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Sulphide-binding processes of *Riftia pachyptila* haemoglobins

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The most distinctive and one of the best studied species found around East-Pacific Rise hydrothermal vents is the giant tube-worm *Riftia pachyptila* Jones, 1981. This "autotrophic" animal is devoid of a digestive system and acquires metabolic energy from the oxidation of sulphide by chemolithoautotrophic bacteria that live symbiotically inside a specific, highly vascularized organ, the trophosome. Therefore, blood transport nutrients is a crucial issue for this animal (review in Childress & Fisher, 1992). *Riftia* supplies its internal symbionts with oxygen and sulphide via extracellular haemoglobins (Hbs), two dissolved in the vascular blood (V1 and V2) and one in the coelomic fluid (C1) (Zal et al., 1996a) (Fig. 1). Indeed, *Riftia*'s Hbs possess an unusual property to bind simultaneously and reversibly oxygen and sulphide on two different sites (Arp et al., 1987). However, in spite of this singular property, the V1 Hb exhibits the typical hexagonal bilayer structure commonly found in annelids (see Toulmond & Truchot, 1993), comprising a total of 180 polypeptide chains with 144 globin chains and 36 linker chains of four different types (Fig. 1 A). The smaller Hbs, V2 and C1, consist only of 24 globin-like chains (Zal et al., 1996a) (Fig. 1 B). Interestingly, as for other Vestimentifera and Pogonophora, there are free cysteine (Cys) residues on two globin-chains common to V1, V2 and C1 (Zal et al., 1996b). Although, free Cys residues have been proposed previously as the sulphide-binding sites of the Hbs in these symbiotic species (Arp et al., 1987), this hypothesis has never been verified. Ten years after the discovery of *Riftia*'s Hbs ability to bind sulphide (Arp et al., 1987), we have now identified the sites involved in this unusual mechanism (Zal et al., 1997, 1998).

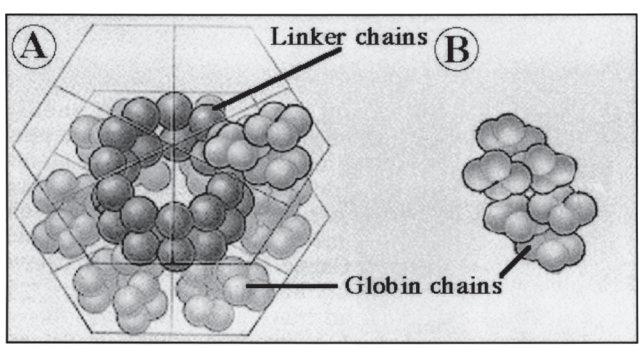

Figure 1. Model of *Riftia pachyptila* Hbs, adapted from Toulmond & Truchot, 1993. (A) Scheme of V1 Hb on top view. Each one-twelfth consists of globin chains, in light grey, associated in four dimers and four monomers. The dark grey central spheres correspond to the linker chains indispensable to the formation of this specific quaternary structure. (B) Scheme of V2 and C1 Hbs. These smaller Hbs consist only of the association of globin chains.

Indeed, recent findings on vertebrate Hbs may help understand the mechanism of sulphide-binding by *Riftia* Hb. Jia et al. (1996) reported the responsibility of the reactive thiol group at position ß93 on mammals and birds ß globin chains. These authors showed that S-nitrosothiol groups (SNO) could bind reversibly on this reactive sulfydryl (SH) forming the so-called S-nitrosohaemoglobin (Fig. 2 A). Similarly we showed recently that reactive thiol groups, highlighted on two globins from *Riftia*'s Hbs, bind sulphide, likely following a similar mechanism forming the so-called S-sulfohaemoglobin by analogy with the term mentioned above (Figs. 2 B, 3 A) (Zal et al., 1997, 1998).

In addition, recent results on stoichiometry between sulphide binding versus Cys contained on *Riftia*'s Hbs and sulphide-binding inhibition experiments, showed that free Cys on globin-like chains and Cys involved in disulphide bridges on linker chains were responsible for the sulphide binding properties of *Riftia* Hbs (Zal et al., 1997, 1998) (Figs. 3 A, B). Indeed, only the participation of linker chains in sulphide binding could explain the observed difference between the binding capacity of V1 Hb and that of V2 or C1 Hbs, since these three Hbs are built with almost the same globin components (Zal et al., 1996a).

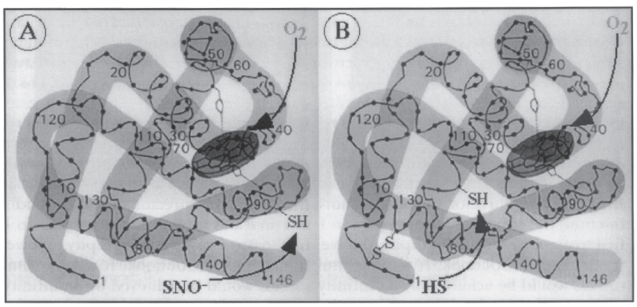

Figure 2. The vertebrate haemoglobin is a tetramer composed of two α and two ß-subunits. In human Hb, each subunit contains one haem, and the ß-subunit (A), also contains highly reactive SH groups (Cys ß93) able to be S-nitrosylated. (B) Similarly, some *Riftia*'s globin chains possess free Cys binding HS^- groups forming a component named S-sulfohaemoglobin as shown for the ß-subunits (adapted from Perutz, 1964).

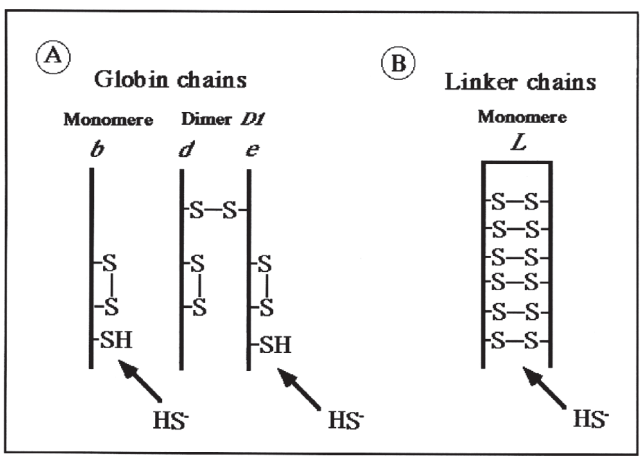

Figure 3. Scheme of sulphide binding mechanisms highlighted by *Riftia*'s Hbs. (A) The free Cys present on *Riftia*'s globin chains b and e are involved in sulphide-binding process by V1, V2 and C1 Hbs. (B) In addition, the larger V1 Hb can form persulphide groups on its linker chains, a mechanism which could account for the higher sulphide-binding potential of this Hb.

In summary, the deep-sea hydrothermal tube-worm *Riftia pachyptila* possesses a multi-haemoglobin system with three different extracellular haemoglobins (V1, V2 and C1 Hbs). Unusually, these Hbs can bind oxygen and sulphide at two different sites, simultaneously and reversibly. Two globin chains common to these three *Riftia* Hbs possess one free cysteine residue and at least one of them is conserved among Vestimentifera and Pogonophora. By selectively blocking the free Cys and using electrospray ionization mass spectrometry experiments, we showed that these Cys are involved in sulphide-binding by *Riftia* Hbs (Zal et al., 1998). Moreover, we also demonstrated that the larger V1 Hb can form persulphide groups on its linker chains, a mechanism which could account for the higher sulphide-binding potential of this Hb (Zal et al., 1998).

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