Biophysical Studies on how the N-terminal Part of Photoactive Yellow Protein Accelerates its Functional Dynamics

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Abstract

Photoactive yellow protein (PYP) is a blue-light receptor protein that regulates negative phototaxis in *Halorhodospira halophila*. Photo-illumination converts PYP into a long-lived intermediate state, called pB. The initial pG state of PYP is recovered spontaneously in ~1 second. Previous work showed that the N-terminal region of PYP is important for the rapid rate of pB decay in the PYP photocycle. Our recent results suggested that electrostatic interactions between the negative charge on the N-terminal part of PYP and the positively charge on its PAS core are important in the acceleration of pB decay. To test this proposal, we designed a PYP mutant in which the N-terminal region is positively charged. Currently mutagenic primers are being used to create this mutant PYP. We have already isolated wild type PYP for comparison with the mutant and found that its photocycle corresponds well to published results. We report these data and describe the plan for the next step.

Introduction

The crystal structure of photoactive yellow protein is important to our experiment; for we hypothesize that if we mutate certain parts in it we ultimately affect the photocycle. PYP consists of two regions: a PAS core containing the chromophore and an N-terminal region. The chromophore in the initial state is deprotonated and has a *trans* configuration. Absorption of visible light triggers chromophore photoisomerization, followed by conformational changes during the formation of the pB signaling state. The Photocycle of Photoactive Yellow Protein is a key factor that allows us to study pyp. Upon absorption of visible light, the C=C bond of the chromophore isomerizes from *trans* to *cis*, which initiates a series of sequential alteration of the hydrogen bonding network as well as conformational changes in the protein. The blue-shifted intermediate denoted pB (PYPM) is a biological signaling state whose chromophore is *cis* and protonated. Finally, the photocycle is terminated when the pG state is recovered. Wild type PYP completes the photocycle within one second (Kumauchi et al. 2008).

Our hypothesis is that the negatively charged N-terminal region interacts with the positively charged PAS core, which facilitates the photocycle.

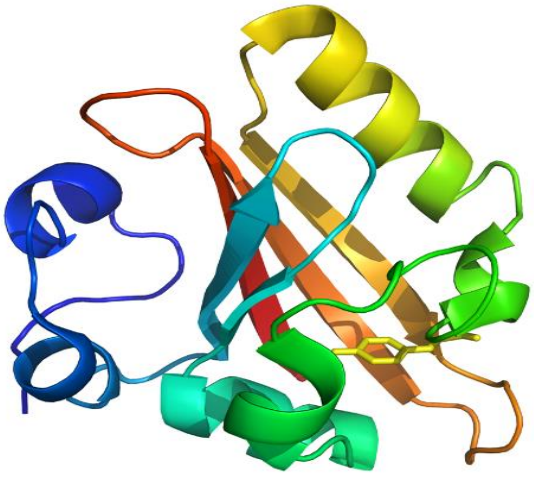
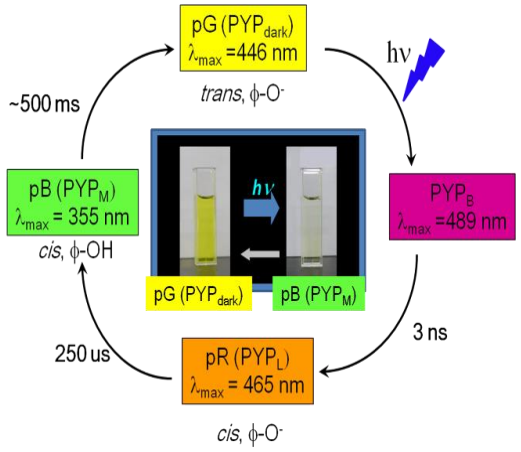


Figure 1- *Crystal structure of PYP*

*=Mentor

If the hypothesis is correct, PYP mutants containing a positive or neutral N-terminal region would have a slower photocycle or not, To test this we use the following steps. 1. Introduce neutral or positively charged residues in the N-terminal region by site-directed mutagenesis. 2. Express, extract and purify the mutant protein. 3. Visible spectroscopy: Is the photocycle rate altered? Fast? Slow? (Harigai et al. 2001, Harigai et al. 2003)



Fiaure 2 *- Photocvcle of PYP*

Experimental Section

Preparation and purification of wild type and mutant type PYP samples was a process that involved multiple different processes and procedures (Imamoto et al. 1995). We prepared wild type PYP and carried out UV/vis spectroscopic measurements. Mutant preparation to introduce multiple positive charged residues in the N-terminal region is ongoing (Mihara et al. 1997). The procedure for protein expression and reconstitution begins with the incubation of *E. coli* in 1 liter of liquid culture for 16 to 20 hours. After incubation we add IPTG, which is a compound that activates the PYP producing gene in the bacteria, and incubation was continued for 4 to 6 hours to allow protein production. The *E. coli* cells are then harvested from the liquid culture through centrifugation of the solution. Once the *E.coli* is collected it needs to be disrupted so that the produced PYP protein can be harvested into samples. This is done by adding *p*CA anhydride (*p*-hydroxycinnamic acid) to the *E.coli*, which is then disrupted further through the process of sonication. Any leftover cell debris is then removed by further centrifugation.

This procedures yields multiple samples of the desired PYP protein. However, the samples also include multiple other protein byproducts of the *E. coli*. Each sample must be thoroughly purified in order to be useful in our experiment. First any major impurities are removed by running the sample through columns containing an anion exchange resin. We then use a Ni-NTA resin which retains our Histidine tagged PYP samples, and allows any other unwanted proteins to filter. The sample is the polished once more using another anion exchange resin. In order to ensure that we have a pure PYP sample that is also sufficiently concentrated we check the UV- visible absorption spectrum measurement using an HP8354 spectrophotometer. Since the PYP has a distinct yellow color it also has a distinct wavelength in the absorption spectrum, allowing us to check its concentration along with any other impurities that may have lasted through purification.

The last step to the experiment is to produce mutant samples of PYP which will allow us to test our hypothesis. First the PYP gene is amplified by PCR with a sense and an anti-sense primer into which multiple positively charge amino acid sequences were introduced. This sequence will serve as our PYP mutation. The PCR amplified DNA fragment is site specifically digested by *Bam*HI and *Pst*I. The digested fragment is introduced to the PQE-80L vector of *E. coli's* plasmid DNA and ligated together. This transforms it into DH-5α. The plasmid

in the *E. coli* colony is then confirmed by colony PCR. The last step is to confirm the DNA sequence in the plasmid DNA. Once the mutant is confirmed it goes through the same collection and purification processes as the wild type PYP samples.

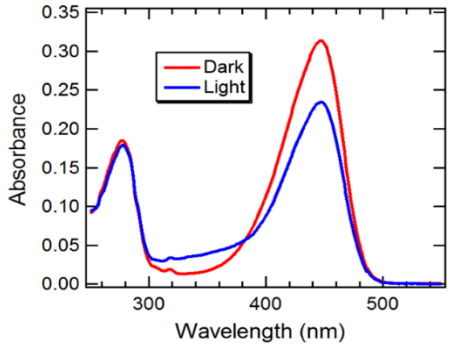


Figure 3- *Absorption spectra of PYP Red: Dark state, Blue: Light illuminated.*

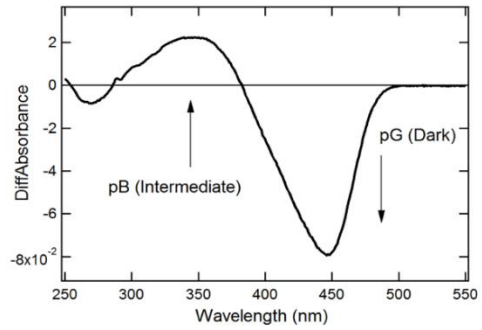


Figure 4 - *Difference spectrum of PYP Light spectrum minus dark spactrum.*

Results & Future Plans

Absorption spectra of wild type PYP were measured in the dark and under the illumination (Figure 3). By exposure to blue light the main pG absorbance band is reduced in intensity, while the mid-wavelength pB species (~350 nm) increased.

The light-induced difference spectrum of wild type PYP was obtained by subtracting the light from the dark spectra (Figure 4). The main band at 446 nm is converted into the blue-shifted pB intermediate (~350 nm). The time-dependence of changes in the absorbance at 446 nm was plotted as a function of time were (Figure 5). The time-course clearly indicates that the absorbance change is light dependent.

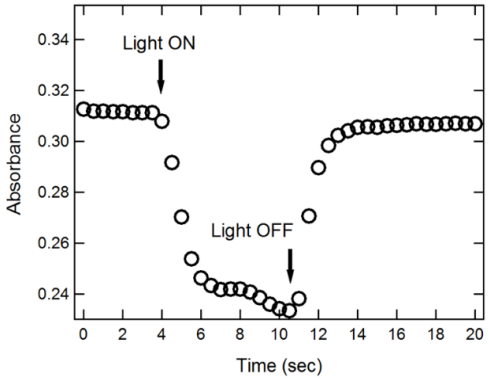


Figure 5 - *pG recovery time course for wild type PYP. The calculated photocycle rate is 350 milliseconds.*

The amount of information we have on wild type photocycles is quite small, because it is difficult to study them. These cycles often occur in fractions of a second and are often hidden by processes taking long periods of time that do not give an accurate measurement of the rate at which the reaction is occurring. This makes it hard to come to any conclusions about the way these cycles occur. Fortunately PYP is a natural occurring photoreactive protein, meaning that we can initiate its photoreactive cycle instantly using a high powered laser giving as an immediate measurement of the rate of the reaction. This makes it much more efficient than other methods and allows us to properly test mutant PYP proteins. By testing multiple mutants of PYP proteins we can try and determine how the structures of these proteins affect their reactions. Using a protein as convenient as PYP we are hoping to answer some questions about proteins in general. If we can learn how the structures of proteins affect their functions than we can

learn how to manipulate them in ways that are beneficial. This means that if our research is successful we can contribute largely to medical research involving proteins in the body such as hormone receptors and many others. If we are correct our research could very well be the first step to multiple future experiments that will open new doors in the fields of microbiology and medicine.

Acknowledgements

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