Phytochemistry 87 (2013) 7–15

Contents lists available at SciVerse ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Review

Non-symbiotic hemoglobins in the life of seeds

Angel J. Matilla *, María del Carmen Rodríguez-Gacio

Department of Plant Physiology, University of Santiago de Compostela, 15782 Santiago de Compostela, A Coruña, Spain

ARTICLE INFO

Article history:
Received 28 March 2012
Received in revised form 13 November 2012
Available online 31 December 2012

Keywords:
Crystal structure
Hexacoordinate and dimeric proteins
Hypoxia in seeds
Non-symbiotic hemoglobins
nsHbs/NO cycle in seeds
Rice nsHbs
Spatial expression of seed nsHbs

ABSTRACT

Non-symbiotic hemoglobins (nsHbs), ancestors of symbiotic-Hbs, are hexacoordinated dimeric proteins, for which the crystal structure is well described. According to the extent of hexacoordination, nsHbs are classified as belonging to class-1 (nsHbs1) or class-2 (nsHbs2). The nsHbs1 show weak hexacoordination, moderate rates of O_2-binding, very small rates of O_2 dissociation, and a remarkably high affinity for O_2, all suggesting a function involving O_2 scavenging. In contrast, the nsHbs2 exhibit strong hexacoordination, low rates of O_2-binding and moderately low O_2 dissociation and affinity, suggesting a sensing role for sustained low (μM) levels of O_2. The existence of spatial and specific expression of *nsHbs1* suggests that nsHbs play tissue-specific rather than housekeeping functions. The permeation of O_2 into seeds is usually prevented during the desiccation phase and early imbibition, generating an internal hypoxic environment that leads to ATP limitation. During evolution, the seed has acquired mechanisms to prevent or reduce this hypoxic stress. The nsHbs1/NO cycle appear to be involved in modulating the redox state in the seed and in maintaining an active metabolism. Under O_2 deficit, NADH and NO are synthesized in the seed and nsHbs1 scavenges O_2, which is used to transform NO into NO_3^- with concomitant formation of Fe^{3+}-nsHbs1. Expression of *nsHbs1* is not detectable in dry viable seeds. However, in the seeds cross-talk occurs between nsHbs1 and NO during germination. This review considers the current status of our knowledge of seed nsHbs and considers key issues of further work to better understand their role in seed physiology.

Contents

* Corresponding author. Address: Department of Plant Physiology, Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, A Coruña, Spain. Tel.: +34 981 593 054.
E-mail address: angeljesus.matilla@usc.es (A.J. Matilla).

0031-9422/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.phytochem.2012.11.016

1. Introduction

Hemoglobins (Hbs) are globular proteins that fix a heme-Fe atom to a proximal histidine (His) via covalent binding (Hoy and Hargrove, 2008). They are not only capable of binding O_2 but they also have affinity for several other small gaseous molecules, including nitric oxide (NO) (Perazzoli et al., 2004, 2006). Hbs are a large ubiquitous superfamily of proteins found in plants, algae, animals, fungi, protozoans, and bacteria (Hardison, 1998; Hebelstrup et al., 2007; Vinogradov et al., 2011a,b) that participate in a wide range of biological functions, such as O_2 transport and storage and NO detoxification. In plants, three major families of Hbs have been described: leghemoglobins (legHbs), non-symbiotic (nsHbs), and truncated (trHbs). Some of their gene sequences have been identified both in very evolved and primitive plants (Garrocho-Villegas et al., 2007). All plants examined contain one or more families of Hbs and they sometimes exist together in a single species, suggesting several functions (Garrocho-Villegas et al., 2007).

LegHbs are present only in legumes and in a few other N_2-fixing species, facilitating O_2 diffusion to N_2-fixing bacteria in root nodules. Also, they metabolize the NO formed during NO_3^-/NO_2^- reduction and assist in N_2 fixation, preventing the O_2-mediated inactivation of bacterial nitrogenases (Ott et al., 2005; Hebelstrup et al., 2007; Hoy and Hargrove, 2008).

The nsHbs are rather different, and are unusual in that their heme prosthetic groups are hexacoordinated in the ferric and deoxyferrous states (Dordas, 2009; Vinogradov et al., 2011a). Although they occur in much lower concentrations than legHbs, they are ubiquitous in the plant kingdom, including symbiotic plants, and are expressed in seeds, roots, leaves, and stems of non-symbiotic dicots as well as monocots (Andersson et al., 1996; Sturms et al., 2011; Hill, 2012). Based on O_2 affinity and sequence similarity, nsHbs are classified as belonging to class-1 (nsHbs1) or class-2 (nsHbs2) (Hoy and Hargrove, 2008; Dordas, 2009; Smagghe et al., 2009). A possible function of nsHbs1 is to modulate the levels of NO and redox potentials (Igamberdiev et al., 2005; Hebelstrup et al., 2007). Although the first nsHb characterized in a monocot was from barley (Duff et al., 1997), Appleby et al. (1988) were the first to postulate that nsHbs could sense O_2 levels. It is now well established that the differences in O_2 affinity between nsHbs1 and nsHbs2 are regulated by the extent of coordination, at least in *Arabidopsis thaliana* (Bruno et al., 2007). The affinity of nsHbs2 for O_2 is two orders of magnitude lower than that of nsHbs1 and similar to that of cytochrome oxidase (Hoy and Hargrove, 2008). NsHbs2 may have a specific function in facilitating O_2 supply to mitochondrial respiration (Smagghe et al., 2009). A barley *nsHbs*–cDNA has been cloned and expressed in *Escherichia coli*, producing a dimeric protein with a native molecular mass of 40 kDa (Duff et al., 1997).

NsHbs expression is induced under several abiotic- or biotic-stress conditions such as hypoxia, nutrient deficiency, and pathogen infection (Taylor et al., 1994; Seregélyes et al., 2003; Ohwaki et al., 2005). In dicots, nsHbs were first detected in root nodules and roots of the non-legume *Parasponia andersonii*, roots of non-nodulating *Trema tomentosa*, and nodules of actinorhizal plants (Garrocho-Villegas et al., 2007; Hebelstrup et al., 2007; Kakar et al., 2011). Genes of nsHbs were first cloned from barley (Taylor et al., 1994) and subsequently from rice (Arredondo-Peter et al., 1997) and are now known from a number of dicots, including soybean (Andersson et al., 1996) and *A. thaliana* (Trevaskis et al., 1997). In addition, nsHbs have been identified in primitive bryophyte species (Arredondo-Peter et al., 2000), where they are involved in the plant responses to hypoxia by helping to maintain the energy status of plant cells through a mechanism alternative to the classical fermentation pathways (Sturms et al., 2011).

The third family of Hb found in plants, the trHbs, are small O_2-binding hemoproteins, known from eubacteria, cyanobacteria, protozoa, and plants, and which are recognized as a separate cluster within the Hb superfamily. In the trHb family, three distinct groups (I, II, and III) can be distinguished with four clusters within group II (Wittenberg et al., 2001). They have a three-dimensional structure containing the four α-helices surrounding the heme group arranged in a sort of α-helical bundle composed of two antiparallel helix pairs (B/E and G/H) (i.e. 2-on-2 arrangement of a α-helices) (Watts et al., 2001) and appear to be ubiquitous in plants (Wittenberg et al., 2001; Vieweg et al., 2005; Garrocho-Villegas et al., 2007; Jokipii-Lukkari et al., 2009). However, the identified plant *trHb* genes are longer than genes encoding 3-on-3 Hbs arrangement of α-helices (3-on-3 Hbs) which are the conventional globin fold in that display and extend loop substituting for the heme proximal F-helix observed in globins (Watts et al., 2001; Jokipii-Lukkari et al., 2009).

While recent evidence points towards nsHbs playing a crucial role in several hypoxic phases of growth and development, their function is far from being elucidated. This review describes the significance and possible functions of nsHbs during plant-stress responses focussing on late seed development and germination (i.e. early imbibition), two phases of seed life characterized by different degrees of hypoxia. First, it is necessary to introduce the unique characteristics of this family of O_2-binding proteins.

2. Evolutionary features of non-symbiotic hemoglobins

Structural analysis of primitive nsHbs and legHbs has revealed some evolutionary changes of legHbs from nsHbs: (i) a transition at the heme prosthetic group from hexacoordinate to pentacoordinate; (ii) a decrease in the length of extended polypeptide linking the B and E helices (i.e. CD-region) and of the N- and C-terminal regions, and (iii) a compaction of the protein into a globular structure (Vinogradov et al., 2011a). Notably, *Hbs* genes from modern maize and ancestral teosinte are highly-conserved and code for identical Hb proteins. This suggests that the domestication from teosinte to maize (*Zea mays*) did not substantially affect the evolution of Hbs in these plants (Arechaga-Ocampo et al., 2001; Gopalasubramaniam et al., 2008). On the other hand, the *nsHb1* of *Mirica gale* (*MgHb*) shows characteristics of *legHb* and *nsHbs1* (bi-functional role). *MgHb* is similar to the *Hb* gene identified in *P. andersonii* but different from that of *Casuarina glauca*, a close actinorhizal relative to *M. gale* (Heckmann et al., 2006). Although the ancestor of plant nsHbs has not yet been identified, recent results indicate that nsHbs may have evolved from algal Hbs, such as nsHbs-like globins from *Micromonas* and *Ostreococcus* (Fernández et al., 2010). *In silico* analyses of protein sequences indicate that the major structural properties of nsHbs originated more than 850 million years ago and have been conserved during evolution to maintain plant function and adaptation. The significant events that occurred during the evolution of land-plant nsHbs were recently elucidated. Phylogenetic analysis has revealed that nsHbs and legHbs evolved from lineages other than trHbs (Hoy and Hargrove, 2008; Garrocho-Villegas et al., 2008; Dordas, 2009). Likewise, the analysis of moss *nsHbs* revealed that primitive land-plant *nsHbs* are interrupted by 3 introns in the same position as in all known land-plant *nsHbs*, thus indicating that ancestral land-plant *nsHbs* were interrupted by 3 introns (Arredondo-Peter et al., 2000; Garrocho-Villegas and Arredondo-Peter, 2008). On the other hand, gene and protein sequence comparison suggests that nsHbs1 and nsHbs2 are monophyletic and that they evolved via a gene duplication event prior to the divergence of monocots and dicots (Garrocho-Villegas et al., 2007; Gopalasubramaniam et al., 2008). In short, nsHbs are very likely to be ancestral to legHbs and hence nsHbs proteins are