Crystal Structure of Giant Hemoglobin from Beard Worm

Pogonophorans and vestimentiferans are marine animals that have no mouth or gut. Their nutrition is provided by endosymbiotic sulfur-oxidizing bacteria living inside their body. These animals live in sulfide-rich environments such as hydrothermal vents. Morphological, embryological and genetic studies suggest that pogonophorans, vestimentiferans, and annelids are closely related. All three groups have giant (400 kDa and/or 3500 kDa) extracellular hemoglobins (Hbs) that are remarkably different in their quaternary structure from well-known mammalian tetrameric assembly.

Pogonophorans have only a ~400 kDa Hb composed of ~24 globins in their blood [1]. Vestimentiferans have three types of Hb; hexagonal bilayer vascular V1 (~3500 kDa), vascular V2 (~400 kDa), and coelomic C1 (~400 kDa). Their molecular mass suggests pogonophoran Hbs are homologous to vestimentiferan V2 Hbs. It has been reported that both pogonophorans and vestimentiferans giant Hbs transport oxygen and sulfide simultaneously. Giant Hbs provide the host with oxygen and endosymbionts with sulfide. Sequence analyses of pogonophoran and vestimentiferan Hbs have shown that their putative sulfide-binding Cys residues are well conserved. On the other hand, annelids have a 3500 kDa extracellular Hb which is thought to be a homologue of vestimentiferan V1. However, annelid giant Hb has no physiological function of sulfide-binding in the case that annelids live in sulfide-free environments.

Pogonophorans and vestimentiferans are quite unique animals in their method of acquiring nutrition, and their Hbs have characteristic assembly and function of transporting sulfide. Little is known about these sulfide binding extracellular Hbs and no vascular giant Hb structure is available, whereas only a moderate resolution (5 Å) whole structure of 3500 kDa hexagonal bilayer Hb from an annelid, its partial reconstructed structure composed of 12 globins [2], and the structure of coelomic Hb (C1) of vestimentiferan *Riftia pachyptila* [3] have been reported. However, the detailed mechanism of sulfide binding is still unclear. To provide further structural insights into the sulfide-binding mechanism of these giant Hbs, especially vascular Hb, we have performed a X-ray crystal structure analysis of vascular giant Hb from pogonophoran *Oligobrachia mashikoi* (Fig. 1(a)).

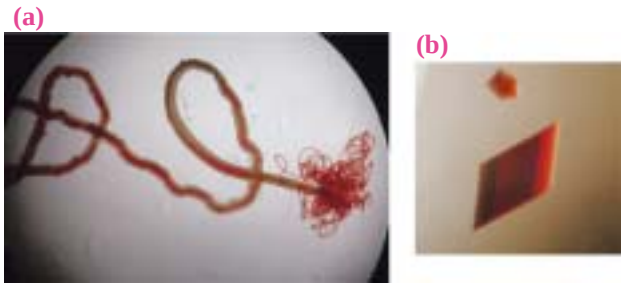

Fig. 1. **(a)** Part of body of *Oligobrachia mashikoi*, and **(b)** crystals of *Oligobrachia* Hb.

Crystals of *Oligobrachia* Hb were successfully obtained (Fig. 1(b)), and X-ray diffraction study was performed at beamlines **BL38B1**, **BL41XU**, **BL44XU**, and **BL45XU** [4,5]. The structure of *Oligobrachia* Hb is composed of 24 globin chains with six copies of each of four individual globin chains, termed A1, A2, B1, and B2. The entire structure is hollow-spherical, with outer and inner diameters of about 120 Å and 50 Å, respectively (Fig. 2). One-half of the *Oligobrachia* Hb molecule (Hb 12mer) is composed of a three-fold trimer of a tetramer. There are an intra-tetramer and an inter-tetramer disulfide bonds per tetramer. This Hb tetramer structure consists of two dimers (A1B1 dimer and A2B2 dimer in *Oligobrachia* Hb). *Oligobrachia* Hb and *Riftia* C1 show the same quaternary structure as that of 24mer and are considered to be a dimer of the 12mer. These 12mer structures are basically similar to annelid reconstructed 12mer. The dodecameric assembly of these Hbs could be a fundamental unit of Hbs from pogonophorans, vestimentiferans, and annelids, not only in 3500 kDa hexagonal bilayer Hbs but also in 400 kDa Hbs.

It has been suggested that conserved Cys residues among pogonophorans and vestimentiferans are involved in potential sulfide-binding sites. The thiol group of these Cys residues are proposed to form a persulfide group (S-sulfohemoglobin). In *Oligobrachia* Hb, both the A1 and B2 subunits possess a common conserved Cys, and the A2 subunit has another conserved Cys. There is a non-conserved Cys at a position different in the B1 subunit from those of other three subunits. Although the obtained structure is a sulfide-free form, the structure of the Hg derivative used for phasing provides significant insights into the sulfide-binding mechanism because Hg atoms specifically bind to free Cys

residues. All four of these free Cys residues are bound by the Hg compound in the Hg derivative crystal. Environments around the conserved Cys residue of the A1 and B2 subunits are revealed to be more suitable for sulfide binding. These sites surrounded by Phe residues are quite similar to each other, and are highly aromatic (Fig. 3). These Phe residues are completely conserved in pogonophorans Hb and vestimentiferan Hb. Two Phe residues come into contact with a Hg atom in the derivative structure, and this strongly suggests that in the native Hb, the sulfide bound to the Cys residue could be stabilized by aromatic-electrostatic interactions with these two Phe side chains. The environment surrounding another conserved Cys of the A2 subunit is also hydrophobic, where Leu and Ile residues contribute. A hydrophobic environment prevents bound sulfides from excessive contact with solvents to avoid undesired oxidation.

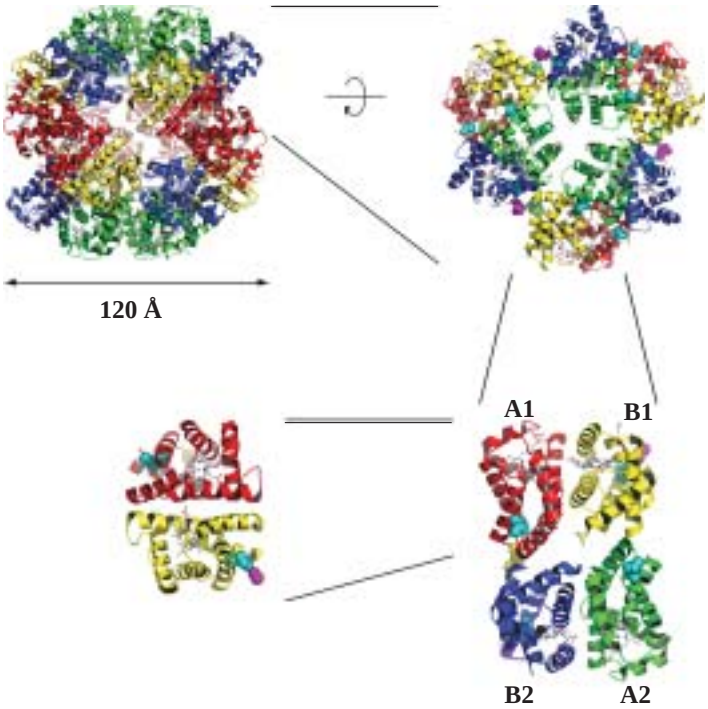

Fig. 2. Structural hierarchy in *Oligobrachia* Hb. Giant Hb is considered to be a dimer of 12mer (upper-left). The 12mer structure is composed of a three-fold trimer of a tetramer (upper-right). This tetrameric assembly has no D_2 symmetry as that in mammalian Hb (lower-right). The Hb tetramer consists of two dimers (lower-left). Space-filling models indicate disulfide bonds.

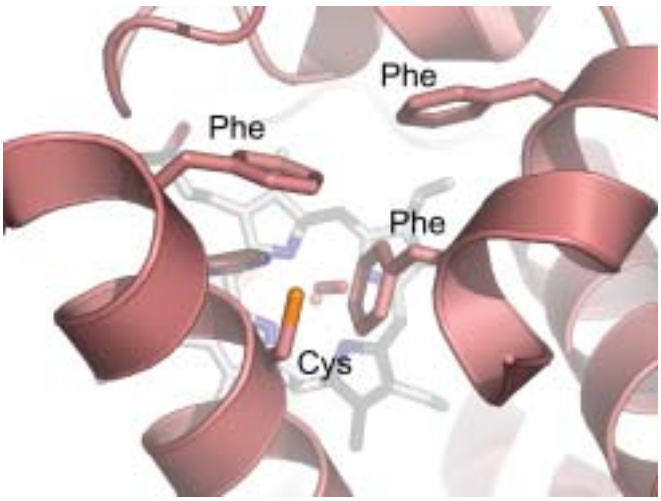

Fig. 3. Potential sulfide-binding site of A1 subunit. The environment around the conserved Cys residue is highly aromatic.

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