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OPTICAL MEMORY POTENTIAL OF PHOTOACTIVE YELLOW PROTEIN

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13. ABSTRACT *(Maximum 200 words)*

This contract targeted improved growth of photoactive yellow protein (PYP) and evaluation of its optical signal processing capabilities. The growth experiments were highly successful. Ectothiorhodopsin halophila bacteria were grown and plenty of PYP isolated for the optical experiments. Then recombinant wild-type aop-protein was grown in E. coli and purified; the chromophore was activated as the thiophenol ester which is very reactive with apo-protein. The reconstituted protein has properties indistinguishable from native protein. Much larger quantities of reconstituted recombinant protein can now be made in E. coli in a shorter period of time than when native protein was prepared in E. halophila. This success has made it possible to make mutants and proteins with variant chromophores, resulting in shifts in the absorption maximum and changes in the photocycle kinetics. These studies are at an early stage and should eventually lead to protein tailored to meet the requirements of photochemical device.

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Abstract

This contract targeted improved growth of photoactive yellow protein (PYP) and evaluation of its optical signal processing capabilities. The growth experiments were highly successful. Ectothiorhodopsin halophila bacteria were grown and plenty of PYP isolated for the optical experiments. Then recombinant wild-type aop-protein was grown in E. coli and purified; the chromophore was activated as the thiophenol ester which is very reactive with apo-protein. The reconstituted protein has properties indistinguishable from native protein. Much larger quantities of reconstituted recombinant protein can now be made in E. coli in a shorter period of time than when native protein was prepared in E. halophila. This success has made it possible to make mutants and proteins with variant chromophores, resulting in shifts in the absorption maximum and changes in the photocycle kinetics. These studies are at an early stage and should eventually lead to protein tailored to meet the requirements of a photochemical device.

The main goal of the optical studies was to assess the potential of PYP as a 3D optical storage medium using two-photon absorption (TPA). Also it was to be compared with bacteriorhodopsin (BR); a large TPA cross section had been reported already for BR, and 3D storage based on it was under study. Extensive measurements were made on PYP in search of TPA using modelocked 200fs pulses as a pump and cw probe light from a xenon arc lamp. The wavelength range 820 to 910nm was investigated without success. The sensitive technique used permitted an upper limit to be placed on the PYP TPA cross section which is more than four orders of magnitude smaller than that reported for BR. This negative result was not anticipated; checking and cross checking this null result took far more time than a measurement of a BR-size cross section would have taken. As a result , pump-probe spectroscopy on PYP to detect intermediate states was not performed because such studies were relevant to this task only if PYP exhibited TPA. Instead considerable effort was made to reproduce the TPA measurement on BR, using the same experimental instrumentation; although a signal very similar to that published was seen, it was identical to that with pure water. Since only one group has reported BR TPA, a verification is essential.

Contents

1.1 OPTICAL CHARACTERIZATION OF PHOTOACTIVE YELLOW PROTEIN

1.1.1 Introduction

Photoactive yellow protein (PYP), was first isolated at the University of Arizona from the extremely halophilic purple phototrophic bacterium Ectothiorhodospira halophila[1]. Many of its properties are similar to that of bacteriorhodopsin which is of interest due to possible applications such as 3D optical data storage[2, 3, 4]. PYP does, however, have several advantages over bacteriorodopsin; PYP is a smaller ($M_r = 14,000$) molecule than bacteriorhodopsin, and it is water-soluble while bacteriorodopsin is membrane bound. This makes it easier for structural analysis, which is important for possible engineering of the molecule. Several new variations of PYP have already been produced with slightly different optical properties from the original version[5].

This motivated us to undertake the investigation of the nonlinear optical properties of PYP for possible uses in nonlinear optical devices. First pump-probe measurements were performed using one-photon excitation. It was found that the absorption peak of PYP at 446nm is almost completely bleached under excitation by a cw argon laser at 458nm. The associated index change was calculated and found to be comparable to that of bacteriorhodopsin. A possible application in 3D optical data storage is dependent on a two-photon process. Therefore two-photon excitation pump-probe spectroscopy was performed to determine the two-photon absorption (TPA) cross section of PYP. No TPA was observed, so only an upper limit for the TPA cross section was found. This limit is 10^4 smaller than the TPA cross section reported for bacteriorhodopsin; consequently, PYP is unsuitable for applications involving TPA.

The PYP samples used in this work were supplied by Savitha Devanathan, Terry Meyer, and Gordon Tollin from the Biochemistry Department at the University of Arizona. The PYP used in the experiments was extracted from E. halophila (strain BN9626) and purified according to Meyer[1].

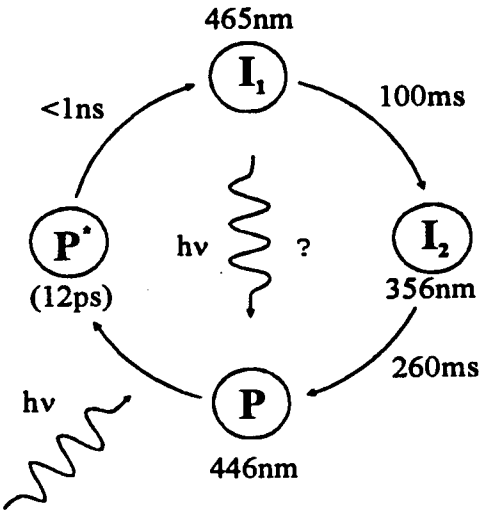

Figure 1.1: Schematic of the photocycle of PYP.

1.1.2 Photocycle of Photoactive Yellow Protein

The photocycle of PYP is shown in figure 1.1. The protein undergoes a bleach and recovery cycle similar to that of sensory rhodopsin[6]. In its ground state (**P**) the protein has a visible absorption maximum at 446nm. After absorption of a photon, it is excited to a short-lived electronic state ($\mathbf{P^*}$); there may be intermediate states with lifetimes shorter than 12ps. The protein then undergoes an electronic transition and relaxes to the red-shifted $\mathbf{I_1}$ state in less than 1ns. A third relaxation to the $\mathbf{I_2}$ state then follows with a 100μs time constant. The protein remains in the $\mathbf{I_2}$ state until it undergoes a conformational change and returns to the ground state, **P**, with a time constant of 260ms.

1.1.3 One-Photon Pump-Probe Spectroscopy

Theory

In a nonlinear one-photon pump-probe experiment using a cw pump, an equilibrium builds up between molecules in the ground state **P** and the long lived excited state $\mathbf{I_2}$. The other states relax so fast that the populations in these states can be ignored. Since the $\mathbf{I_2}$ state has negligible absorption at 446nm, the population difference of the ground state can be found by measuring the bleaching of the 446nm absorption peak.

Assuming an average pump intensity I_0 for the probe cross-section through the length L of the PYP sample, the population densities $N_{\mathbf{P}}$ and $N_{\mathbf{I_2}}$ of the **P** and the $\mathbf{I_2}$ states, respectively, are given by the rate equations:

$$\frac{dN_{\mathbf{P}}}{dt} = -\frac{(1-e^{-N_{\mathbf{P}}\epsilon_p L})I_0}{\hbar\omega L} + \frac{N_{\mathbf{I_2}}}{t_d} \tag{1.1}$$

$$\frac{dN_{\mathbf{I_2}}}{dt} = -\frac{dN_{\mathbf{P}}}{dt} \tag{1.2}$$

where ϵ_p is the absorptivity of PYP at the pump wavelength, ω is the optical frequency of the pump beam, and t_d is the decay time between the excited state and the ground state. In steady state, we get:

$$N_{\mathbf{I_2}} = \frac{I_0 t_d (1-e^{-N_{\mathbf{P}}\epsilon_p L})}{\hbar\omega L}. \tag{1.3}$$

The absorption coefficient of the ground state is given by

$$\alpha = N_{\mathbf{P}}\epsilon \tag{1.4}$$

where ϵ is the extinction coefficient (natural log base) of PYP. Using $N_{\mathbf{I_2}} + N_{\mathbf{P}} = N_{\mathbf{P}}^0$, where $N_{\mathbf{P}}^0$ is the total concentration of molecules, and multiplying equation (1.3) by ϵ give

$$\alpha_0 - \alpha = \frac{\epsilon t_d (1-e^{-\alpha_p L})}{\hbar\omega L} \tag{1.5}$$

where α_0 is the absorption coefficients of the ground state without excitation and α_p is the absorption coefficient at the pump wavelength with excitation. This equation can now be solved numerically to find the absorption as a function of pump intensity.

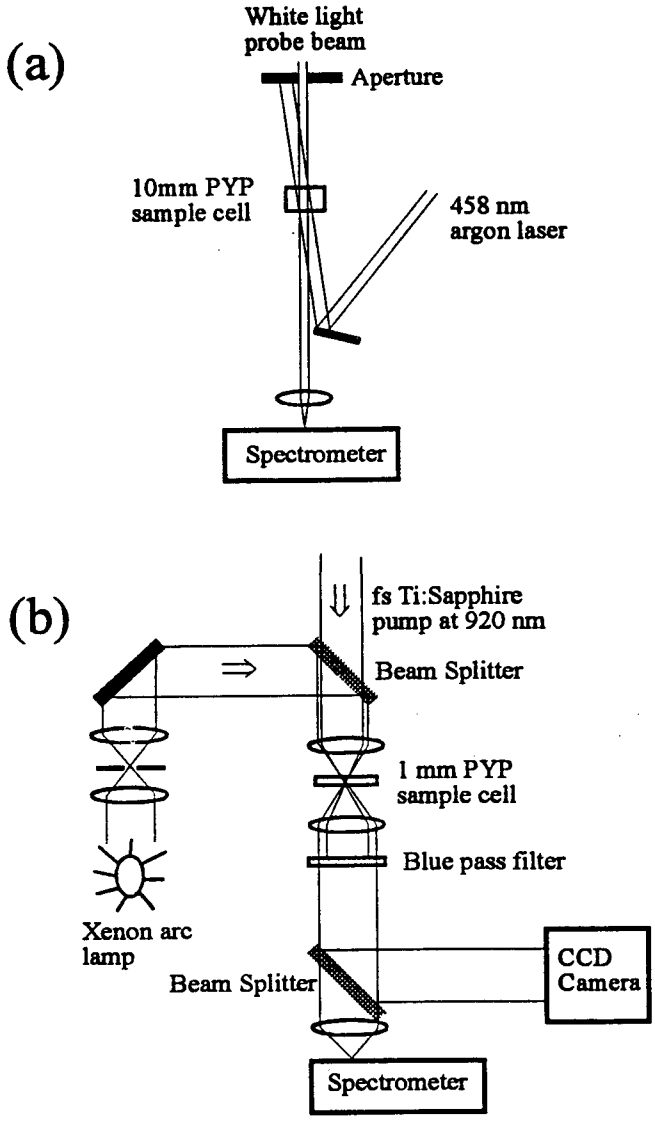

Figure 1.2: (a) and (b) show the experimental setups used in pump-probe experiments on PYP for one-photon and two-photon absorption respectively.

Experimental setup

The setup used in the one-photon-absorption measurements is shown in fig. 1.2(a). The probe and pump light counterpropagated through a quartz sample cell, so that the pump beam could be eliminated from the probe beam. The 1/e pump beam radius was 1.9mm, and the probe radius was 0.5mm. The sample cell had a 10mm path length and contained a 10μM concentration PYP solution. The 458nm line of a cw argon laser was used as the pump.

Experimental results and discussion

The absorption spectra of the PYP sample for different pump powers are shown in fig. 1.3(a). A complete bleaching of the 446nm absorption peak occurs as the pump power increases. The absorption completely recovers after the pump beam is turned off. The increase in absorption at 356nm indicates the population buildup of the $\mathbf{I_2}$ state.

Fig. 1.3(b) shows the index change associated with the bleaching shown in 1.3(a). The index change is calculated from the absorption change using the Kramers-Krönig relations[7]. The maximum index change was about $1.4{\cdot}10^{-6}$ for our sample. Due to the many similarities between the photocycle of PYP and bacteriorhodopsin, it is natural to compare the measured index to that reported for bacteriorhodopsin[8]. Although the reported measurements were done on a film of bacteriorhodopsin and not a solution, the index change per molar concentration change can be compared for the two cases. An absorption change at the absorption peak of about -1.6 OD and a maximum index change of $4{\cdot}10^{-3}$ were reported for a 25μm film of bacteriorhodopsin pumped with 20mW/cm^2. Using the extinction coefficient of 52mM^{-1}cm^{-1} (10 base log) for bacteriorhodopsin[9], this corresponds to a molar concentration change of 12.3mM giving 0.33M^{-1} for the index change per molar concentration change. In our measurements we had a maximum absorption coefficient change of -0.53cm^{-1}. The extinction coefficient of PYP is 45.5mM^{-1}cm^{-1}[10] (10 base log), giving a corresponding concentration change of 5.06μM. The maximum index change was $1.4{\cdot}10^{-6}$, giving an index change per molar concentration change of 0.28M^{-1}, almost as large as that of bacteriorhodopsin. This ratio is, as expected, independent of the pump intensity in our measurements.

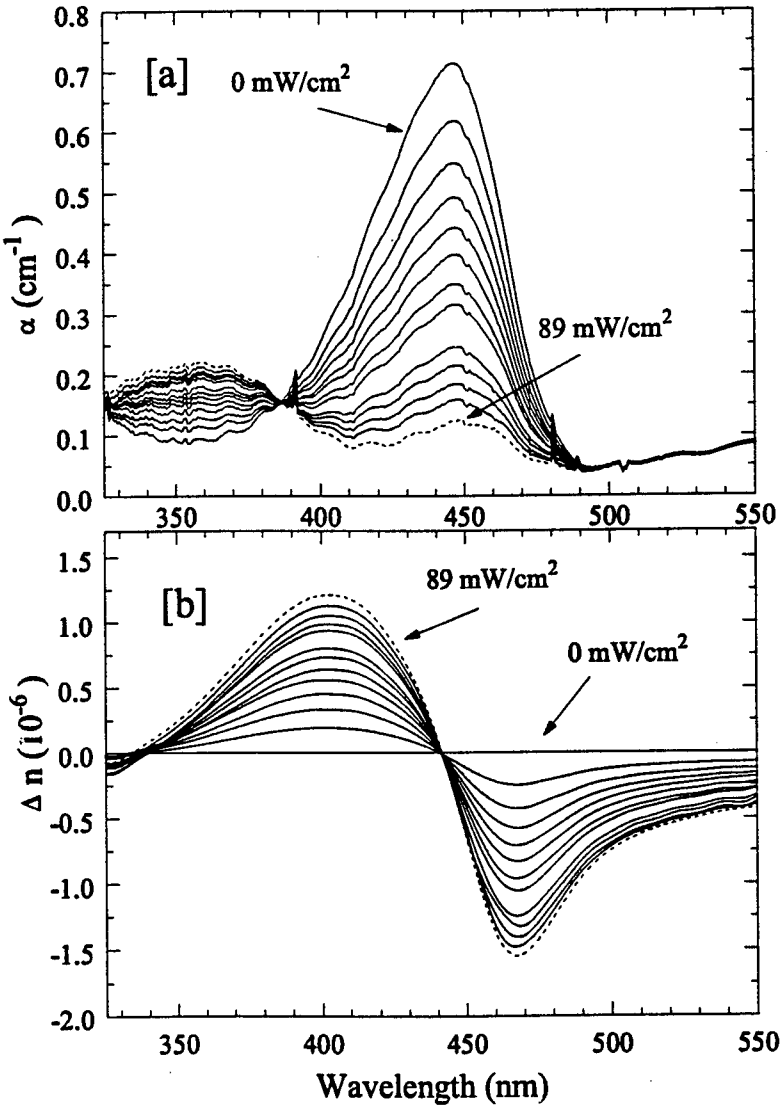

Figure 1.3: Measured absorption of a PYP sample as a function of pump laser intensity. Pump wavelength is 458nm. (b) Calculated (using Kramers-Krönig relations) refractive index change associated with the absorption change observed in (a).

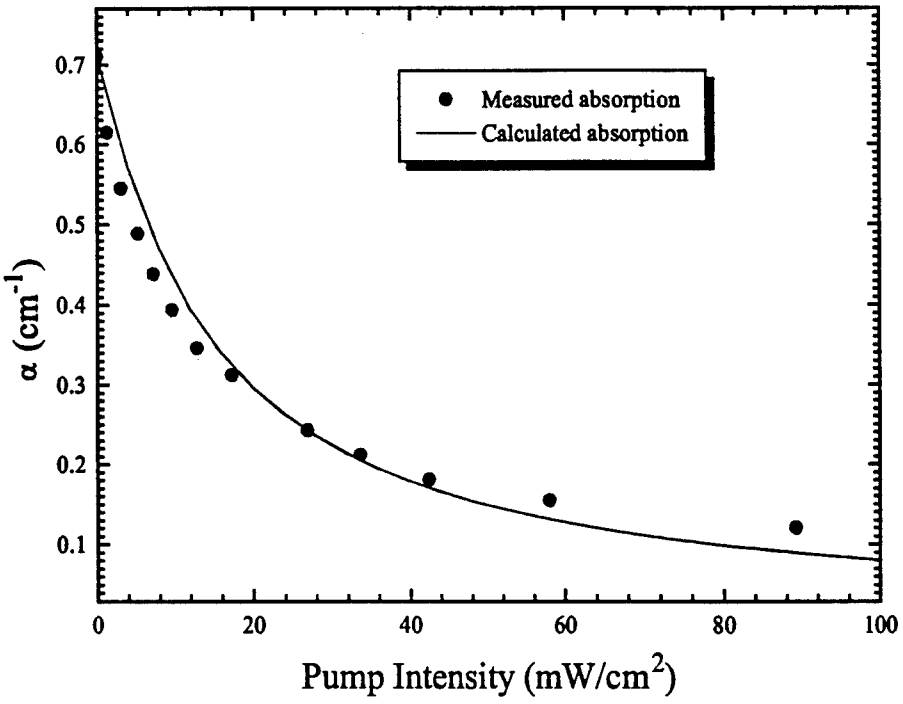

Figure 1.4: Measured absorption for different pump powers as function of pump intensity. Pump wavelength is 458nm.

Fig. 1.4 shows the measured absorption as a function of pump intensity. The theoretical curve is calculated using $\alpha_p = 0.77\alpha$ found from the absorption spectrum in figure 1.3(a). There is a good agreement between the calculated and measured absorption showing that our two level model works well in the cw regime.

1.1.4 Two-Photon Pump-Probe Spectroscopy

Theory

A two-photon absorption (TPA) process involves the simultaneous absorption of two photons and excitation of the medium to an energy level with an energy equal to the sum of the photon energies. Since the probability of having two photons at the same spatial position goes like the square of the intensity, TPA excitation depends on the square of the pump power. Since TPA is less allowed than one-photon absorption, a high pump intensity is therefore required to observe TPA. Using the same cw approach as for the one-photon excitation is not practical, since high enough intensity can not be obtained cw; even if it could be, such a high intensity would lead to strong thermal effects. A quasi-cw excitation is, however, practical using a high-repetition-rate mode-locked laser and a cw probe beam at 446nm. As long as the repetition rate is high compared to the lifetime of the excited state, an accumulation of excitation will occur from all the pump pulses during the lifetime of the excited state, leading to an equilibrium buildup of population that can be probed cw. Ps or fs pulses can achieve high peak intensities ideal for TPA with negligible thermal effects. Thermal effects are negligible for ps or fs pulses. TPA excitation spectroscopy is treated in detail by Birge[11].

The number of photons absorbed via two-photon excitation at time t during the time increment Δt is given by

$$^2N^{abs}_{\Delta t}(t) = N_{\Delta t}(t)A_2 \tag{1.6}$$

where

$$A_2 = 1 - exp[-F(t)S\delta_\lambda CL] \approx F(t)S\delta_\lambda CL \tag{1.7}$$

and

$$N_{\Delta t}(t) = \frac{P(t)}{\hbar\omega}\Delta t \tag{1.8}$$

Here $F(t)$ is the photon flux at time t, S is the photon correlation parameter ($= 1$ for coherent light), δ_λ is the two-photon absorptivity (cm^4 sec molecule^{-1} photon^{-1}), C and L are the concentration and path length, respectively, and $P(t)$ is the laser power at time t. Assuming a gaussian pulse shape spatially and temporally and integrating over time, one finds for the beam-center ground-state fractional population change Δ_1 due to one single pulse:

$$\Delta_1 = \frac{S\delta_\lambda E^2}{\sqrt{2\pi}\pi^2 w_0^4 \tau (\hbar\omega)^2} \tag{1.9}$$

where w_0 is the 1/e radius of the intensity of the beam, τ is the 1/e half width of the pulse intensity in time, and E is the total energy in one pulse given by

$$E = \frac{P_{av}}{\nu_L} \tag{1.10}$$

where P_{av} is the average power of the mode-locked pulse train from the laser and ν_L is the repetition rate of the pulses.

Since the repetition rate of the pulses is high compared to the excited state lifetime of PYP, we can write for the steady state relative population change of the ground state

$$\Delta_{ss} = \Delta_1 \nu_L t_d \tag{1.11}$$

where we have assumed no saturation effects on the ground-state population. Since the absorption at $\lambda = 446$nm is linearly dependent on the ground-state population, the relative change in population Δ_{ss} is equal to the relative change of the absorption. Equations (1.9) through (1.11) can be used to find δ_λ by measuring the relative absorption change at $\lambda = 446$nm.

Experimental setup

The setup used for the two-photon pump-probe experiment is shown in fig. 1.2(b). Probe light from a xenon arc lamp was spatially filtered and then focused onto a 1mm path length quartz spectroscopic cell containing a 43μM solution of PYP. 200fs (τ = 100fs) pulses with an 82MHz repetition rate from a Spectra-Physics Tsunami mode-locked fs Ti:Sapphire laser with a tunable wavelength from 820 to 910nm were combined with the probe light through a beam splitter and then focused onto the spectroscopic cell. The

spot size of the pump was made larger than the probe spot size by focusing and recollimating the pump beam so that all of the probe beam was within the pump beam throughout the sample length. The spatial overlap of the pump and probe and the spot sizes were monitored on a CCD camera by imaging the focus of the beams in the sample cell onto the camera. The pump beam was blocked using blue glass bandpass filters, and the probe beam was sent through a spectrometer to an optical multi-array detector. Scattered pump light leaking through the bandpass filter was subtracted by taking a background with the probe blocked but not the pump. The 1/e pump beam radius was measured to be 30μm and the probe radius to be 15μm. Thermal convection, observed in the sample when high pump powers were applied, introduced some drift in the observed probe absorption spectrum and limited the determination of the smallest observable bleaching of the absorption to about 1%.

Experimental results and discussion

Fig. 1.5 shows the absorption spectrum measured with the TPA setup. It is evident from the figure that there is no observable bleaching of the absorption peak even for our highest average pump power of 300mW at 890nm. From the figure it is clear that the bleaching could not exceed 1%; using equation (1.9) through (1.11) this results in an upper limit of the TPA cross section of PYP of

$$\delta_\lambda < 3.5 \cdot 10^{-52} \quad \text{cm}^4 \text{ s molecule}^{-1} \text{ photon}^{-1} \tag{1.12}$$

The pump wavelength was scanned in the whole tuning range from 820 to 910nm of the laser, but no observable bleaching was found.

1.1.5 Conclusion

In summary, PYP experiences a strong nonlinear bleaching of the 446nm absorption peak associated with the ground state absorption when excited via one-photon 458nm absorption. The bleaching and the corresponding calculated index change are found to be very similar to reported values for bacteriorhodopsin. In contrast, we find no TPA in PYP, whereas a relatively large TPA cross section ($3{\cdot}10^{-48}$ cm^4 s molecule^{-1} photon^{-1}) has been reported for bacteriorhodopsin[13]. We are able to place an upper limit of $3.5{\cdot}10^{-52}$ cm^4 s molecule^{-1} photon^{-1} for the TPA cross section of PYP,

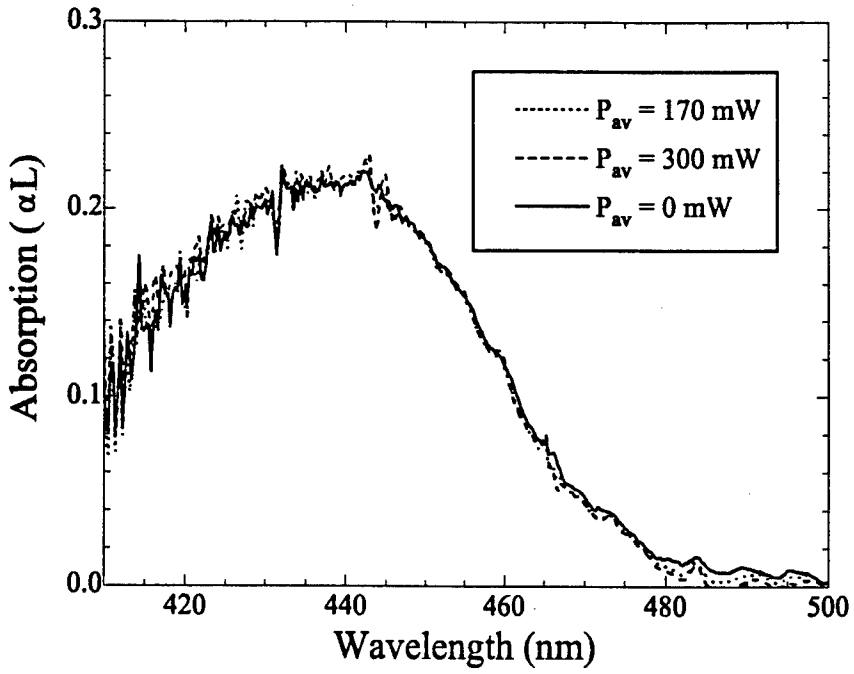

Figure 1.5: Measured absorption for different pump powers showing no evidence of bleaching due to two-photon absorption. Pump wavelength is 890nm.

i. e. 10^{-4} less than that reported for bacteriorhodopsin. Thus PYP is not a good candidate for applications involving TPA, such as a 3D optical memory, but it may be useful for one-photon nonlinear applications where TPA is detrimental.

1.2 TPA MEASUREMENTS ON BACTERIORHODOPSIN

1.2.1 Time Integrated Using Femtosecond Pulses

After doing the TPA measurements on PYP, we repeated the measurement on bacteriorodopsin (BR) with the same setup. We pumped at 890nm with up to 300mW average power (pump beam radius = 30μm). This wavelength should have a fairly large TPA cross section according to Birge's plot of TPA cross section vs. wavelength although the peak is at longer wavelength. Again, no bleaching was observed.

This method of measuring the TPA cross section is not as good for BR for two reasons: First, the lifetimes of the first and second intermediate stages **M** and **O** are 1ms and 10ms respectively. Therefore there is less accumulation of population; i.e. there are fewer excitation pulses within a lifetime. This reduces the sensitivity by at least 26 in comparison with PYP.

Second, the absorption associated with state **M** is blue shifted, thus reducing the absorption of the original absorption peak at 568nm. However, since the lifetime of this state is only 1ms it's contribution is small. The absorption of the **O** state is red shifted, but still has considerable absorption at 568nm. It will therefore not contribute very much to a reduction in the original absorption. It is difficult to evaluate the size of this effect since the absorption spectra in Birge's paper [12] for the different states are taken at different temperatures and do not show relative scales. Probing at 632nm does not work either. The **M** state reduces the absorption at 632nm and the **O** state increases it. According to Prof. George Atkinson it just about cancels if one integrates over time. So the only way is to time-resolve the 632nm probe.

Also at the time we tried BR in the TPA setup, we were not aware of the importance of light-adaptation. This would have reduced the efficiency of the excitation because some of the molecules are in a state that does not

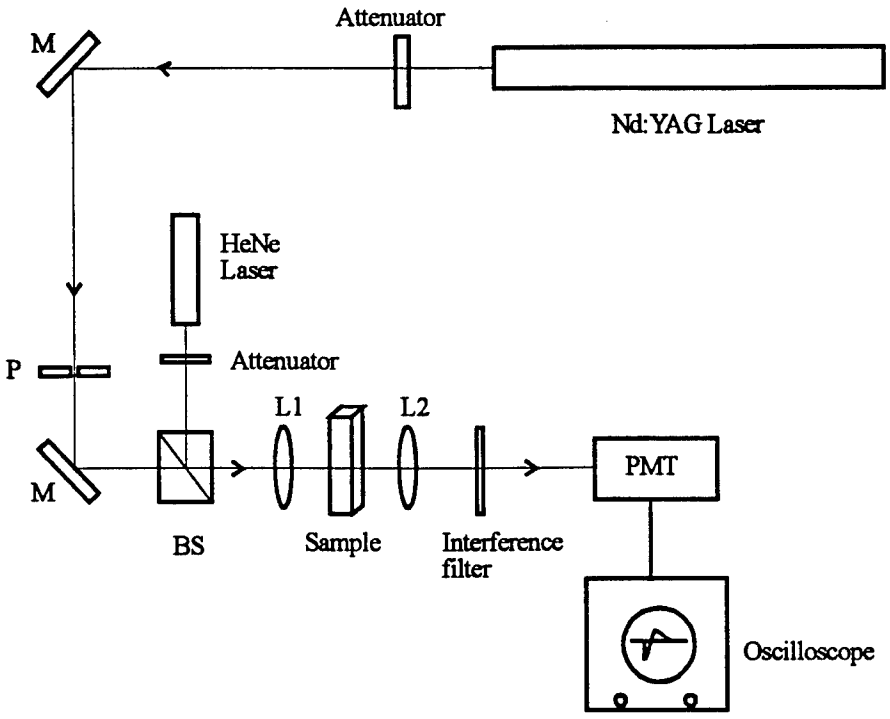

Figure 1.6: Setup used in the TPA measurements on BR using ns pulses.

go through the photo-cycle.

Because of all these factors, the fs measurements on TPA in BR were inconclusive.

1.2.2 Time Resolved Using Nanosecond Pulses

Birge et al. [13] used an 8-ns, 0.4mJ laser pulse to irradiate BR in D_2O in order to measure the TPA spectrum of BR. Using the transmission of the 632.8nm line of a He-Ne laser, they determined the TPA signal as a function of time, enabling them to calculate the TPA cross sections of BR to be 290 and 120GM in the double resonance states. These numbers have not been reproduced by any other groups as far as we know.

We have repeated the Birge et al. experiment using BR in D_2O using the same type of Nd:YAG laser with the same pulse length, repetition rate, and pulse energy; see figure 1.6. We even obtain a curve of probe transmission versus time that agrees with theirs; see figure 1.7. However, when the BR sample was replaced by pure water, the probe transmission was identical. From this we conclude that using BR in a liquid host and strong nanosecond pulses results in stronger thermal effects than two-photon-absorption effects. We suggest that BR in a solid polymer host irradiated by picosecond laser pulses should minimize thermal effects and permit a measurement of the TPA of BR. This is an important measurement not only to clarify the 3D optical potential of BR but also the BR state assignments based on the Birge et al. TPA measurements.

1.3 BIOCHEMICAL ASPECTS

We have made considerable progress toward biochemical characterization of PYP and have essentially achieved our goal which was to make large quantities of native protein, to purify and reconstitute recombinant protein, and to make mutants and chromophore variants with altered photochemical properties.

Our initial goal was to make sufficient native protein to carry out the optical studies. This was accomplished early in the grant period and the optical studies are ongoing. Our second goal was to purify recombinant wild type apo-protein grown in E. coli, develop methods for activating the chromophore and reconstituting the apo-protein. We succeeded in activating the chromophore as the thiophenol ester which is very reactive with apo-protein. In addition, two other methods have been developed by other research groups, all of which result in reconstituted protein with properties indistinguishable from native protein. Much larger quantities of reconstituted recombinant protein can now be made in E. coli in a shorter period of time than when native protein is prepared in E. halophila. Success in this endeavor has also allowed us to begin making mutants and proteins with variant chromophores.

We have characterized two mutant proteins, E46Q and R52A, in collaboration with Dr. Libby Getzoffs group in La Jolla, CA and have submitted a paper for publication. Glutamic acid 46 is buried in the protein interior and is hydrogen bonded to the chromophore hydroxylate. The ionization

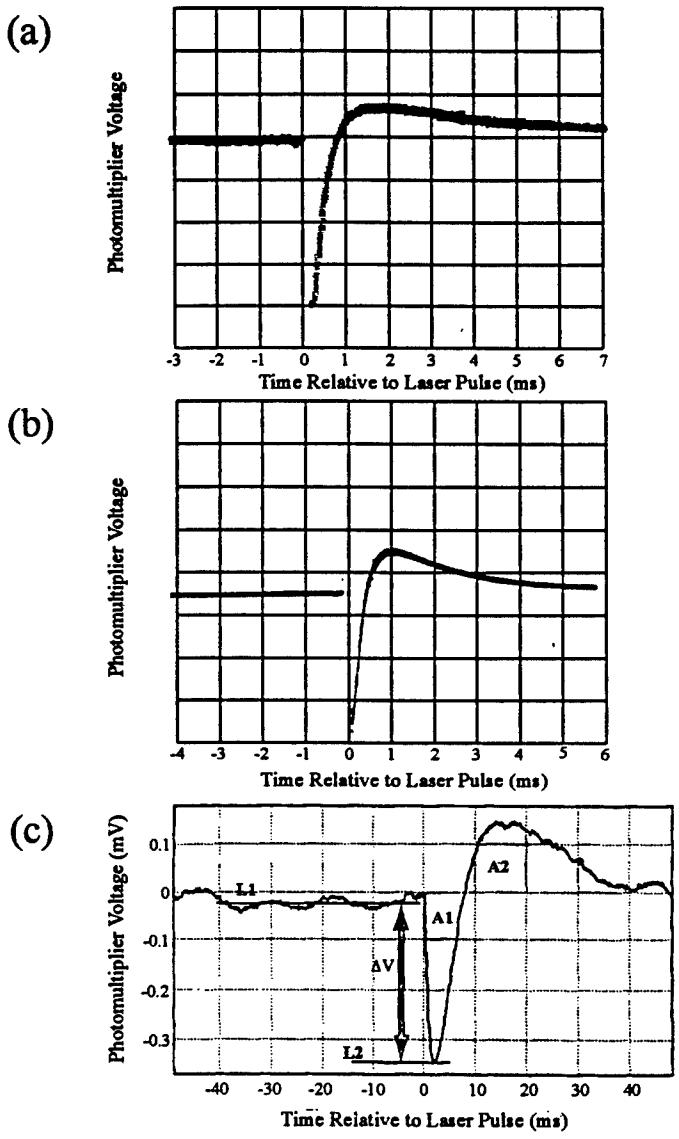

Figure 1.7: (a) 632nm probe signal vs. time trough a BR sample after excitation by a 0.4mJ 1.06μm YAG ns pulse. (b) Same as (a) but with pure water replacing the BR. (c) is taken from Birge

constants for Glu46 and for the chromophore hydroxyl are shifted more than six pH units from their normal solution values of 4.5 and 9.0 due to their apparent interaction in the active site. The E46Q mutant (carboxyl replaced by an amide) was designed to test the role of Glu46 in stabilizing the chromophore in the ionized state. The wavelength maximum of the E46Q mutant was shifted 16 nm to the red, to 462 nm. Apparently, the hydroxyl is still ionized in the mutant and the weaker H-bond allows more negative charge to be concentrated on the chromophore. The kinetics of the photocycle are quite different from those of the wild type protein. The second phase of the bleach reaction is much faster than wild type. At low pH, the recovery or return to ground state is about the same as wild type, but there is a dramatic 700-fold increase with pH (pK = 8) as shown in figure 1.8.

Based upon the large effect of pH on the photocycle of E46Q, we reinvestigated the effect of pH on the photocycle of native PYP as shown in figure 1.8. Previously, we had examined kinetics at only a few pH values and saw small effects which did not seem at that time to be very important. We now know that the bleaching reaction is fastest at pH 5 and slows 3-fold with pH (pK $\leq$ 5.7). The recovery reaction is more complicated, it is minimal at pH 5 and 10 and maximal at pH 8 (pKs = 6.4 and 9.4) and shows a 16-fold variation in rate constant. Thus, the E46Q mutant is very different from wild type PYP. We believe that the functional group responsible for the pK 8 in E46Q is Tyrosine 42, which also H-bonds to the chromophore. This will be tested by future mutagenesis.

We expected much more dramatic effects with mutant R52A for two reasons. First of all, Arg52 blocks solvent access to the chromophore and must swing out of the way during the photocycle. Secondly, Arg52 was thought to interact with the chromophore to form a stabilizing ion pair. The small shift in the absorption spectrum from 446 to 452 nm in R52A (see figure 1.9) indicates that Arg52 has a minimal role in stabilizing the negative charge on the chromophore through formation of an ion pair in the wild type protein. The kinetics of the bleaching reaction during the photocycle of R52A are faster than wild type and recovery is slower, although the effect of pH is essentially the same. This indicates that the principal effect of the R52A mutation is on protein stability, i.e. Arg52 probably has no direct effect on the properties of the chromophore.

We made protein with two variant chromophores, cinnamic acid (CA-PYP) and 3,4-dihydroxy-cinnamic acid (DH-PYP). The absorption maxi-

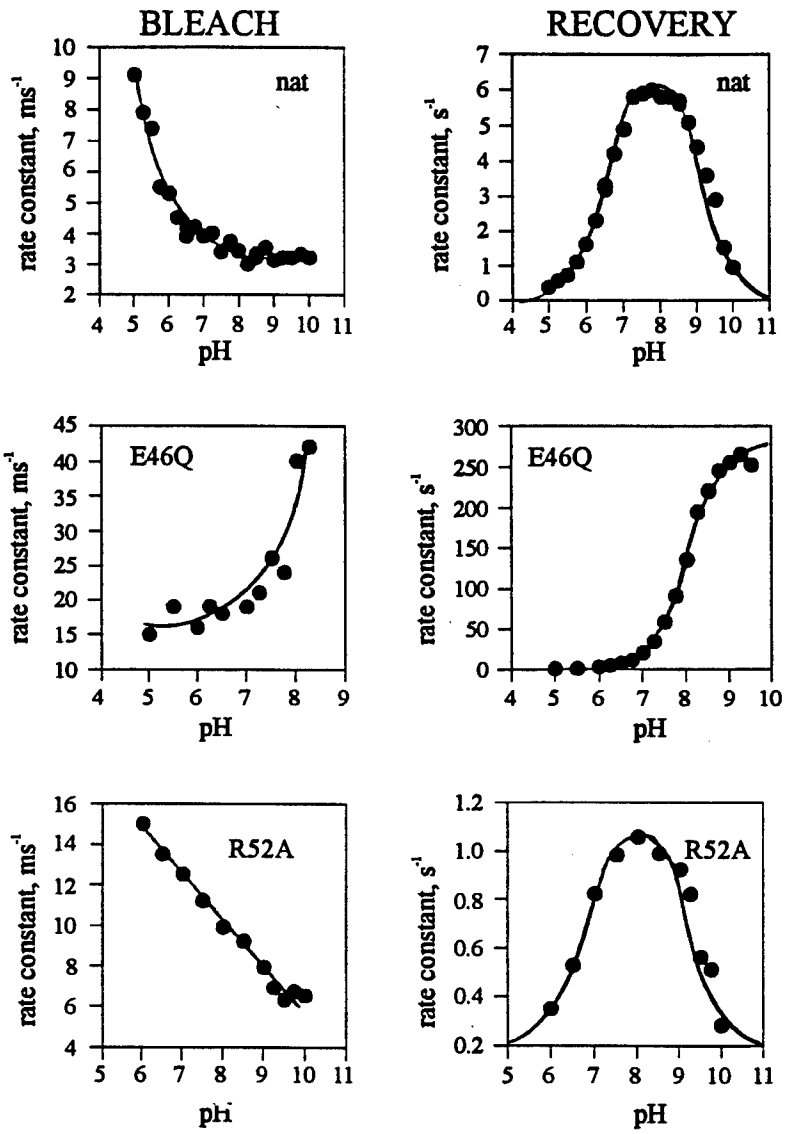

Figure 1.8: Effect of pH on the bleach and recovery rates of native PYP and the E46Q and R52A mutant proteins.

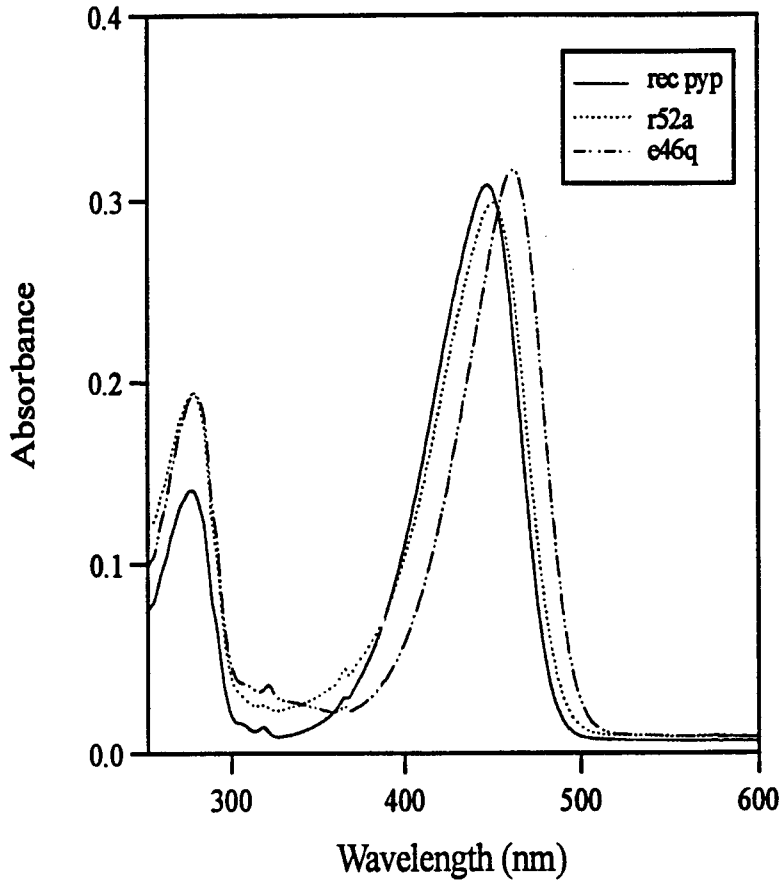

Figure 1.9: Absorption spectra of reconstituted PYP and the mutants R52A and E46Q.

mum of the CA-PYP variant is near 325 nm which is not red-shifted very much from that of free chromophore, indicating that the 4-hydroxylate group of the native chromophore is essential for the large red-shift in native protein. The DH-PYP variant is red-shifted to 458 nm (see figure 1.10) and is partially bleached in the ground state. The kinetics of the bleaching reaction are too fast to measure with our present setup and recovery is about 20-fold slower ($0.2\ s^{-1}$) than native PYP. Our results suggest that the main effect of adding the 3-hydroxyl group is to destabilize the protein. This is probably because the active site is too small to accommodate a larger chromophore. We now intend to add variant chromophores to the mutant proteins. If the effects on protein stability are additive, we would not expect to be able to make the R52A DH-PYP variant at all. If the variant is stable, there may not be an additive effect on kinetics if the steric hindrance is eliminated through mutation. It will be interesting as well to see whether the red-shifts due to mutation and to chromophore variation are additive in both R52A and E46Q.

Our results to date are encouraging in that we have been able to affect both the absorption maximum and the photocycle kinetics through mutation and chromophore variation. These studies are still at an early stage and should eventually result in protein tailored to meet the requirements of a photochemical device.

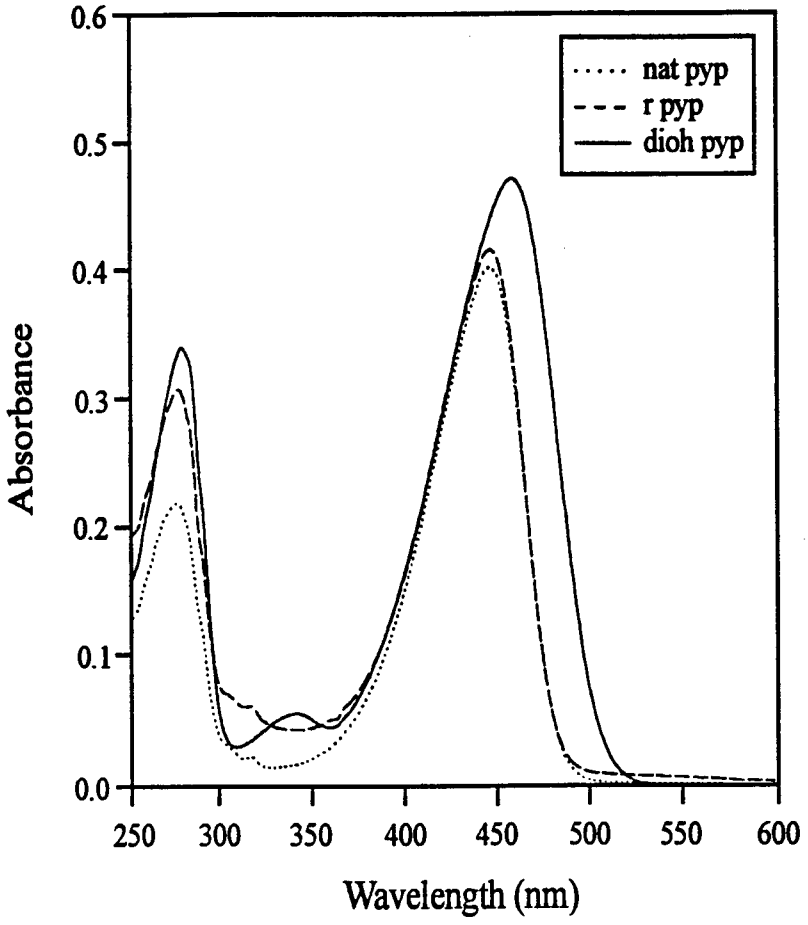

Figure 1.10: Absorption spectra of native PYP, reconstituted PYP, and DH-PYP.

1.4 PUBLICATIONS

O. Lyngnes, H. M. Gibbs, C. F. Li, S. B. Devanathan, T. E. Meyer, G. Tollin, M. A. Cusanovich, "One-Photon and Two-Photon Pump-Probe Spectroscopy of Photoactive Yellow Protein", submitted to Journal of Nonlinear Optical Physics & Materials, (1996).

U. K. Genick, S. B. Devanathan, T. E. Meyer, I. Canestrelli, E. Wiliams, M. A. Cusanovich, G. Tollin, E. D. Getzoff, "Active-Site Mutants Implicate Key Residues for Control of Color and Light Cycle Kinetics of Photoactive Yellow Protein", submitted to Biochem. (1996).

S. Devanathan, U. Genick, E. Getzoff, T. E. Meyer, M. A. Cusanovich, G. Tollin, "Preparation and Properties of a 3,4-Dihydroxy-Cinnamic Acid Variant of the Photoactive Yellow Protein", to be submitted for publication.

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