Bacteriorhodopsin Encapsulated in Transparent Sol–Gel Glass: A New Biomaterial

Shuguang Wu,† Lisa M. Ellerby,† J. S. Cohan,† Bruce Dunn,*,‡ M. A. El-Sayed,*,† J. Selverstone Valentine,*,† and Jeffrey I. Zink*,†

Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, California 90024, and Department of Material Science, University of California, Los Angeles, Los Angeles, California 90024

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The photosynthetic membrane protein of bacteriorhodopsin (bR) was encapsulated in an optically transparent and porous silica matrix using a modified sol–gel procedure. The absorption spectra and the kinetics of the photocycle characteristic of the proton pumping function of bR were studied systematically throughout the different stages of the glass formation process. This new biomaterial was characterized by means of its optical absorption, circular dichroism (CD), and Raman spectra; its photocycle kinetics; the characteristic activation parameters of its photocycle; and its deionization and cation regeneration properties. The global trimeric bR structure, the local structure of the retinal chromophore, and the proton pumping function of bR were not affected by the encapsulation process. It was also found that the bR glass formed allowed transport of small ions such as Ca^{2+} in to and out of the glass medium, and those ions were found to affect the properties of the protein just as they do in aqueous suspensions. The bR protein was found to bleach if delipidated prior to encapsulation. These observations as well as analysis of the CD spectrum suggest that the bR is encapsulated along with its membrane lipids. These results taken together suggest that this optically transparent system offers a potentially useful new bR-containing material for optical imaging and optically based ion-sensoring devices as developed and proposed for other bR-based systems.

Introduction

New sol–gel procedures are now available for encapsulation of enzymes and other proteins in optically transparent, porous silicate glasses,[1,2] and it has been demonstrated that the encapsulated biomolecules retain their characteristic spectroscopic properties[1] and enzymatic activities[2,3] inside the glass matrix. New biomaterials prepared in this fashion show promise for use as optically coupled biosensors. The enzymes and other proteins initially chosen for encapsulation by this new sol–gel procedure were water soluble and relatively resistant to denaturation by the alcohol that was generated during the preparation of the sol–gel.[1,2] However, many of the proteins of interest for incorporation in new biomaterials are either lipid soluble, alcohol sensitive, or both. It was important, therefore, to determine whether the sol–gel method could be extended to the encapsulation of such proteins. Bacteriorhodopsin (bR) is a membrane-bound, alcohol-sensitive, light-transducing protein which has considerable potential for technical applications and thus provides an interesting system for encapsulation by the sol–gel method.

Bacteriorhodopsin is found in the purple membrane of *Halobacterium halobium*.[4,5] It consists of 248 amino acid residues in seven trans-membrane α helices and has a molecular weight of 26 000 D. Upon the absorption of light, bR undergoes a photocycle that pumps protons across the membrane, resulting in a pH gradient which is used as a proton-motive force for the synthesis of ATP from the ADP. The photocycle consists of a cyclic sequence of intermediates of lifetimes varying from 0.4 ps to several milliseconds (or longer for modified bR) (see Figure 1). During the photocycle, proton translocation is accomplished through the deprotonation step of the protonated Schiff base (the formation of M intermediate) and the subsequent reprotonation of the Schiff base (the decay of M intermediate).

In the past decade, in addition to extensive studies to elucidate the molecular mechanism of the proton pump of bR and to understand the relationship between its structure and biological function,[4,5] many papers have discussed the potential use of this light-transducing protein as an active component in optically coupled devices.[6–11] Some of the most unique properties of this protein include its high thermal and photochemical stability, its efficient conversion of light energy into electric energy, the large variation in the time of formation, and decay of the different intermediates of its photocycle (see Figure 1), and its photochemical properties such as its high quantum

† Department of Chemistry and Biochemistry.
‡ Department of Material Science.

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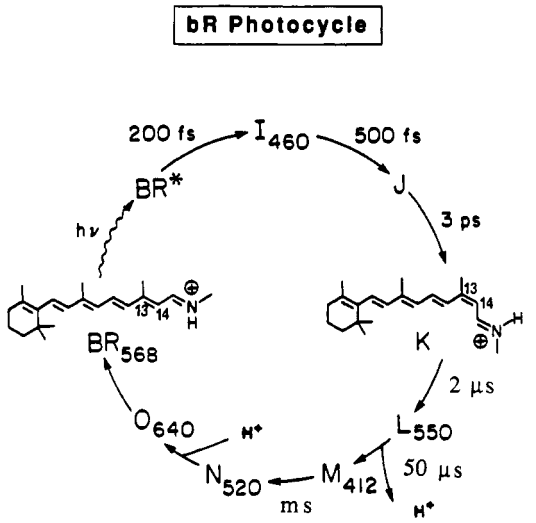

Figure 1. Scheme of the photochemical cycle of bacteriorhodopsin. The photointermediates formed during the cycle are abbreviated by single letters. Index numbers represent the absorption maxima of the intermediates. The deprotonation and reprotonation steps of the Schiff base are indicated by the release and uptake of the proton during M formation and decay processes.

yield and the broad frequency distribution of the ground-state absorption spectra and its photointermediates. In addition, genetic engineering of bacteriorhodopsin has been used to change some of the properties of bR proteins in order to meet the specific requirements of different technical applications.[7] Possible technical applications of bR to the molecular electronics area and optical information processing have been recently reviewed.[6-8]

Several different approaches have been taken to prepare new materials containing bR. Preparations incorporating bR in thin films formed using Langmuir–Blodgett or in polymer matrix using spin coating techniques have been successful,[9,10] and a photocell based on the use of optically thin film of bR in which the photoinduced current is shown to have great sensitivity to changes in light intensity has recently been developed by Miyasaka et al.[11] In the same paper, the imaging-detecting and processing potential of this bR-based biological photoreceptor were demonstrated.[11]

In the present paper, we describe a newly modified sol–gel procedure which resulted in the successful encapsulation of bR in optically transparent silicate glass. In addition, we describe spectroscopic, photochemical, and chemical studies of the purple bR glass that suggest strongly that the bR protein is encapsulated intact with its accompanying lipids and that the essential structure and photochemical and chemical reactivity of bR are retained in the glass.

Experimental Section

The silica sol was prepared following the procedures described previously[1,2] by sonication of tetramethoxysilane (TMOS, Aldrich, 15.25 g), deionized water (3.38 g) and 0.04 M HCl (0.22 g) in a ice cooled ultrasonic bath for 30 mins.[1] The silica sol (1.5 mL) was mixed with a 74 μM bR suspension (1.0 mL) in 0.01 M sodium phosphate buffer, pH 7.0 for the encapsulations. The silica sol (1.5 mL) was also mixed with phosphate buffer only and served as the baseline correction sample for the spectroscopic measurement. The bR-doped sol was quickly transfered to polystyrene cuvettes and then stored at 4 °C for aging and drying. During the aging process, the samples were washed with buffer solution twice a day in order to remove alcohol as it was formed and thereby to prevent the protein from denaturing and bleaching. After aging for approximately 2 weeks, the samples were exposed to air for drying at 4 °C. Usually 2–3 weeks was needed for the drying process. Complete drying resulted in partial bleaching of the samples; the drying process was therefore terminated when ~80% of the solvent by weight had evaporated. The resulting samples were then stored in buffer solutions.

The deionization of a bR-glass sample (λ_{max} = 568 nm) was carried out by dialysing the solid sample in double-distilled H_2O with the cation-exchange resin of Bio-Rad AG50W-x4 (Richmond, CA) in a Teflon beaker (ion-free) until a complete blue sample (λ_{max} = 602 nm) was formed, usually in ~3–4 h. The regeneration of the purple bR-glass was performed by soaking the blue sample in the cation-containing solution (e.g., Ca^{2+}) under a constant stirring. This process often took 3–5 h, depending on size of the cations involved.

Steady-state absorption spectra were taken with a Hewlett-Parkard 8451 diode array spectrometer. The CD spectra in bR in aqueous suspension and glass matrix were measured by using a JASCO-J-600 spectropolarimeter.

Resonance Raman spectra of bR in the glass matrix were obtained by using the 514.5-nm line of a Coherent Ar^+ laser. The scattered light was collected at a 90° angle and analyzed with a Spex 1402 double monochromator, an RCA C31034 photomultiplier tube, and a Stanford Research Systems photon counter. The laser beam was passed through the front face of the glass sample, parallel to the monochromator slit. The monochromator was stepped in 3 cm^{-1} increments with a dwell time of 10 s/point.

The transient absorption measurement was carried out by using the experimental setup described previously.[12] The photolyzing light was the 580-nm pulses from an N_2 laser pumped dye laser and had an energy of ~50 μJ with a temporal width of 0.5 ns. The 405-nm probe light was the output of a 100-W Hg arc lamp (Pek Labs 401, Sunnyvale, CA). The wavelength-selected probe beam was detected by a photomultiplier tube (RCA 1P28A, Lancaster, PA) and recorded with a transient digitizer (biomation 8100 waveform recorder; Gould Inc., St. Clara, CA), which was interfaced to the personal computer. The signals were averaged 1000–2000 shots and then converted to transient absorption in optical density. Sample temperatures were regulated using a water bath (Neslab RTE100D; Dublin, CA) and measured with a digital thermometer (Fiseer Scientific NBS LCD; Pittsburgh, PA).

Results and Discussion

Spectroscopic Characterization of bR Immobilized in the Glass Matrix during the Sol–Gel Process. The purple membrane protein of bacteriorhodopsin has a characteristic absorption band at 568 nm due to the retinal chromophore which is covalently bound to the apoprotein of bR through a protonated Schiff base (PSB) linkage. Upon denaturation by heating or adding denaturing reagents, the purple membrane starts to bleach and loses its retinal chromophore and thus its proton pumping activity. Therefore, the studies of this unique absorption band in the visible region are often utilized to characterize the structure and function of this protein. For this reason, the effect of encapsulation on the spectroscopic properties of this protein was monitored by optical spectroscopy throughout the process of the gel formation.

Figure 2 shows the absorption spectra of the retinal chromophore in bR in aqueous suspension (A), just before and during the sol–gel processess, i.e., gelation (B), aging (C), and during the different stages of drying (D–F). Comparison of the spectrum of bR in aqueous solution with those obtained during the gelation, aging, and drying

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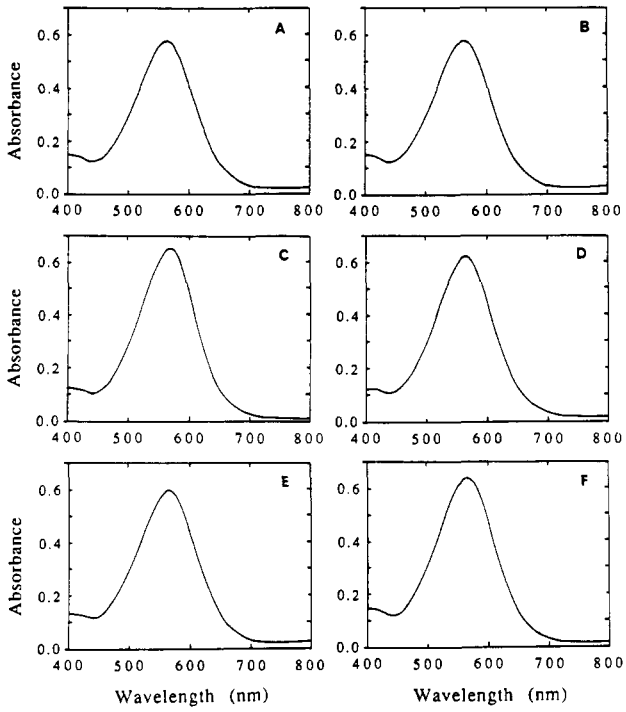

Figure 2. Absorption spectra of the retinal chromophore of the purple membrane bR in aqueous suspension (A) and in the gel during the different stages of its formation; gelation (B, 4 h after preparation), aging (C, 7 days after gelation), and drying (D–F, 7, 14, and 21 days after aging, respectively).

of the gels indicates that the absorption coefficient of the chromophore and its band shape do not undergo any significant changes. These results clearly indicate that the color of the protein and its spectroscopic properties are preserved during the glass formation.

To study the importance of the membrane bilayer in forming the glass matrix, we also attempted to form the glass matrix using delipidated bR (solubilized by Triton X-100 detergent) in the silica gel. Under those conditions, the bR protein started to denature and lose its purple color during the first 30 min of the gelation process, suggesting that the native lipid environment of bR plays an important role in stabilizing the protein structure as it is encapsulated in the glass medium.

Electron diffraction studies[13] have shown that the protein in bR is arranged in a two dimensional trimeric structure in the native lipid membrane. The trimeric structure of bR can be identified by its well-known biphasic CD spectrum in the visible region of the retinal absorption. Although the origin of this biphasic CD band is not well understood,[14,15] it is generally accepted that the biphasic band shape is directly associated with the presence of the trimeric bR structure in the membrane. Therefore, we can use this unique feature of bR CD spectrum to probe the global structure of this membrane protein. Figure 3 shows the CD spectra of the retinal chromophore of bR in the aqueous suspension (top) and the 80% dried sol–gel glass (bottom). It is obvious that the biphasic CD band is observed for bR in glass matrix as well as in solution. This result indicates that the entity of bR trimeric

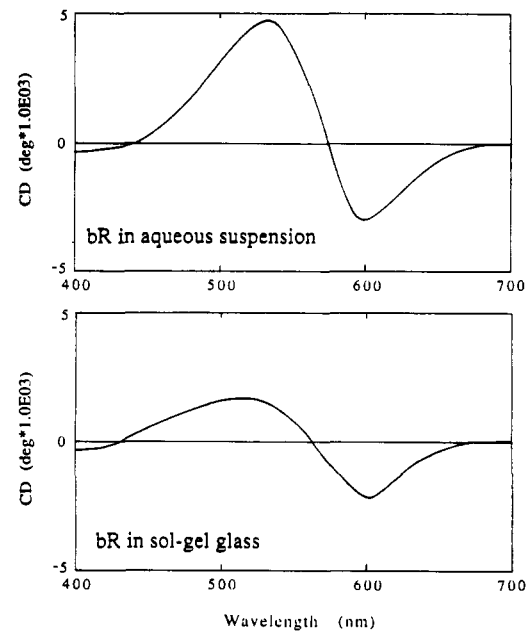

Figure 3. CD spectra of the retinal chromophore of the purple membrane bR in the aqueous suspension (top) and the sol–gel glass (bottom).

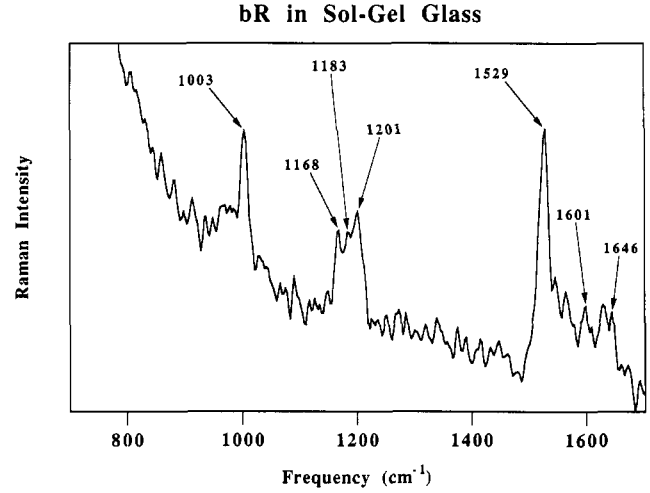

Figure 4. Resonance Raman spectra of the purple membrane bR in sol–gel glass. Spectra were obtained with a 200 mW, 514.5-nm excitation which is focused on the front surface of the glass sample.

structure is also preserved during the formation of the glass matrix.

Resonance Raman spectroscopy has been used as an in situ probe of the local structural changes of the retinal chromophore in bR.[16,17] The resulting vibrational spectra are very sensitive to the chromophore structure and to the interaction between the chromophore and its environment. To study the effect of the encapsulation of bR in glass matrix on the local structure of the retinal polyene chain, the steady-state resonance Raman spectra of bR glass were taken using 514.5-nm excitation as shown in Figure 4.

According to the well assigned Raman spectra of ground-state bR,[16] an intense ethylenic C=C stretching vibration appears in the region of 1500–1600 cm^{-1} (peak at 1529 cm^{-1}). The weak peaks in the 1100–1400-cm^{-1} region are

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assigned to C–C single bond stretching and vinyl hydrogen in-plane rocking; this part of the spectrum is referred to as the "fingerprint region" because these bands are sensitive to the cis–trans configuration of the C=C double bonds as well as the conformation of the C–C single bonds. The spectra in the 800–1050-cm^{-1} region are assigned to hydrogen out-of-plane (HOOP) and CH_3 rocking modes.

The Raman spectrum of bR in glass in Figure 4 resembles that observed for bR in aqueous suspension reported previously[16] except that some of the fine structure in the fingerprint region were not well resolved in the bR glass sample. Also, the spectra below 900 cm^{-1} were too weak to be observed in our experiment. The low signal-to-noise ratio in bR glass sample and the small differences in the spectra between the bR glass and aqueous suspension may arise from our low spectral resolution, probably due to the glass light scattering and small amount of stationary build-up of the photointermediates of the photocycle since the bR glass sample is not spun during the data acquisition. The C=C stretching frequency (at 1529 cm^{-1}) is the most intense and is in good agreement with that expected from the correlation between the wavelength of the optical absorption spectrum and the C=C stretching frequency.[17] The conformation fingerprint, the bands at 1201 and 1168 cm^{-1}, are similar in both systems. The weak band at 1190 cm^{-1} belongs to the 13-cis isomer. This observation suggests the presence of small amounts of the other intermediates in the cycle (or a small amount of dark-adapted bR). The strong intensity of the HOOP band at 1003 cm^{-1} is similar to that observed in solution and suggests similar degree of nonplanarity of the retinal system. The band at 1643 cm^{-1} suggests that the Schiff base nitrogen that binds the retinal system to the protein backbone is protonated, just as in aqueous suspension.

From the structural studies by absorption, CD, and resonance Raman spectroscopies, it can be concluded that the encapsulation of bR protein in the silica glass matrix does not affect the global structure of the protein nor the local structure of the retinal chromophore in this protein.

It should be pointed out that the native bR in the silica glass starts to bleach when the dryness of the sample reaches about 80%. Similar behavior was observed when films of bR were prepared under different conditions of humidity. We conclude that a certain degree of hydration is required to maintain the structure and function of the bR protein. It is a known fact that completely dehydrated bR films loses its proton pumping ability (i.e., loses its biological function).[18]

Proton-Pumping Activity of bR Immobilized in Sol–Gel Matrix. When the retinal chromophore absorbs the photon (λ_{max} = 568 nm), a photochemical cycle is initiated followed by the formation of a number of photointermediates on different time scale as shown in Figure 1. As a result of the photocycle, proton gradients are generated across the membrane bilayer. By extensive time-resolved spectroscopic studies (see references cited in refs 4 and 5), X-ray crystallographic structure analysis,[13] and systematic mutagenesis studies,[19] a great deal of understanding of the molecular mechanism of the proton pump of bR has been achieved. It has been known that the proton pumping from the inside of the protein to the extracellular surface of the membrane and the proton uptake by the protein from the outside of the membrane are directly related to the deprotonation and the reprotonation of the Schiff base within the protein.[4,5] Furthermore, it was also found that the rate of deprotonation and reprotonation of the Schiff base during the cycle are directly correlated to the formation and decay of one of the intermediates called M_{412} (λ_{max} = 412 nm). Therefore, the kinetics of the formation and decay of the M intermediate are usually studied in order to monitor the proton pumping function of bR.

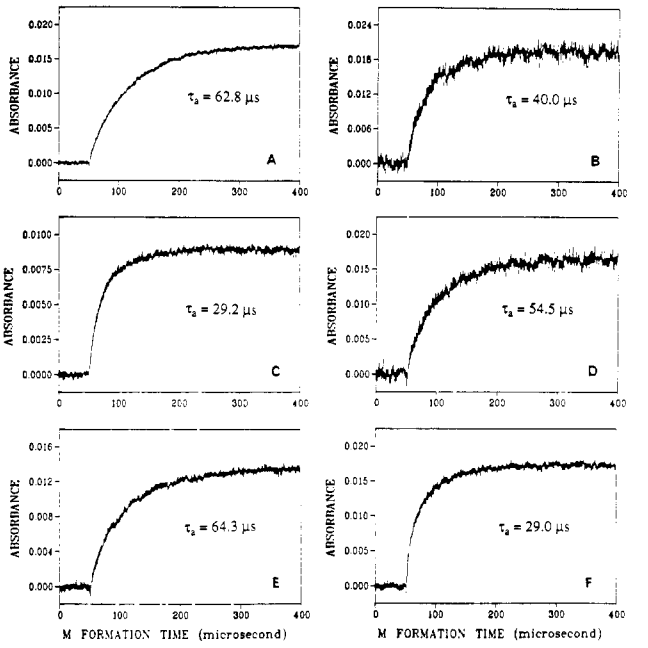

Figure 5. Formation kinetics of M intermediate in the photocycle of the purple membrane bR in aqueous suspension (A) and in the gel during the different stages of its formation; gelation (B, 4 h after preparation), aging (C, 7 days after gelation), and drying (D–F, 7, 14, and 21 days after aging, respectively). Kinetic parameters are summarized in Table I. Only average formation times are shown for each kinetic curve.

To test the effect of encapsulation on the proton pumping kinetics of bR in sol–gel glass, we monitored the kinetics of the M formation and decay during the entire processes of the gel formation. Figures 5 and 6 show the formation and decay kinetics, respectively, of the M intermediate monitored at 405 nm for the native bR in aqueous suspension (A) and at various stages of the sol-gel process, i.e., gelation (B), aging (C), and drying (D–F). From Figures 5 and 6, the following observations can be made: (1) bR retained its proton pumping function when it was embedded in the sol–gel glass matrix throughout the entire history of the gel formation. (2) During different stages of the gel formation bR had different kinetic behaviors as compared with bR in aqueous suspension for both M formation and decay process. (3) It seemed that the encapsulation of bR in the glass modified the M decay process (the reprotonation process of the Schiff base) much more profoundly than the M formation process. The bR in the glass which was 80% dry (Figure 6F) had ~10 times longer M lifetime than that in aqueous suspension (Figure 6A) while the M formation time was decreased by only a factor of 2–3 (Figure 5F,A). All the kinetic parameters for both M formation and decay are summarized in Table I.

Observation 1 clearly indicates that the light-transducing protein bR could be immobilized in a sol–gel glass matrix with retention of its proton pumping function. This result

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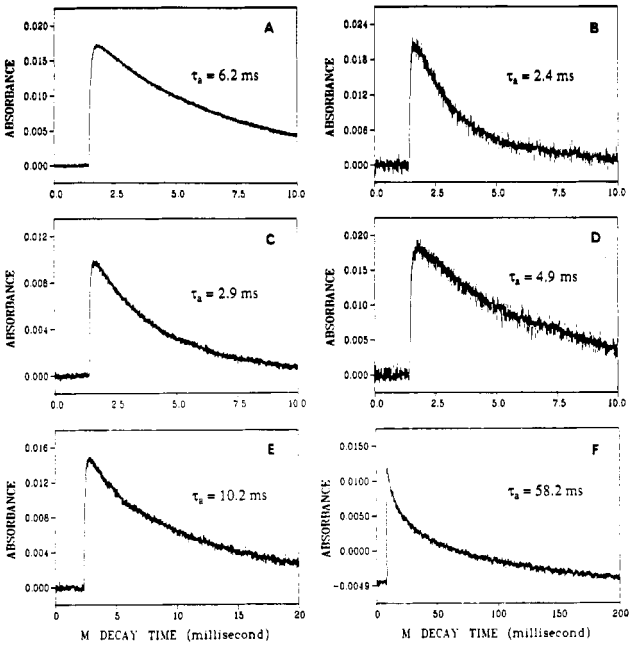

Figure 6. Decay kinetics of M intermediate in the photocycle of the purple membrane bR in aqueous suspension (A) and in the gel during the different stages of its formation; gelation (B, 4 h after preparation), aging (C, 7 days after gelation), and drying (D–F, 7, 14, and 21 days after aging, respectively). Kinetic parameters are summarized in Table I. Only average decay times are shown on each kinetic curve.

is consistent with our observation that the electronic absorption spectrum due to retinal was retained throughout the gel formation processes (Figures 2–4). These findings suggest that, in addition to bR in polymer films prepared previously, bR protein encapsulated in sol–gels should be considered as a candidate biomaterial with potential applications in molecular electronics and optical imaging processes.

The observed differences in the kinetics of proton pumping (observation 2) reflect that different chemical and/or physical environments are presented to the bR protein during the different stages of glass formation. It is known that the initial step in gel formation requires an acid catalyst and that a condensation reaction follows forming a silanol (Si–OH) and methanol. The silanol and methoxy groups then react to form siloxane (Si–O–Si) group producing water and methanol as byproducts. These processes continued to occur during the gelation and the aging stages. Further removal of solvent continues until the drying stage is complete and a rigid transparent material is formed. It is apparent from our results that changes in the environment of bR during the different stages of glass formation strongly influenced the photocycle kinetics. The fact that the photocycle kinetics were much more sensitive to the gel formation process than were the absorption spectra suggests that probing of the kinetic behavior of bR protein is more useful than conventional absorption spectroscopy in characterizing and monitoring the active site changes due to changes in the environment of the protein.

Observation 3 presents an important practical aspect in that the lifetime of the M intermediate in the glass matrix is increased by more than ~10 times relative to that in the aqueous suspension. The bR → M or M → bR process has been used in halogram recording.[7,8,20] Increasing the M lifetime gives rise to an increased photosensitivity or bleaching potential for the initial bR state or M state. Currently, the increase in the M lifetime is realized by using genetic engineering,[20] e.g., a substitution of the Asp96 residue by another amino acid residue. The observed large increase in the lifetime of M upon immobilization of bR in glass matrices suggests that these bR glasses may provide yet another promising biomaterial in addition to the films of bR mutants are currently in use.[7,8,20]

Studies of the Activation Parameters of the M Formation and Decay of bR Immobilized in the Sol–Gel Glass Matrix. We have further determined the activation parameters of M formation and decay for bR in the glass. Figure 7 shows the Arrhenius plots of the rate constants for both the M rise and decay at several different temperatures, and the derived activation parameters are summarized in Table II. The activation energy (63.6 kJ/mol) and entropy (61.3 J/kmol) for bR glass for the slow component of the M formation are very similar to those obtained for bR in aqueous suspension (E_a = 61 kJ/mol and ΔS_a = 60 J/kmol). On the other hand, for the M decay slow component, these parameters are much smaller for bR glass (E_a = 24.5 kJ/mol and ΔS_a = −142.0 J/kmol) than for bR in aqueous suspension (E_a = 73 kJ/mol and ΔS_a = 31 J/kmol). These results are consistent with the observed differences in the kinetic measurement of M formation and M decay for both bR in glass matrices and in aqueous suspension suggesting that the reprotonation channel (M decay) is modified more than the deprotonation channel (M formation) by the immobilization of bR in the glass. The protein conformation changes caused by glass formation may account for this difference in the energetic and entropic effects for both the M formation and the M decay process. The much reduced activation energy and the negative value of the entropy of activation for the reprotonation process are similar to those observed in bR suspension at high pH.[21] The reprotonation was suggested to involve a diffusion controlled process involving H_2O (or OH^-).

Effect of the Deionization and the Cation Regeneration on its Absorption Spectrum for bR Immobilized in the Sol–Gel Matrix. The glass pores in dried gels usually has an average diameter less than 100 Å. Thus, it should be possible for ions to readily pass through the porous glass medium. A well-washed purple membrane contains 3–4 mol of Mg^{2+} and ~1 mol of Ca^{2+}/mol of bR.[22] It is known that bR protein changes its color from purple to blue (i.e., the absorption maximum shifts from 568 to 602 nm) upon the deionization of the protein.[23] Meanwhile, the formed blue bR loses its proton pumping activity.[24] By the addition of cations such as Ca^{2+}, Mg^{2+}, and organic cations, the purple color of bR and its proton-pumping properties can be restored.

The purple bR-glass was immersed in a solution containing an ion-exchange resin under constant stirring for 4 h. A transparent blue bR-glass was formed, suggesting the depletion of the metal cations that control the retinal

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Table I. Formation and Decay Times of M Intermediate for bR in Aqueous Suspension and in the Gel during Different Stages of the Glass Formation at 23 °C[a,b]

	M formation					M decay				
	τ_{fast} (μs)	A_{fast} (%)	τ_{slow} (μs)	A_{slow} (%)	τ_{ave} (μs)	τ_{fast} (ms)	A_{fast} (%)	τ_{slow} (ms)	A_{slow} (%)	τ_{ave} (ms)
A	7.0	10	69	90	62.8	4.9	91	19	9	6.2
B	13.0	36	55	64	40.0	1.4	93	15	7	2.4
C	12.0	51	47	49	29.2	2.6	53	3.2	47	2.9
D	12.6	20	65	80	54.5	4.8	82	5.5	18	4.9
E	24.0	35	86	65	64.3	1.7	17	12.0	83	10.2
F	6.2	40	44	60	29.0	8.9	40	91.1	60	58.2

[a] A = bR in aqueous suspension; B = gelation (4 h after preparation); C = aging (7 days after gelation); D–F = drying (7, 14, 21 days after aging stage). [b] Average formation and decay time tave is calculated according to equation: $\tau_{ave} = A_{fast}\tau_{fast} + A_{slow}\tau_{slow}$.

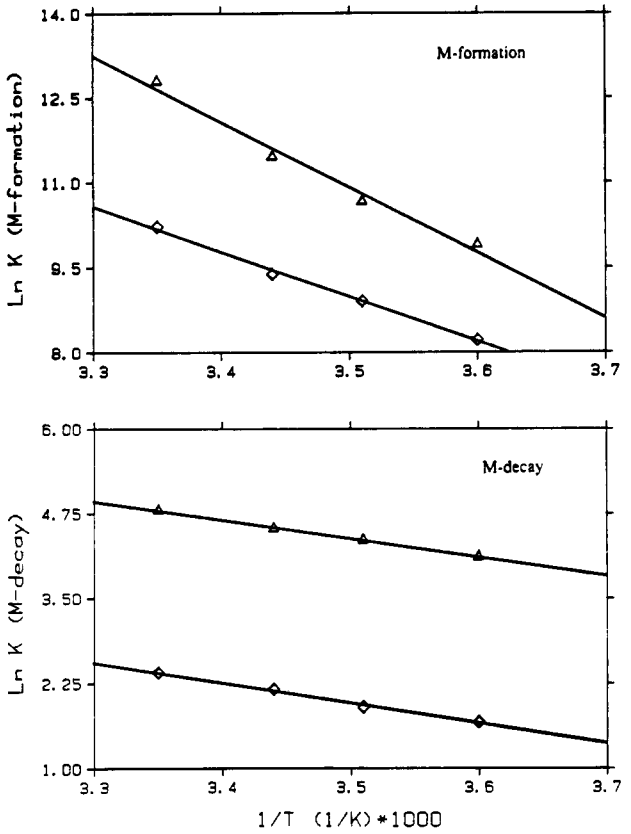

Figure 7. Arrhenius plots of the M formation (top) and M decay (bottom) in the photocycle of the purple membrane bR in the glass matrix. The derived activation parameters are summarized in Table I. (△) and (◆) are for fast and slow components, respectively.

Table II. Activation Parameters of the M Formation and M Decay for Native bR in Sol–Gel Glass at 25 °C

	$E_a^{\ddagger}$ (kJ/mol)	$\Delta H_a^{\ddagger}$ (kJ/mol)	$\Delta S_a^{\ddagger}$ (J/K mol)	$\Delta G_a^{\ddagger}$ (kJ/mol)
bR in glass matrix				
M formation slow component	66	64	61	48
M formation fast component	96	94	183	42
M decay slow component	25	22	−142	67
M decay fast component	23	20	−129	61
bR in aqueous suspension				
M formation slow component	61	59	60	43
M decay slow component	73	71	31	64

[a] $\Delta H_a^{\ddagger}$, $\Delta S_a^{\ddagger}$, and $\Delta G_a^{\ddagger}$ are estimated from the Wynne-Jones and Eyring equation: $k = kT/h \exp(\Delta S_a/R - \Delta H_a/RT) = kT/h \exp(-\Delta G_a/RT)$.

color and PSB function. Figure 8 shows the absorption spectra of the purple bR-glass (A) and its corresponding deionized blue form (B). After soaking the blue bR-glass in concentrated solution of $CaCl_2$ for ~3 h, a complete conversion to the purple form was achieved (Figure 8C).

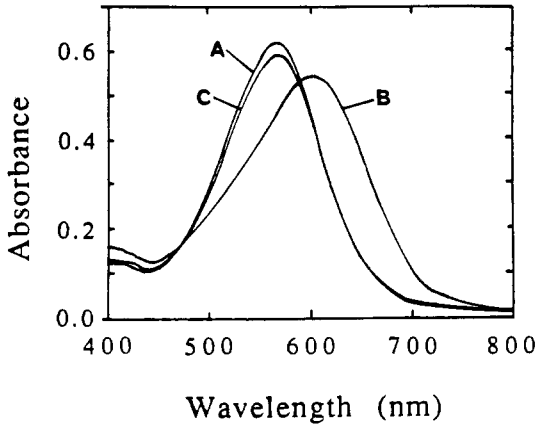

Figure 8. Absorption spectra of the retinal chromophore of the purple membrane bR immobilized in the glass matrix (A), its deionized form (B), and its Ca^{2+}-regenerated form (C).

The Ca^{2+}-regenerated purple bR-glass is found to regain its proton pump function. In addition to Ca^{2+}, we also used other cations such as tetraethylammonium (TEA; $(CH_3CH_2)_4N^+$) to regenerate the blue bR-glass in order to probe the general porous properties of the glass medium. It was found that like Ca^{2+}, the TEA cation can also restore the purple color of bR in the glass matrix. Together with its photochromic shift and photocycle properties, the deionization and cation regeneration properties of bR-glass should extend its use as a potential cation sensor.

Conclusions

It has been demonstrated that bR immobilized in a transparent sol–gel glass matrix can be made by a modified sol–gel technique. The optical and photocycle properties have been measured and found to be retained throughout the gel formation processes. The activation parameters of the M formation and M decay for bR in the glass matrix have been determined and compared with those obtained for bR in aqueous suspension. Besides the thin film of bR, this optically homogeneous material should extend the potential use of bR as a component in new biomaterials. Together with its unique photochemical properties and its high stability, the observed increase in M lifetime of bR photocycle in the glass and its deionization and ion-regeneration properties make this system an attractive material for potential use in optical imaging processing and optically based solid-solid ion sensoring.

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