

Variable ultrafast optical nonlinearity in bacteriorhodopsin achieved through simple chemical treatment

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ABSTRACT

Tunability of the third-order optical nonlinearity and optical limiting action of bacteriorhodopsin (BR) was achieved by altering the native structure through simple surfactant treatment like cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS) and Triton X-100. The variation in spectral position and intensity of the UV-visible absorption spectrum was due to aggregation (hyperchromic lipid absorption-280 nm), solubilization (redshift in chromophore absorption—600 nm, formation of retinal—396 nm) and monomerization (blueshift in chromophore absorption-553 nm) effects upon interaction with anionic, cationic and non-ionic surfactants. Further, changes in the Raman vibrational modes confirmed the alteration in C=C ethylenic chain and 13-cis retinal on surfactant treatment. Surfactant dose-dependent assay of nonlinear optical parameters was evaluated by using the Z-scan technique with ultrashort laser pulse excitation (800 nm, \sim 150 fs, 80 MHz). The closed aperture data demonstrated self-defocusing nature and notably higher monomerization (0.15–0.25 mM) effects endorsed sign reversal (self-defocusing to self-focusing) of the nonlinear refractive index coefficient. Bare BR exhibited saturable absorption, while reverse saturable absorption (RSA) was observed for all surfactant-treated samples (expect for 0.20 mM CTAB and 1.0 mM SDS). The observed RSA and optical limiting was ascribed due to two-photon absorption process. Interestingly, we observed that aggregation enhanced the nonlinear refraction, monomerization improved nonlinear absorption (NLA), and solubilization reduced the nonlinear optical (NLO) response of BR. The maximum nonlinear refractive index ($n_2 = 8.78 \times 10^{-19} \text{ m}^2/\text{W}$) was observed



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for 0.20 mM CTAB, while the NLA coefficient ($\beta = 4.40 \times 10^{-12} \text{ m/W}$) and low optical limiting threshold (538 µJ/cm²) were observed to be highest for 0.25 mM TX-100 sample. The influence of aggregation, monomerization and solubilization effects of purple membrane in achieving the variation of the NLO coefficients is discussed.

Introduction

In the photonics community, bacteriorhodopsin (BR) is considered to be one of the most promising lighttransducing proteins comprising photoactive retinal chromophore and seven transmembrane helices of purple membrane (PM) [1–3]. Persistent attention has been particularly shown toward native BR, which has established properties such as high quantum efficiency, wide range absorption, extremely large optical nonlinearity and remarkable stability against high energy photons, chemical and thermal environment [4, 5]. Under ultrashort pulse excitations, BR exhibits fast optical response (short intermediate and relaxation lifetime) due its chirality and retinal chromophores [6, 7]. A huge number of technical NLO applications of BR were proposed and processed during last two decades which includes neutral-type logic gates, nonlinear optical filters, reversible holographic media, spatial light modulators, two-photon volumetric memories, real-time holographic imaging systems, opto-genetics, tunable image switching and most importantly optical limiters for laser safety [8–15]. In particular, demonstration of high-speed (ns) optical switching and light controlled spin filtering has emphasized the urgent need to fine tune the properties of this special bio-material [16]. A small perturbation in the crystalline structure of BR can yield shifting in electron density of the molecule leading to substantial changes in dipole moments, absorption band and refractive index [17]. An investigation on mutation and nanohybrid formation with BR has been intensively performed for tuning the optical properties which offers application ranging from medicine to electronics [18–21]. In this sense, the optical characteristics of BR can be modified by simple environmental treatment like the change of pH, temperature, external electric field and site dependent chemical interaction [22, 23]. In BR the retinal chromophore is the key molecule for light active photocycle mechanism which is covalently bound via Lys-216 to the seven helical structures. Due to the polar nature of the lipids in helical structure, one can easily modify the structure through simple chemical treatment without denaturing the protein moiety [24]. Hence, addition of surfactant like chemicals has added advantage of reduced intermediate photocycle lifetime resulting in shorter relaxation time and faster optical response. Therefore, understanding the tunability of nonlinear optical (NLO) properties through structure modification will be an interesting subject of investigation for the realization of advanced photonic devices such as optical switching and broad-band optical limiters. It has been established that interaction of various surfactants with lipids having polar nature can result in delipidation, solubilization, and monomerization leading to structure modification in PM [18, 25]. These effects were highly allied with the steric hindrance of hydrophobic tails and charged heads of the surfactants. Previous reports have proposed that nonionic surfactants can lead to delipidation or monomerization of PM without momentous denaturing of BR. In particular non-ionic surfactant, such as Triton X-100, solubilizes the trimeric state into monomeric state of PM and then produced monomerization effect [24]. Ionic surfactants can manipulate the photocycle of BR which primly depends on the charges of the hydrophilic heads and the length of the hydrophobic tails. Cationic surfactant such as CTAB interacts electrostatically with the lipids, resulting in aggregation of PM. Furthermore, anionic surfactant like SDS can hydropobically interacts with PM leading to solubilization of helices [25]. Therefore, surfactant treatment can alter the fast isomerization dynamics (fast optical response, shortlived lifetime, and photo-stability) of BR which in turn modifies the NLO response and polarizability [6, 7, 26, 27]. In recent years, usage of ultrashort nearinfrared pulsed lasers with high repetition rate in medical and industrial sector has increased to great extent [28]. Thermal nonlinearity arising due to such excitations has to be properly understood for the realization of non-local NLO phenomena such as spatial soliton propagation and shock waves [29]. Although measurement of nonlinearity and optical limiting actions of BR were demonstrated using cw and nanosecond pulsed lasers [13, 30–33], investigations in the ultrashort pulse regime for structure-modified BR still remain unexplored. This article reports the surfactant dose-dependent investigation of BR under ultrashort laser (800 nm, ~150 fs, 80 MHz) excitation to disentangle the roles of surfactants in changing the optical absorption of BR and achieving tunability of nonlinear optical coefficients via structure modification of PM.

Materials and methods

Preparation of structure-modified bacteriorhodopsin

Bacteriorhodopsin was isolated by aqueous two-phase separation (ATPS) technique as proposed by Shiu et al. [34], and the detailed procedure followed was reported elsewhere [35]. The isolated PM was made lightadapted by keeping the sample under sunlight for 1 h. The structure-modified samples were prepared by treating the light-adapted PM with three different surfactant like anionic (SDS, Sigma-Aldrich, 99%), cationic (CTAB, Sigma-Aldrich, 99%) and non-ionic (TX-100, Sigma-Aldrich, Bio-molecular Grade). Four different concentrations of each surfactant-treated sample were prepared, and all were below critical micelle concentration (CMCs) of surfactants [25]. Initially during surfactant treatment, phosphate buffer solution was used to control the pH (7.4) of PM and constituent BR concentration was controlled at 20 µM by spectroscopic OD (560 nm) measurement. Surfactant-treated BR samples were named as S1 (1 mM), S2 (2 mM), S3 (3 mM), S4 (4 mM) for SDS, C1 (0.05 mM), C2 (0.10 mM), C3 (0.15 mM), C4 (0.20 mM) for CTAB, and T1 (0.10 mM), T2 (0.15 mM), T3 (0.20 mM), T4 (0.25 mM), for TX-100, respectively.

Physical measurements

The freshly prepared samples were taken in a 1-cm cuvette (20 μ M in water) and were subjected to UV–visible absorption measurements to confirm the structural changes of PM with the interaction of surfactants. Spectrum was recorded using Shizmazu, UV-Pharma 1700 spectrophotometer between 200 and

800 nm. Raman measurement was carried out using Renishaw inVia Raman microscope with spectrometer attachment using 785-nm laser excitation. Initially the samples were fixed on the sample stage of the Raman spectrophotometer using 96 well plates upon which 200 µl of each sample was loaded into separate wells. In order to attain finest vibrational modes of each Raman spectrum, the power adjustment of the input laser beam was optimized between 50 and 100% of the input power for various samples. Third-order optical nonlinearity of the sample solutions of PM (20 µM in water) was measured by Z-scan experiments using ultrashort pulse laser excitation. To consider the sample as thin medium during calculations, the samples were taken in 1-mm cuvette and the Rayleigh range was designed to be larger than the sample thickness. Femtosecond laser pulses were obtained from an oscillator (Chameleon, Coherent) that delivered \sim 150 fs pulses with a repetition rate of 80 MHz at 800 nm. The pulses were tunable in the 700-950 nm spectral range. Initially 70-80% linear transmission at 800 nm was maintained for all the samples by varying the solution concentration. In order to achieve preeminent data, the experiments were repeated several times and the data were used for obtaining the thirdorder nonlinear optical coefficients from the theoretical fits made based on Sheik-Bahae formalism [36].

Results and discussion

Structural modification assay—UV–visible absorption and Raman

UV-visible absorption spectroscopy has been comprehensively employed in exemplifying the electronic transition of the aromatic and retinal residues of BR in the UV–Vis region, respectively. Spectrally hushed surfactants were chosen, and hence, the observed corresponding absorption shifts of each sample will provide information on the structure modification of PM. For native BR, the characteristic absorption band at 568 nm can be attributed to lightadapted state of all-trans retinal whose extinction coefficient was estimated to be $62,700 \text{ M}^{-1} \text{ cm}^{-1}$. Intense band at 260-280 nm endorse the presence of constituent BR which has aromatic residues of 11 tyrosines and 8 tryptophans [37]. Earlier reports have demonstrated that Triton X-100 (neutral surfactant) can lead to monomerization of PM by solubilizing the



trimeric state into monomeric state [24]. Also CTAB (cationic surfactant) results in aggregation of PM through electrostatic interaction, while SDS (anionic surfactant) creates solubilization of helices via hydrophobic interaction. The recorded UV-visible spectra provided further confirmation on the change in electronic state due to various interaction of surfactant with BR. UV-visible absorption spectrum of surfactant-treated sample is shown in Fig. 1a, c, and significant absorption shift was observed on each surfactant treatment. Under CTAB treatment (Fig. 1a), retinal absorption (568 nm) did not get much altered while lipid absorption (280 nm) was greatly enhanced which provides the indication of aggregation of PM patches. Gradual addition of CTAB (0.05–0.20 mM) increases the lipid aggregation which was confirmed by hyperchromic effect in lower wavelength (260-280 nm). Here cations of CTAB can electrostatically interact with lipid moiety of the purple membrane (PM) which results in charge neutralization. This results in the reduction in solubility leading to significant PM aggregation [25]. For SDS (Fig. 1b), the band at 568 nm gets depleted and shifted to 600 nm, when the concentration is gradually increased up to 3.0 mM leaving the band at 280 nm unchanged. Further increasing the concentration to 4.0 mM, the band at 600 nm disappeared and formation of new band at 390 nm was observed, indicating the formation of free retinal [25]. Here, under SDS interaction, 12-carbon hydrophobic tail and sulfate hydrophilic head of anionic SDS solubilize PM suspensions via surface hydrophobic interaction leading to the denaturing of PM and generation of free retinal. TX-100-treated PM absorption spectrum is shown in Fig. 1c in which the retinal band was blueshifted from 568 to 553 nm. which demonstrates the transformation of trimeric state to monomeric state of native PM. Blueshift in



Figure 1 UV-visible absorption spectra of a CTAB, b SDS and c TX-100-treated PM.

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retinal absorption resulted in drop of concentration of all-*trans* retinal state from 94 to 84%, which is an evidence for the occurrence of monomerization [38]. As the concentration of TX-100 was increased (0.1–0.25 mM), depletion on lipid region (260 nm) takes place which is also a sign of monomerization effect. Thus UV–visible absorption confirms that surfactant treatment of BR resulted in aggregation (CTAB), solubilization (SDS) and monomerization (TX-100) of PM suspensions.

The most precise structural information of biomolecules can be extracted from resonance Raman spectra with cw laser excitation. Raman spectra of surfactant-treated PM suspensions are shown in Fig. 2a–c. Each spectrum was compared with native PM suspensions, and the spectral shifts due to surfactant addition were discussed in detail. Generally the fingerprint regions for wild BR lie around 1100–1400 cm⁻¹ and in the present case, the bands at 1005 and 1208 cm⁻¹ can be assigned to tryptophan which acts as the back bone of PM [39]. The strongest lines in the spectra at 1524 cm⁻¹ pointed up the presence of ethylenic (C=C) stretching vibrations of the retinal polyene chain which helps in the ion transportation. The presence of *13-cis* configuration was confirmed by the peaks at 1172 and 1204 cm⁻¹ [40]. The bands near 1400 and 1414 cm⁻¹ correspond to C–H bending of retinal methyl groups present in the BR configuration [35]. No peaks that correspond to other impurities or residues were observed in the spectrum, confirming the purity of BR.

CTAB-treated BR spectrum (Fig. 2a) depicted no alteration in basic back bone structure, and blueshift in ethylenic (C=C) stretching vibrations was observed only at concentration of 0.20 mM. The intensity of main bands at 1524 and 1167 cm⁻¹ diminished with increase in concentration confirming the alteration of ethylenic (C=C) stretching and 13-*cis* vibrations due to charge neutralization. SDS treatment leads to solubilization of PM suspensions which was assured by



Figure 2 Raman spectra of a CTAB-treated PM, b SDS-treated PM, c TX-100-treated PM.



the disappearance of ethylenic (C=C) stretching vibrations and 13-*cis* configuration (Fig. 2b). Gradual addition of SDS leads to complete solubilization of PM which resulted in spectral disappearance of tryptophan, ethylenic (C=C) stretching, aromatic and aliphatic side chain vibrations. Thus, no Raman vibrational modes were observed for BR treated with SDS (2-4 mM). For TX-100-treated samples (Fig. 2c), no considerable spectral disappearance was observed in Raman spectrum; however, small spectral shift (2–5 cm⁻¹) along with spectral intensity variations was observed in the entire region. Thus, Raman analysis gives additional shore up and clear structural modification information on surfactant-treated PM suspensions.

Nonlinear absorption (NLA)

The recorded open-aperture (OA) patterns of stripped and surfactant-treated BR samples under femtosecond IR excitation are shown in Fig. 3a–c. In the

data presented in Figs. 3, 4, 5 the scattered symbols represent the experimental data, while the solid lines correspond to the theoretical fits made for two-photon absorption process based on Sheik-Bahae formalism. The error bars represent the uncertainties in (a) estimation of spot size at focus thereby leading to errors in estimation of the peak intensity (b) fitting errors (c) input laser energy fluctuations (d) detector calibration, etc. It is interesting to be observed that wild BR exhibited saturable absorption (SA) behavior, while all the surfactant-treated BR demonstrated reverse saturable absorption (RSA) with exemption for samples C4 and S1 which replicated the behavior of native BR. BR consists of the protein matrix and all-trans retinal, a chromophore which is responsible for its nonlinear optical properties. Earlier studies of BR illustrated same behavior (SA) recorded with various wavelengths and intensities [4, 15, 26]. The maximum aggregation (C4) and minimum solubilization (S1) constrains the photon interaction and, hence, resembles the NLA behavior of wild BR. In



Figure 3 OA Z-scan data of a CTAB, b SDS, c TX-100-treated BR, d schematic diagram of 2PA process. *Open symbols* represent the experimental data, while the *solid lines* represent theoretical fits to the data.

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Figure 4 CA data of PM treated a CTAB, b SDS, c TX-100. Open symbols represent the experimental data, while the solid lines represent theoretical fits to the data.

general the OA patterns provide insight information about the nonlinear absorption mechanisms like twophoton absorption (2PA), excited state absorption (ESA), free carrier absorption (FCA) [29]. Hence the obtained experimental data were fitted with nonlinear transmission equation for open-aperture mode as proposed in Shiek-Bahae formalism [36].

$$T_{\text{OA}(n\text{PA})} = \frac{1}{\left[1 + (n-1)\beta_n L_{\text{eff}} (I_{00}/(1 + (z/z_0)^2)^{n-1})\right]^{1/(n-1)}}$$
(1)

where β_n is the effective multi-photon absorption (MPA) coefficient [n = 2 for two-photon absorption (2PA), n = 3 for three-photon absorption (3PA) etc.], I_{00} is the input irradiance, and $L_{\text{eff}}\left(=\frac{1-e^{Lz_0}}{\alpha_0}\right)$ is the effective path length. For all samples, the experimental data were found to be best fitted for 2PA case and the extracted nonlinear absorption coefficient (β) is presented in Table 1. The process of 2PA can be explained as follows: BR has several intermediate

photocycle states which can deliver sophisticated photon interaction under high energy fs excitation. Here the electrons simultaneously absorbs two photons of input beam (800 nm, 1.55 eV) to move to a virtual state (3.10 eV) and then relaxes to M state of BR (410 nm, 3.02 eV) via non-radiative transition. This mechanism is schematically represented in Fig. 3d with proposed transition of $E_0 \rightarrow (2PA) \rightarrow$ $E_1 \rightarrow$ (non-radiative) $\rightarrow E_2$. The estimated nonlinear absorption coefficient of wild BR was $0.31 \times$ 10^{-12} m/W and found to be higher than known amino acid derivatives like tryptophan, tyrosine, histidine and proline [41]. The higher value of nonlinear absorption coefficient of BR was due to (1) elongated π -conjugated system (2) π -electron delocalization and (3) polar and polarizable residues in the protein [14]. It is interesting to note that the surface-modified BR possessed higher β value than wild BR. Enhancement of nonlinear absorption due to surfactant treatment arises mainly due to the perturbation in amino acid sequence of PM and polyene





Figure 5 Optical limiting curves of, a CTAB, b SDS and c TX-100-treated samples, d schematic representation of NLO response. *Open symbols* represent the experimental data, while the *solid lines* represent theoretical fits to the data.

chain of retinal chromophore. All mechanisms mentioned above are directly related to the degree of delocalization of π -electrons of the polyene chain, which get altered on surfactant treatment.

From the data presented in Table 1, it is evident that monomerization (TX-100) has increased and solubilization or denaturing (SDS) has reduced the NLA action of BR. Although aggregation (CTAB) has improved the NLA coefficient than native BR, it suppressed the NLA behavior with increase in strength of aggregation (concentration).

Nonlinear refraction (NLR)

Closed aperture (CA) Z-scan experiments were carried to determine the sign and magnitude of nonlinear refractive index of bare and surfactant-treated BR. Nonlinear refractive index mainly depends on electronic polarization, molecular reorientation effect, nonlinear scattering, excited state refraction, free carrier refraction and thermal effect. The obtained CA pattern of native and surfactant-treated PM is shown in Fig. 4a–c. All the samples along with wild BR show peak-valley pattern (negative nonlinearity, self-defocusing) except for T2, T3 and T4 which shows valley-peak pattern (positive nonlinearity, self-focusing).

It is well known that estimated third-order nonlinear optical properties strongly depend on the input laser properties used for excitation. Gnoi et al. [44] and Makhal et al. [45] have demonstrated that the cumulative thermal effects contribute to the overall nonlinearity when excited with high-repetition-rate laser pulses. To extract fast nonlinearities from the data obtained with femtosecond pulses with high repetition rates and to avoid accumulated thermal effects, one needs to chop the input pulses appropriately. When the pulse repetition rate is higher than thermal diffusivity of sample, molecules cannot attain ambient temperature before the arrival of the next pulse. Thus, due to the involvement of Kerr

| Sample code | $n_2 \times 10^{-19}$ (m ² /W) | $\beta \times 10^{-12}$ (m/W) | $ \begin{array}{l} {\rm Im}\; \chi^{(3)} \times \; 10^{-21} \\ ({\rm m}^2/{\rm v}^2) \end{array} $ | Re $\chi^{(3)} \times 10^{-20}$ (m ² /v ²) | $\chi^{(3)} \times 10^{-20}$ (m ² /v ²) | $\chi^{(3)} \times 10^{-16}$ (esu) | Limiting onset $(\mu J/cm^2)$ |
|----------------|--|----------------------------------|--|--|---|------------------------------------|-------------------------------|
| BR | 4.59 ± 0.23 | 0.31 ± 0.16 | 0.37 ± 0.19 | 0.88 ± 0.04 | 0.88 ± 0.04 | 1.85 ± 0.09 | _ |
| C1 | 4.85 ± 0.24 | 1.10 ± 0.06 | 1.13 ± 0.06 | 0.93 ± 0.05 | 0.93 ± 0.05 | 6.42 ± 0.32 | 695 ± 35 |
| C2 | 5.47 ± 0.27 | 0.92 ± 0.05 | 1.12 ± 0.06 | 1.05 ± 0.05 | 1.05 ± 0.05 | 5.38 ± 0.27 | 823 ± 41 |
| C3 | 5.65 ± 0.28 | 0.70 ± 0.04 | 0.85 ± 0.04 | 1.08 ± 0.05 | 1.08 ± 0.05 | 4.11 ± 0.21 | 1012 ± 51 |
| C4 | 8.78 ± 0.44 | 0.43 ± 0.02 | 0.52 ± 0.03 | 1.68 ± 0.08 | 1.69 ± 0.08 | 2.63 ± 0.13 | _ |
| T1 | 4.33 ± 0.22 | 0.30 ± 0.02 | 0.36 ± 0.02 | 0.83 ± 0.04 | 0.83 ± 0.04 | 1.79 ± 0.09 | 858 ± 43 |
| T2 | 4.24 ± 0.21 | 2.00 ± 0.10 | 2.44 ± 0.12 | 0.81 ± 0.04 | 0.84 ± 0.04 | 11.66 ± 0.58 | 708 ± 35 |
| T3 | 3.82 ± 0.19 | 3.70 ± 0.19 | 4.51 ± 0.23 | 0.73 ± 0.04 | 0.85 ± 0.04 | 21.57 ± 1.09 | 659 ± 33 |
| T4 | 3.51 ± 0.18 | 4.40 ± 0.22 | 5.37 ± 0.27 | 0.67 ± 0.03 | 0.86 ± 0.04 | 25.65 ± 1.28 | 538 ± 30 |
| S1 | 3.11 ± 0.16 | 0.36 ± 0.02 | 0.44 ± 0.02 | 0.59 ± 0.03 | 0.59 ± 0.03 | 2.11 ± 0.11 | _ |
| S2 | 2.63 ± 0.13 | 0.20 ± 0.01 | 0.24 ± 0.01 | 0.50 ± 0.03 | 0.50 ± 0.03 | 1.19 ± 0.06 | 875 ± 44 |
| S3 | 2.59 ± 0.13 | 0.17 ± 0.01 | 0.21 ± 0.01 | 0.49 ± 0.02 | 0.49 ± 0.02 | 1.01 ± 0.05 | 1012 ± 51 |
| S4 | 1.93 ± 0.10 | 0.16 ± 0.01 | 0.19 ± 0.01 | 0.37 ± 0.02 | 0.37 ± 0.02 | 0.94 ± 0.05 | 1218 ± 61 |

Table 1 Third-order NLO coefficients of untreated and treated PM samples

components, the order of observed nonlinearity is several orders higher than the previous case. In the present case, we did not have the necessary chopper or pulse-picker (or true 1 kHz pulses) to measure/ evaluate only the electronic nonlinearity. In the reference 45, the authors obtained two-photon absorption coefficient of $(5.4-6.2) \times 10^{-10}$ m/W with MHz excitation and $(9.5-15) \times 10^{-12} \text{ m/W}$ for a phthalocyanine complex indicating a two orders of magnitude difference between high-repetition-rate and low-repetition-rate pulses. Even in reference 44 data two orders of magnitude difference was observed for 2PA coefficient (toluene) for the cases of with and without thermal contribution. Furthermore, our recent studies on two phthalocyanine molecules [46] performed at 800 nm with fs, MHz pulses and 1.5 ps, kHz pulses clearly demonstrated again a two orders of magnitude difference in the 2PA coefficient. We also expect similar order of magnitude difference in our case. However, further studies only will confirm the accuracy of this statement. Furthermore, Khurgin et al. [47] have demonstrated that when the optical fields are concentrated into the volumes on the scale of few 10's of nm, the speed of the thermo-optical effects approaches ultrafast time (picosecond) scale. They also argue that this can be accomplished with the use of plasmonic effects in metal nanoparticles in combination with the thermo-optic material leading to phase shifts sufficient for ultrafast all optical switching. The variation in nonlinear transmittance under closed aperture mode can be obtained by the relation [29].

$$T_{\rm CA} = 1 \pm \frac{4\Delta\phi_0\left(\frac{z}{z_0}\right)}{\left[1 + \left(z/z_0\right)^2\right] \left[9 + \left(z/z_0\right)^2\right]}$$
(2)

where $\Delta \phi_0$ is the phase change of laser beam and by fitting procedure, and the nonlinear refractive index (n_2) can be calculated as follows

$$n_2 = \frac{|\Delta\phi_0|\lambda}{2\pi I_{00} L_{\rm eff}} \tag{3}$$

The estimated values of nonlinear refractive index (n_2) are summarized in Table 1. Monomerization effect produced shift in nonlinear refractive index from negative to positive. Compared with BR, monomerization (TX-100) and solubilization (SDS) effect has reduced the nonlinear refractive index (n_2) and the NLR process get suppressed with increase in concentration of these surfactants. However, the nonlinear refractive index (n_2) was enhanced upon CTAB treatment and nonlinear refractive index gets increased as function of aggregation (C1–C4). Hence, aggregation effect delivers higher NLR behavior than the monomerization and solubilization effect; hence, CTAB can be a better entrant for the improvement of NLR behavior of BR.

With ultrashort laser pulse excitation, aggregation (CTAB) of BR molecules enhanced NLR and suppressed NLA process. As depicted in UV–Vis and Raman studies, enhancement of lipid absorption (280 nm), alteration of ethylenic stretching (1524 cm⁻¹) and 13-*cis* vibrations (1167 cm⁻¹) due to charge neutralization have enhanced NLR due to

aggregation of PM patches. Also cationic surfactant treatment (CTAB) has induced the light scattering [23] leading to change in nonlinear refractive index and thus increasing NLR process. Neutral surfactant (TX-100) treatment creates the formation of M state faster leading to quicker thermal exchange which in turn enhanced the nonlinear absorption. Thus, monomerization (TX-100) of BR has resulted in monomeric state which leads to improved NLA and reduced NLR behavior. These can be further confirmed by UV-visible and Raman studies, which shows the denaturing of PM and generation of free retinal (blueshift in retinal band from 568 to 553 nm) through disappearance of tryptophan, ethylenic (C=C) stretching, aromatic and aliphatic side chain vibrations via surface hydrophobic interaction. Anionic (SDS) surfactant treatment solubilizes aromatic amino acid sequence of the native structure leading to inferior nonlinear optical absorption. And solubilization (SDS) has denatured the BR which was evidenced through disappearance of C=C stretching vibrations and 13-cis configuration escorting to reduced NLA and NLR action. The addition of surfactants thus leads to structural changes (alters the intermediate life time) in bacteriorhodopsin which is observed on optical absorption spectra [23, 25].

Optical limiting

Optical limiting curves were extracted from the OA patterns of z-scan experiment and the position-dependent fluence was evaluated with relation [29]

$$F(z) = \frac{4\sqrt{\ln 2} \left(\frac{E_{\rm in}}{\pi^{3/2}}\right)}{\omega(z)^2} \tag{4}$$

where F(z) is the input fluence, E_{in} is the laser energy and ω_o laser beam radius at the focus.

The optical limiting behavior of each sample was examined and the obtained results are shown in Fig. 5a–c. It was observed that the transmittance rapidly decreased with input fluence expect for pure BR, C4 and S1 samples (which shows saturable absorption). For all reverse saturable samples, the observed limiting can be ascribed due to two-photon absorption process. Also due to high repetition rate, thermal effects leading to self-defocusing (wild BR, CTAB, SDS-treated BR) and self-focusing (TX-100treated BR) effect contributes to the optical limiting behavior. The estimated limiting threshold was found to be $\sim \mu J/cm^2$ and hence has potential application in safety devices against micro-Joule energy ultrashort pulsed lasers used in femtosecond laser-assisted cataract surgery (FLACS), material processing and micromachining [28]. A sample with higher nonlinear absorption coefficient produces stronger limiting behavior with lower limiting threshold. Among the structure-modified BR, monomerization effect shows the minimum limiting (538 μ J/cm²) values followed by aggregation and solubilization. Reports show that laser damage threshold of BR molecule estimated via passive mode locking experiment (514 nm, 64 fs, 10 nJ laser excitation) was 1 GW/cm² which is sufficient for attaining efficient optical limiters for high energy ultrashort pulses [42]. Also monomerized and aggregated BR molecules can manifest the additional feature of synthesis flexibility to form hybrid BR films with higher NLO coefficients. Thus, realization of preferentially oriented chemically modified BR films can be the cutting-edge materials in laser safety devices as it can yield higher NLO performance systems.

Table 1 summarizes the tunability of third-order NLO coefficients of BR achieved through structural modification. The variation in the NLO parameters of surfactant-treated BR with its native was primly due to the structure modification induced through aggregation (CTAB), monomerization (TX-100) and solubilization (SDS) effect (Fig. 5d). With ultrashort laser pulse excitation, aggregation of BR molecules enhanced NLR and suppressed NLA process, while monomerization of BR has resulted in monomeric state leading to improved NLA and reduced NLR behavior. And solubilization has denatured the BR escorting to reduced NLA and NLR action. From the nonlinear absorption coefficient (Im $\chi^{(3)}$) and nonlinear refractive index (Re $\chi^{(3)}$), the nonlinear optical susceptibility of the samples was estimated and given in Table 1. For all the samples, the optical susceptibility was found to in the order of 10^{-20} esu, which is found to be seven orders $(2.1 \times 10^{-27} \text{ esu})$ higher than previously reported (1064 nm, 100 ps, 400 Hz) value of native BR [43]. In all the cases, Re $\chi^{(3)}$ was found to be higher than Im $\chi^{(3)}$ demonstrating the dominance of nonlinear refraction compared to nonlinear absorption. Aggregation followed by monomerization effect provided stronger nonlinear optical susceptibility than native BR, while solubilization reduced it. Among the samples, the maximum nonlinear absorption coefficient and nonlinear refractive index was observed for T4 $(\beta = 1 \times 10^{-12} \text{ m/W}, \text{ three times than BR})$ and C4 $(n_2 = 8.8 \times 10^{-19} \text{ m}^2/\text{W}$, two times than BR). respectively. This clearly indicates that monomeric state has stronger NLA than trimeric state of BR and aggregation has promoted stronger photon interaction, leading to thermal effect and thus enhanced NLR. Thus, it can be concluded that, with tunable NLO properties and low limiting threshold, structure-modified BR can yield nonlinear absorption (TX-100), nonlinear refraction (CTAB)-based optical limiters for human safety against femtosecond lasers used in medical and industrial sectors.

Conclusions

Structural modification of bacteriorhodopsin was successfully made by simple dose varying surfactant treatment. From the UV-visible absorption spectrum, enhancement in lipid absorption at 280 nm confirms the aggregation (cationic CTAB), blueshift in chromophore absorption to 553 nm pointed out the monomerization (non-ionic TX-100), and redshift in chromophore absorption maximum to 600 nm along with formation of peak at 390 nm due to free retinal solubilization(anionic illustrates the SDS)effect induced in native BR. Raman vibrational modes clearly provide strong evidence on aggregation, solubilization and monomerization via spectral disappearance of tryptophan, ethylenic (C=C) stretching and shift in 13-cis vibrations. Ultrafast Z-scan experiment reveals that the surfactant treatment has modified the nonlinear optical response of BR and thus tunability of NLO coefficient was achieved. Open-aperture Z-scan data showed that wild BR exhibited saturable absorption (SA) behavior, while all the surfactant-treated BR seize reverse saturable absorption (RSA) with exemption for samples having maximum aggregation (0.20 mM CTAB) and minimum solubilization (1.0 mM SDS). The observed nonlinear absorption was ascribed to be originating from two-photon absorption with proposed transition of $E0 \rightarrow (2PA) \rightarrow E1 \rightarrow (\text{non-radiative}) \rightarrow E2$. In closed aperture Z-scan mode, all the samples along with wild BR depicted peak-valley pattern (negative nonlinearity, self-defocusing) except for T2, T3 and T4 which depicted valley-peak pattern (positive nonlinearity, self-focusing). Also sign reversal from negative to positive nonlinear refractive index was observed in TX-100 treatment above 1.5 mM concentration. Upon CTAB treatment, the induced index and decreased nonlinear absorption coefficient with increase in concentration of CTAB, while in the case of TX-100, nonlinear absorption coefficient was found to be increasing and nonlinear refractive index was decreasing with concentration. Hence, monomerization induced enhancement in nonlinear absorption and suppress the nonlinear refraction process. For SDS-treated samples, both nonlinear absorption and nonlinear refraction were found to be decreasing with concentration due to solubilization effect. For all reverse saturable samples, the observed limiting behavior can be ascribed due to two-photon absorption process. Also due to high repetition rate, thermal effects leading to self-defocusing (wild BR, CTAB, SDS-treated BR) and self-focusing (TX-100treated BR) effect also contribute to the optical limiting behavior. Our results establish that CTAB treatment provides strongest nonlinear refraction $(8.8 \times 10^{-19} \text{ m}^2/\text{W})$ and TX-100 provides strongest nonlinear absorption $(4.40 \times 10^{-12} \text{ m/W})$ along with lowest limiting onset values (538 μ J/cm²). Thus, structure-modified BR can offer nonlinear absorption (TX-100) and nonlinear refraction (CTAB)-based optical limiters for human safety against ultrashort lasers used in medical and industrial sectors.

aggregation effect has increased nonlinear refractive

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