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Photoactive yellow protein's choromophore site, its subsequent structure, and the modification of it

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The bacteria genera *Halobacterium*, *Ectothiorhodospira*, and *Rhodobacter* possess a unique protein in it that responds to the effects of light absorption, in which the protein will absorb a photon, flex, and then release a photon of its own as the protein changes shape. This unique property can be seen in several different types of proteins spanning several different genera and families of prokaryotes; however it will be specifically the properties of The Photoactive Yellow Protein (PYP) that will be discussed and reviewed in length in this paper. The most common technique that has been used to determine the shape and structure of this protein is most commonly x-ray crystallography. The utilization of this technique provides many unique possibilities as well as challenges, as a generally accepted all-encompassing set of conditions does not exist for proteins; each specific protein of differing amino acid chains will have its own unique set of variables that produce the most effective crystallization. Also, by modifying the presence of an additional and severable tracking and collection mechanism on the protein, these improved isolation abilities allow better purification of the PYP as well as its subsequent encoding genes.

Introduction

The overall mechanism and precise structure of PYP in vivo is still being researched, however the theory that is currently accepted is that at the terminal end of a protein's α-helices is a unique structure that is becomes excited when a sufficiently strong source of blue colored light excites it and then releases a corresponding photon as the electrons in the outermost shell return to their low-energy state [1]. The most likely reason that its reactive-color affinity is blue is due to the fact that this is closer to an ultraviolet wavelength, and thus has more possibility of ionization. This temporary oscillation in the protein's electron distribution potentially causes a section of it to essentially actuate and torque itself as the changing electrostatic levels of the protein change. The significance of this is that the proteins conformation changes, and that before the protein can release another stimulus of photons it must undergo a refractory period that reorients the actuation of the photoactive yellow protein's choromophore into its initial state which will thereby allow it to be a high-energy state once again. The duration of this period of time it takes for it to reset is typically around 2 – 3 seconds in its natural state; however modification of the protein's amino acid sequence has shown that this photocycle can be dramatically shortened or lengthened almost a hundred fold [1]. The specific reaction occurs at a 4-hydroxycinnamyl choromophore anion which is then attached to a Cysteine-69 chain via a thioester bond [1,2]. The specific wavelength emitted by a purified strain of PYP will absorb the largest portion of light from the 446nm range, which is in tandem agreement with the idea of blue light absorption by the bacteria genera themselves.

In a laboratory setting, the ideal ratio level of absorption is between the 446nm wavelength that the PYP absorbs, and a 275nm wavelength is expected to be around .5. The precise value that is most accurately sought after is in fact .46 rather than .5 because this

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indicates a higher degree of purification, while still not being too concentrated to utilize in crystallization procedures. Specifically the formula designed to figure the ratio out is [275nm – 550nm]/[446nm – 550nm] = 0.5 [3]. The significance of the light absorbed ~275nm wavelength range is that this is the typical range that non-photoactive protein absorb light, DNA, and other biological molecules inhibit. Also, in the equation the purpose of including the 550nm into the calculations is to introduce a baseline for the equation to subtract any absorbance discrepancies in the spectrometer itself.

Recent Progress

To view a more accurate representation of the PYP's physical structure, x-ray crystallography is the preferred method to locate the precise position of the atoms that compose the PYP and in turn its choromophore. Almost an infinite number of variables can created to isolate precise conditions for the formation of the PYP crystals, such as pH, Precipitating agent, concentration of precipitating agent, temperature, solution medium, etc. However, in one study it was found that a solution of saturated Para-Chloromercuribenzoic acid, with the pH adjusted to 10 gave a completeness of 99% of the protein successfully imaged [1]. The resolution of image was very good too, as a resolution distance of 2.2 Å could be determined. As an example, when using 10 mM KO_sCl_6 with the pH adjusted to 8.5 as the precipitating agent, only 88% of the protein's structure could be imaged [1]. Even at such a low level of imaging, the resolution was only 2.8 Å. Other compounds used in crystal formation were Potassium tetracyanoplatinate ($K_2Pt(CN)_4$) with pH 7, Potassium Dicyanoaurate ($KAu(CN)_2$) with pH 7, Erbium Acetate ($Er(CH_3COO)_3$) with pH 7. Polyethylene glycol (PEG) as well as Ammonium Sulfate ($(NH_4)_2SO_4$) [1] were also used as precipitating agents for the crystal formation process, however were not listed in the studies. In other experiments there were several heavy atom compounds as well; however these had less impressive results.

Since PYP is an organic protein, the most common method of producing and harvesting PYP is to grow it in an obtainable microorganism. The most common method of this collection is modifying an *E. Coli* bacteria's DNA to code for PYP production. This is most typically accomplished with introducing a plasmid that has a modified pET-16b [2] vector that will now code specifically for a protein, which then the bacteria can spread via Lateral gene transfer. After this proliferation of *E. Coli* reaches a specific point, the cells are isolated and then lysed with either cavitation bubbles via ultrasonic vibrations, or a chemical lysing agent that with remove their cell walls. The entirety of the cell's contents now resides in the resulting solution and can be further isolated via Nickel-ion/Anion-exchange [1] chromatography columns, pre-saturation Ammonium Sulfate precipitation, and kDa Dialysis [3].

Discussion

There are several potential uses for the complete understand of PYP's mechanisms and actions. The capability of PYP to be used as both an intracellular communicator, as well as many other molecular biology topics is very clearly understood. The Specific structure of PYP is strongly analogous to PAS domain Proteins (PAS Standing for Per {standing for period circadian protein} Arnt {standing for aryl hydrocarbon receptor nuclease translocator protein} Sim {standing for single-minded protein})[4]. The significant trait these have in common is a typical wavelength that excites their structure. The three sources of PAS domain are very common in both bacteria as well as humans, which lends their use involved in biomedical research understandings. [4]

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