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Could structural similarity of specific domains between animal globins and plant antenna proteins provide hints important for the photoprotection mechanism?

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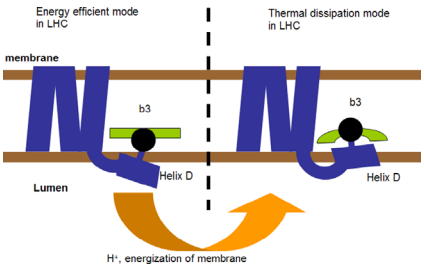
HIGHLIGHTS

- A conformational two-state mechanism for qE is proposed.
- The mechanism relies on conformational changes of helix D and b3.
- Helix D is a sensor of lumen energization.
- Chlorophyll b3 is a quenching center.

GRAPHICAL ABSTRACT

A working model is suggested for the site and mechanism of qE.



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ABSTRACT

Non photochemical quenching is a fundamental mechanism in photosynthesis, which protects plants against excess excitation energy and is of crucial importance for their survival and fitness. In the last decades hundreds of papers have appeared that describe the role of antenna regulation in protection or the so called qE response. However, the exact quenching site is still obscure. Previously overlooked features of the antenna may provide hints towards the elucidation of its functionality and of the quenching mechanism. Recently it was demonstrated that the catalytic domain of human myoglobin that binds the pigment (*i.e.* heme) is similar in structure to the domain of the light harvesting complex II of pea that binds Chl *a* 614 (former known as b3). In addition, it is well accepted that conformational changes of the chlorophyll macrocycle result in reversible changes of fluorescence (the lowest fluorescence corresponds to non planar macrocycle). Here we put forward a hypothesis regarding the molecular mechanism that leads to the formation of a quenching center inside the antenna proteins. Our main suggestion is that a conformational change of helix H5 (known also as helix D) forces conformational changes in the macrocycle of Chl *a* 614 is implicated in the ΔA535 absorbance change and quenching during photoprotective qE. The specific features (some of them similar to those of heme domain of globins) of the b3 domain account for these traits. The model predicts that antenna proteins having b3 pigments (*i.e.* LHCII, CP29, CP26) can act as potential quenchers.

1. Introduction

Photosystem II (PSII) is known as the "engine of life" on earth (Barber, 2003). It produces (i) proton motive force used for ATP synthesis, (ii) oxygen and (iii) reducing power in the form of

Abbreviations: LHCII, light-harvesting complex II; Chl, chlorophyll; qE, energization quenching; zea, zeaxanthin

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NADPH. However, a missing link in the understanding of PSII function is the rapid auto-regulatory loop of its down-regulation or more specifically, the down regulation of its antenna. Photosynthetic antenna complexes of higher plants have a dual role. A. They absorb photons, transferring excitation energy to the reaction centers for photochemical utilization B. With increasing light intensity they rapidly (within seconds) switch into a more dissipating state (energy trap), which safely converts excess energy into heat. In a mechanical analogy, this energy trap is equivalent to the breaks of the car (not the emergency break), which can lead to either a slow or sudden decrease of kinetic energy. In other words, antennas have the intriguing ability to regulate the amount of trapping according to the external environment (Krüger et al., 2014). This down-regulation mechanism known as qE (or energization quenching), protects the photosynthetic apparatus from photodamage (Niyogi, 1999). The qE response is activated by the low pH of the thylakoid lumen, which activates proteins PsbS (Li et al., 2000), violaxanthin deepoxidase (VDE) (Demmig-Adams, 1990; Niyogi et al., 1998), and other light harvesting complexes (Walters et al., 1996; Bassi et al., 1999). VDE produces zeaxanthin (Zea), which together with lutein, and PsbS, are necessary for full amplitude of qE in vivo (Jahns et al., 2009). In addition to the protonation of PsbS and the formation of zeaxanthin, the PSII antenna undergoes a rearrangement that facilitates quenching of chlorophyll excitations (Betterle et al., 2009; Johnson and Ruban, 2011). During the qE response an increase of the absorbance at ~535 nm is evident and well established (Li et al., 2000; Bilger et al., 1989; Johnson and Ruban, 2014). Nevertheless, the exact pigment(s) or more generally put, the origin of this peak is/are still unknown. The two *in vivo* signals, fluorescence quenching at~685 nm and absorbance at ~535–540 nm are linearly correlated *in vivo* (Krause, 1973).

Although many of the essential components of qE are known, the exact sequence of events that lead to quenching remains elusive and an area of active research (Ruban et al., 2007; Ahn et al., 2008; Holzwarth et al., 2009). At least four hypotheses appeared over the years for photoprotective qE. One school of thought gave emphasis to PsbS role and deepoxidation of violaxanthine (see for example Zaks et al., 2012). Another group suggested that Chl 8 (formerly called Chl b3 also called Chl 614 in Liu et al., 2004) dissipates energy interacting with the extended π electrons of Zea (Standfuss et al., 2005). Barros and Kühlbrandt (2009) suggested that Zea binds to the PsbS monomers, which in turn interact with the antenna proteins. Horton's group and coworkers suggested that a conformation change is triggered by a twist of neoxanthin and Lutein gets closer to Chl 1, 2 and 7 and forms the dissipation sink (Pascal et al., 2005 and Ruban et al., 2007). Lastly, Liu et al. (2004) suggested that the small lumen-exposed helices [helix 2 and helix 5 (called also helix D)] get protonated and re-orient Chl 8 and Chl 14 (b3) to promote energy transfer to Zea which dissipates excitation energy (Dietz, 2012). The present contribution will attempt to consolidate aforementioned concepts into a unifying hypothesis using knowledge from the fields of coordination chemistry and globin biochemistry. In this article, we will compare the structure and mode of action of globins with that of antenna proteins and a novel hypothesis about the qE mechanism will be presented (b3 and helix D conformational changes).

1.1. Chlorophyll antenna proteins

The LHC-II family comprises several Chl-protein complexes with similar polypeptide sequences, structure and function (Jansson, 1999). The main LHC-II has three polypeptide components (Lhcb1, b2 and b3) of about 232 amino acids (Standfuss et al., 2005) and each LHC-II polypeptide binds 14 molecules of Chl (Chl *a* and *b*) and four carotenoids of three different kinds. Noteworthy is that Chl optical properties change upon binding to the apoprotein. For example, Chl 614 (former called b3) has a blue shifted absorption maximum of about 659 nm (Rogl et al., 2002). Besides LHCII, minor antenna complexes (Chlorophyll Protein 29 [CP29], CP26, and CP24) are encoded by *lhcb4*, *lhcb5* and *lhcb6* genes, respectively, and are found as monomers (Jansson, 1999; De Bianchi et al., 2008). Although most research on non-photochemical quenching (NPQ) focuses on LHCII, evidence exists that also the minor antenna complexes (CP29, CP26 and CP24) might act as quenchers (Mozzo et al., 2008). Plants lacking the individual proteins Lhcb4, Lhcb5 or Lhcb1/2 demonstrated that none of these complexes are strictly required for NPQ (Andersson et al., 2001, 2003) although in the absence of all of them NPQ is marginally active (Briantais, 1994; Havaux et al., 2007). Only the mutant lacking Lhcb6 is significantly affected in NPQ (Kovacs et al., 2006) but this effect is ascribed to a limitation of proton pumping (De Bianchi et al., 2008 Ioannidis and Kotzabasis, unpublished). These data strongly suggest that more than one Lhcb complex contributes to NPQ, in line with the proposal that multiple quenching sites are distributed throughout Photosystem II (PSII) (Horton et al., 2005). LHCII structures from pea and spinach are available at atomic detail (Liu et al., 2004; Standfuss et al., 2005) and more recently CP29 structure from spinach was also solved (Pan et al., 2011). This made pioneering researcher groups to suggest various sites as the quenching site of qE (Liu et al., 2004; Standfuss et al., 2005; Pascal et al., 2005). The many different suggested sites indicate *per se* that the matter is still under debate and the quencher still unknown.

1.2. Globins

Globins are heme-containing proteins involved in binding and/or transporting oxygen. These proteins all incorporate the globin fold, a series of eight alpha helical segments (A–H). Two prominent members of this family include myoglobin and hemoglobin, whereas protoglobin is a member recently found in Archaea (Nardini et al., 2008). Myoglobin is a water-soluble globular protein of about 150 amino acids long with one prosthetic group (heme). The oxygen affinity of myoglobin (monomeric) is not so sensitive to pH and ionic strength, but that of mammalian hemoglobin's (heterodimer) is lowered by H^+, Cl^-, CO_2, and 2,3-D-diphosphoglycerate (Perutz et al., 1987). "Sophisticated techniques (such as single crystal X-ray analyses of deoxy and oxyglobins, chemical, spectroscopic and magnetic studies) revealed a quite complex mode of action for the globins. Fe size is slightly larger than the hole in the tetrapyrrolic ring. Thus, it is located about 0.4 A outside the ring center forcing the ring to bend slightly. Oxygen binding to Fe redistributes the electron cloud in Fe and reduces the size allowing insertion in the ring center" (reviewed in Perutz et al., 1987). In other words, the reason for metal shift is the change in molecular orbital structure arising from the change in the coordination state of the metal: penta-coordinated iron lies 0.5 A out of plane while hexacoordinanted lies in plane. More particularly, upon oxygenation the porphyrins flatten, the Fe-N porphyrin bond contract, thus moving the Fe's toward the porphyrin planes. In consequence, the proximal histidines come 0.5–0.6 A closer to the porphyrin planes in oxy form than in deoxy form (reviewed in Perutz et al., 1987). It can be seen that upon oxygenation, helix F shifts towards the heme and to the right carrying the FG segment with it. Its worth mentioning that a great number of globins and mutated globins are crystallized and their 3D structures are available nowadays in protein data banks. Hence, the helix F shift is well documented. Furthermore, a convenient way to follow this contraction is through visible spectroscopy. It is well established that globins have two