The Bioluminescence and Fluorescence of *Aquorea aquorea*

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Literature Seminar

Introduction

The jellyfish *Aquorea aquorea* has contributed two major proteins, aequorin and green fluorescent protein, to modern science. In 1955, Davenport and Nicol[1] reported that the light producing cells of *Aequorea aquorea* fluoresced green when irritated with ultraviolet light. Five years later, Shimomura and co-workers[2] tried to isolate the protein responsible for this observation. During their investigation, they described a protein from the jellyfish that emitted blue light in the presence of calcium. They concluded that the jellyfish must contain two proteins that are responsible for the green fluorescence, as no intramolecular transition was observed within the blue bioluminescent protein that could account for the green fluorescence, seen in the jellyfish's native state. Later, a protein extract from the jellyfish was isolated that could produce the green fluorescence observed by Davenport and Nicol.[1,3] The protein responsible for the fluorescence is called green fluorescent protein (GFP).

As mentioned earlier, GFP was first discovered as a companion protein to aequorin. The first measurements of the fluorescence spectrum of aequorin and the fluorescent spectrum of GFP were reported by Johnson et al.[4] Aequorin's bioluminescent spectrum is broad, with a peak at 475 nm, while the fluorescent spectrum of GFP was sharp, with a peak at 508nm. The GFP of *Aequorea aequorea* transmutes the blue bioluminescence from the photoprotein, aequorin, into green fluorescence. GFP probably evolved not to just shift the output of color mainly to boost the overall efficiency of emission, as bioluminescent efficiencies of photoproteins are quite low. If the

photoprotein excited state energy can be efficiently transferred to GFP, the organism can in theory generate more light for a given energy cost.[4]

GFP has changed from a nearly unknown protein to a commonly used tool in biochemistry, medicine and molecular biology. A search of the 1994 literature found only 10 publications with GFP in the title or abstract, a similar of the Feb. 2001 literature found 106 publications.[4] Bioluminescent and fluorescent proteins have repeatedly appeared during the course of evolution in different organisms, where they serve biological functions important to the organisms that possess this unique trait. These functions, that differ among the organisms, utilize fluorescence in more than one way. The different recognized functions may be classified as defensive (to deter predators), offensive (to aid in predation), and communication (mating and courtship).[4-5] In terms of the total number of different species, the emission of bioluminescence is rather rare. Although fluorescent proteins are found in numerous organisms of the coelenterates, the jellyfish *Aequorea aequorea* is the focus of this paper as it was the first isolated, and it has been the most characterized and studied.

Aequorin

In the investigation of the fluorescence of *Aequorea aquorea,* Shimomura and co-workers[2] isolated a single polypeptide chain of about 22kDa that they named aequorin. Further investigation, found the photoprotein necessary for the fluorescence of GFP as energy transfer takes place between the two proteins.[4,6-7] The protein is believed to be a monomer containing four helix-loop-helix motifs, of which three domains can bind calcium. The molecule has been shown to contain a substrate, 2-

hydroperoxy-coelenterazine, which is tightly but not covalently bound to the protein through a hydrogen bonding network.[6,7-8]

The crystal structure of aequorin has been solved with a resolution of 2.3 angstroms. The protein's scaffold consists of four EF-hand domains arranged in pairs to form the globular protein. Each of the EF-hands is arranged back-to-back, forming short stretches of β-sheet, as seen in other calcium binding proteins such as calmodulin. A hydrophobic surface is present in each half of the protein formed by the EF-hands. This arrangement of the EF-hands forms two facing cups that comprise the hydrophobic binding cavity for coelenterazine. This binding cavity is situated in the center of the protein and is formed by the residues originating from each of the EF-hand domains.[8-9]

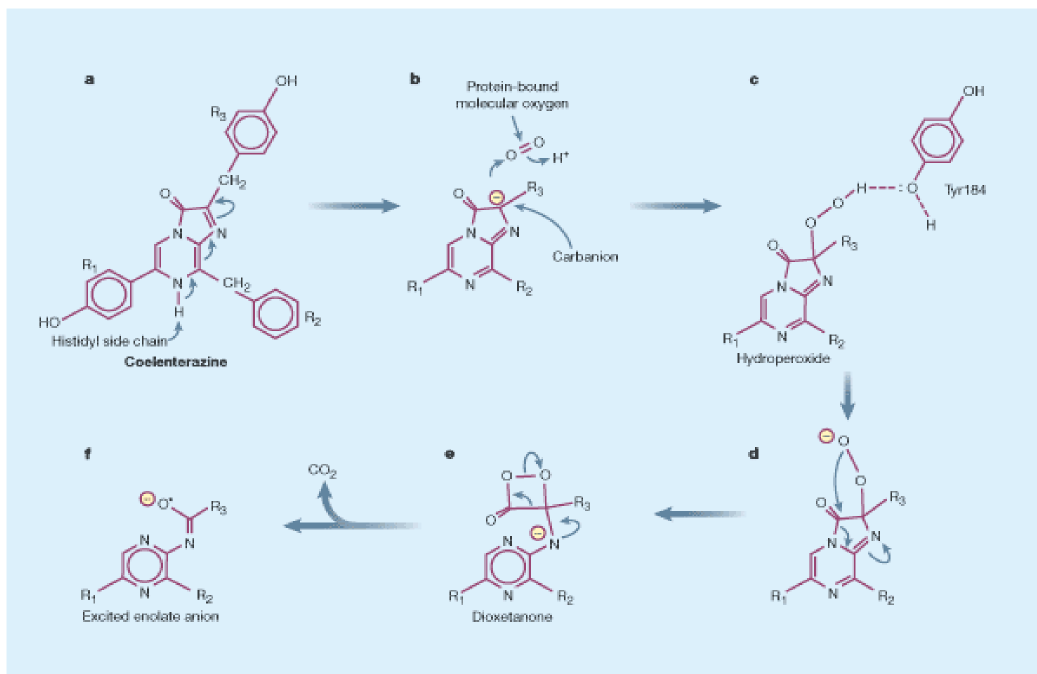

Figure 1. The proposed mechanism of the bioluminescense of aequorin. (Prendergast, F.G. (2000) *Nature* **405,** 291-293)

Aequorin is a member of a unique class of bioluminescent proteins, photoproteins, in which both the substrate, coelenterazine, and oxygen are already bound

to the protein.[4,9] The addition of calcium causes a conformational transition within the protein, triggering an intramolecular reaction and resulting in light emission. The intramolecular oxidative decarboxylation of coelenterazine, suggested by McCarpa and Chang[9], occurs through several intermediate steps (Figure 1). Coelenterazine reacts with oxygen to produce hydroperoxide, and this is followed by deprotonation to form dioxetanone, a strained four-member ring. The dioxetanone undergoes scission to yield CO_2 and an excited enolate anion. The hydrogen network that stabilizes the coelenterazine plays an important role in explaining how the addition of calcium can serve as the "trigger" to the intramolecular reaction. The hydroperoxide, the oxidative decarboxylation intermediate of coelenterazine, is stabilized in solution by an H-bond to Tyr190. In turn Tyr190 is H-bonded to His175 where it plays a important role in the protein's active site. The binding of calcium causes the H-bond between Tyr190-His175 to become stronger, increasing the electrostatic contribution. The hydroperoxide will protonate tyrosine which will allow the peroxy anion to undergo the irreversible nucleophilic addition to the C-3 carbon of coelenterazine to form the committed dioxetanone intermediate. The formation of the dioxetanone intermediate provides the energy, ≥ 70 kcal/mol of exergonicity, necessary for the blue fluorescence of aequorin (λ_{max} = 469 nm).[7-9]

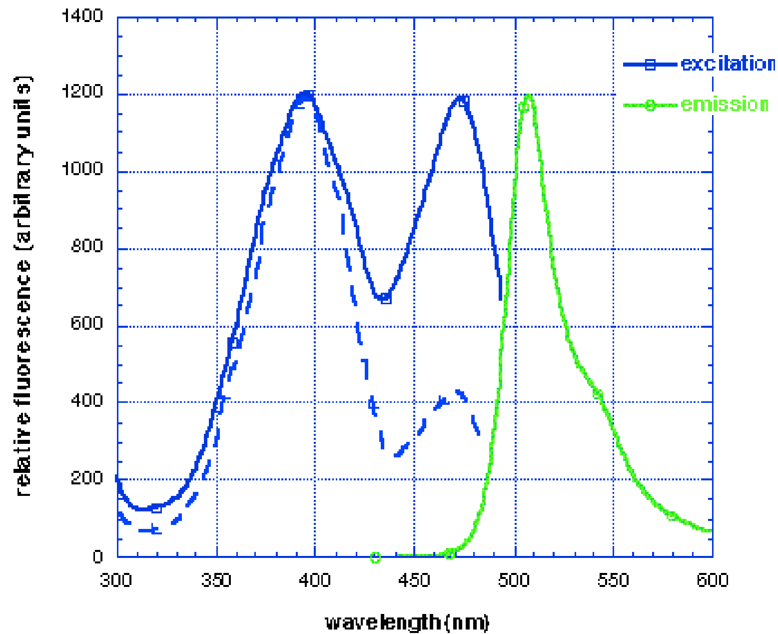

Figure 2. The excitation and emission spectra of *Aquorea aquorea* GFP.

GFP

Green fluorescent protein, GFP, is a spontaneously fluorescent protein isolated from the Pacific jellyfish *Aequorea aequorea.* The protein's primary role is to transduce, by energy transfer, the blue bioluminescense of aequorin into green fluorescent light.[4] GFP is excited by the energy from aequorin and has been shown to increase the intensity of the light given off by a factor of 3.[10] The protein has been crystallized as a dimer, although many monomeric structures of GFP have been solved. GFP is not an obligate dimer, dimer formation is highly dependent on the growth conditions of the crystal. The crystal structure of GFP has been solved to a resolution of 1.9 angstroms and has been shown to be comprised of 238 amino acids.[11-12] The protein possesses the shape of a cylinder, comprised of 11 strands of β-sheet with an α-helix inside and short helical

segments at the ends of the cylinder. The β-sheets form the walls of the cylinder, and the α-helix runs diagonally through the cylinder. The chromophore is situated in the center of the β-sheets where it is linked by the α-helix stretch that runs through the center of the cylinder. Short α-helical sections of the protein form the ends of the cylinder. This unique fold represents a new class that has been named the β-can.[4-6,10] The enclosing of the chromophore within the "can" protects it from the quenching of oxygen and the attack of hydronium ions.

Of the 20 natural occurring GFP's, most of the knowledge about the physical characteristics come from the work on *Renilla* and *Aquorea.* All known GFPs are acidic, dense, globular proteins with monomer molecular weights of 27kDa. Nearly all GFPs are stable non-dissociable dimers (unless in dilute aqueous solution where they exist as monomers) that retain this dimeric characteristic unless denatured under harsh conditions. The observation of dimerization between two monomer GFP's is consistent with most, but not all, of the crystal structures currently available. The dimer contacts are fairly tight consisting of a core of hydrophobic side chains from each monomer and an abundance of hydrophilic contacts (Figure 3). The higher the concentration of GFP the more likely dimerization is to occur. Slight rotation around the long axis of the "beta-can" can induce a switch from predominantly hydrophobic contacts at the interface of the dimer to predominantly hydrophilic contacts. This rotation has shown to have no effect on the individual structures of the dimer, although the absorption spectrum undergoes large changes: the extinction coeffcient of a peak near 475 nm (major excitation peak) is decreased fourfold to fivefold.[5] The high concentration that favors dimerization lowers the chromophore's ability to absorb and get excited by the blue fluorescence of aequorin.

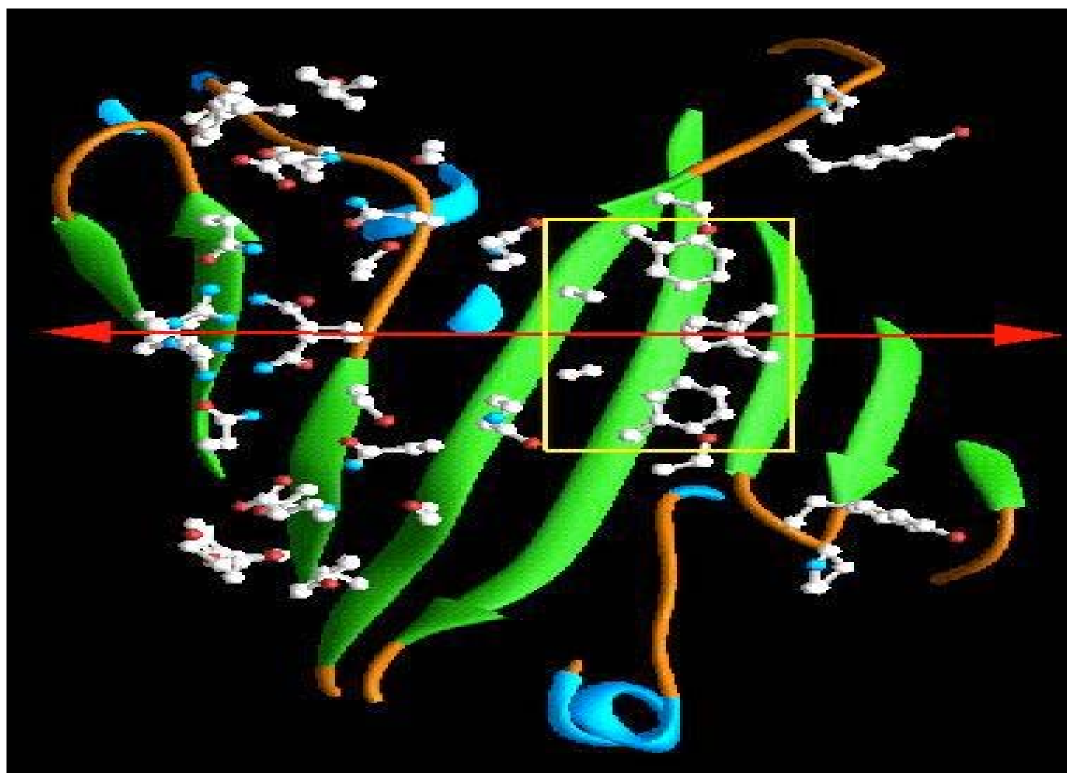

Figure 3. Dimer contact region between two monomer GFPs. (http://www-bioc.rice.edu/Bioch/Phillips/Papers/gfpbio.html)

The chromophore of GFP was first isolated from a papain digest in 1979 by Shimomura and coworkers.[13] The visible absorption and fluorescence of GFP arises from a 4-(*p*-hydroxybenzylidene)-imidazolin-5-one chromophore, which is formed post-translationally by a cyclization of Ser65, Tyr66, Gly67 and dehydrogenation of tyrosine (Figure 5). The autocatalytic cyclization of the chromophore has been postulated to begin by the nucleophilic attack of the carbonyl group of Ser65 by the amino group on Gly67. After the ring formation, the loss of water forms the imidazolin-5-one intermediate. The last step in the chromophore formation involves the oxidation of the Tyr66 side chain by atmospheric oxygen. The posttranslational chromophore formation occurs in part because of the very short intramolecular distances between the carbonyl carbon of Ser65 and the amide nitrogen of Gly65.[4-5] The chromophore forming residues of the immature GFP are

preorganized in a “tight turn” with the distance from the carbonyl carbon to Ser65 and the amide nitrogen of Gly66 being only 2.9 angstroms apart. The 11-stranded β-can structure enforces this “tight turn”, restricting the space of the chromophore-forming region so that the residues are kept at the correct distances for the autocatalytic cyclization of the chromophore.[15-16]

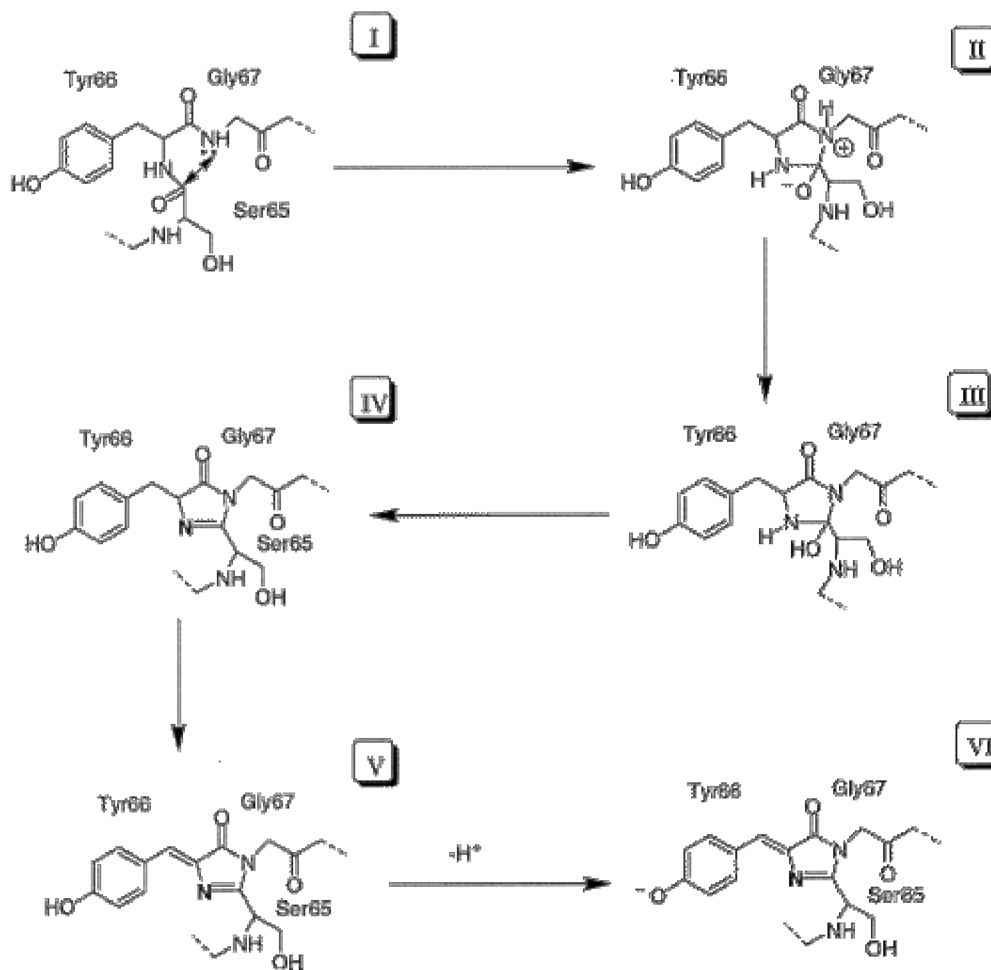

Figure 4. Proposed mechanism for the formation of the chromophore of GFP. (Zimmer, M. (2002), *Chem. Rev.* **58**, 759-781)

Conclusion

Despite aequorin's and GFP's simple structures, many questions still remain unanswered about their chromophore formation and their photochemical properties. The answers to these questions will undoubtaly spur new innovative techniques for their use, as more applications utilizing both of these proteins are developed.

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