from 660 nm to 640 nm. The disappearance of the 650 nm band and the large blueshift in fluorescence spectrum clearly indicate that APC in sol-gels loses its native trimer aggregation structure. Because absorption spectra of the monomer and its subunits are identical, it is not possible to identify the aggregation state of APC in sol-gel from the absorption spectrum. However, the fluorescence intensity of APC in fresh gel is about 58% of APC in buffer. This value is very close to the 61% value observed in PC. This resemblance suggests that APC dissociates into subunits upon entrapment in sol-gel. During the drying process, the absorption spectrum is broadened and the OD decreases, suggesting that while APC is not denatured in fresh and aged sol-gel, a large fraction of APC denatures in the drying process. This is probably due to the shrinkage of the pore size during the drying process or to the loss of water's stabilization of the tertiary structure of this protein. However, rehydration of the dried sol-gel did not restore the spectroscopic properties of APC. This suggests that the shrinkage of the sol-gel irreversibly denatures the APC. The final OD of dried APC doped sol-gel is only 50% of the fresh gel. This value is much smaller than that observed in PC, indicating that a much larger fraction of APC lose their native structure during the drying process.

The results from phycobiliproteins encapsulated in sol-gel matrices indicate that while PE undergoes only minor conformation changes in sol-gel, PC and APC dissociate into subunits in fresh and aged gels and are partially denatured in the drying process. The dimensions of PE hexamer, $(\alpha\beta)_6\gamma$, are approximately 120×60 Å. The monomer and subunits of PA and APC have a much smaller size. Although the average pore size was not measured on the sol-gel glasses prepared here, published values of average pore size are typically less than 100 Å [24], which is comparable to the dimensions of the protein. Since PC and APC dissociate into subunits in sol-gel matrices, the dimensions of these proteins are much smaller than that of the hexamer of PE. If the dimension of the pore size is involved in determining the stability of the protein entrapped, one would expect that PC and APC, being smaller proteins, would be less susceptible to denaturation due to the shrinkage of the pore size. However, our results indicate that the larger hexamer structure of PE is more resistant toward the pore size shrinkage than that of small subunits of PC and APC. Since it is known that the PE hexamer is a very rigid structure due to the presence of the γ linker peptide, our results suggest that the stability of protein in sol-gel matrices depends more on the rigidity of the protein structure

due to the presence of the linker peptide than the size of the molecule.

It is also interesting to note that the effects of sol-gel on the phycobiliproteins increase from PE, PC, to APC, which is similar to their organization in phycobilisomes. The stability of PE can be attributed to the linker peptide unit that stabilizes the aggregation structure. However, why PC is more resistant to denaturation during drying than APC is not clear. One clear difference that exists between APC and the other two phycobiliproteins may be relevant here. APC interacts with the thylakoid membrane in the intact phycobilisome. The structural feature of APC that make this interaction possible may render it more susceptible to sol-gel denaturation. More research is required to elicit the interaction between the sol-gel environment and phycobiliproteins. For example, a measurement and comparison of the effect of sol-gel encapsulation on different subunits of phycobiliproteins will give us more insight into the interactions involved so that we may identify ways to improve the stability of the proteins in a sol-gel matrix.

Sol-gel encapsulated phycobiliproteins have potential applications for fiber optic biosensors. Fiber optic biosensors have received considerable attention due to the advantages of remote sensing capability and ease of miniaturization [25, 26]. A fiber optic sensor uses an optical fiber as the waveguide medium to transmit excitation light to the sensing material and to transmit the signal back to the detector. In addition, the optical fiber can also serve as the sensing element by directly immobilizing recognition molecules (e.g., antibodies, enzymes, etc.) on the fiber surface. Immobilization of biomolecules on the surface of a fiber is one of the critical issues for the successful development of fiber optic sensors. Therefore, a methodology that can effectively immobilize biomolecules onto a fiber surface while retaining their inherent molecular recognition properties in a stable environment is very desirable for biosensing. A number of groups have reported fiber optic biosensors based on the sol-gel technique [17, 27–29]. Phycobiliproteins are widely used as fluorescent tags in fluorescence immunoassay because of their extremely high absorbance coefficients, intense fluorescence emission, and large Stokes shifts [12]. Phycobiliprotein conjugates have been widely used in homogeneous energy-transfer immunoassays [12]. For example, Kronick and Grossman have developed an immunoassay to determine the concentration of human IgG by using the energy transfer between the fluorescein-labeled human IgG and phycoerythrin-labeled antibody [30]. They have demonstrated that energy transfer efficiency as high as 80% could be achieved. The resulting fluorescence spectrum of PE immobilized coated on the fiber surface by the sol-gel technique, based on evanescent wave excitation, indicates that it is possible to develop an optical fiber biosensor using sol-gel encapsulated PE. The simplicity of sol-gel encapsulation, together with the high stability of PE in sol-gel glass [31], suggests that PE doped sol-gel glass is a promising material for biosensors.

Summary

Phycobiliproteins (PE, PC, and APC) are encapsulated in optically transparent sol-gel matrices. Absorption and fluorescence spectroscopies show that the effects of sol-gel entrapment on the protein conformation and aggregation states are significantly different for these three phycobiliproteins. While PE only undergoes minor change in its native structure when entrapped in sol-gel, significant changes in conformation and aggregation state occur when PC and APC are entrapped in sol-gel matrices. A thin film of sol-gel encapsulated light transducing PE has been successfully coated on an optical fiber surface. The strong fluorescence from the evanescent wave excitation from PE coated optical fiber illustrates the potential of sol-gel encapsulated phycobiliproteins for biosensor applications.

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