

RESEARCH NOTE

The Myoglobin and the Hemoglobin of *Biomphalaria glabrata*, an Evidence of Gene Duplications

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Among invertebrate animals, hemoglobins and myoglobins (globins) occur in most but not all phyla and display a great variability in their primary and quaternary structure more than in their vertebrate counterparts (AF Riggs 1991 *Am Zool* 31: 535-545). The invertebrate globins were divided in five distinct groups based on their structure: (1) single chain with one heme-binding domain; (2) single chain with a truncated heme-binding domain (with 100-120 residues instead of the common ~ 150 residues domain); (3) chimerical, one heme-binding domain (with the globin domain attached to unrelated proteins); (4) chimerical, two heme-binding domains covalently linked and (5) chimerical, multi heme-binding domain globins (SN Vinogradov 1993 *Comp Biochem Physiol* 106B: 1-26). The widespread occurrence in eukaryotes and prokaryotes suggests that the globins descended from a monomeric, single chain, single-domain globin existed prior to the time of divergence of eukaryotes and prokaryotes at 1,500-2,000 million years ago (M Goodman et al. 1988 *J Molec Evol* 27: 236-249).

In our laboratory we have partially character-

ized the myoglobin and hemoglobin of *Biomphalaria glabrata*.

Myoglobin - The myoglobin of *B. glabrata* was purified to homogeneity from the radula of the animal. The protein has a characteristic spectrum of heme proteins in the Cyanomet state, it does not show any evidence of polymerization in gel filtration and has a molecular weight of 16 kDa in SDS-PAGE. The circular dichroism analysis showed a high content of alfa-helices, characteristic of "globin-folded" proteins. The sequence information from gDNA and cDNA of this myoglobin was extensively analyzed and interpreted under an evolutionary and structural point of view as well as its oxygen binding properties (S Dewilde et al. 1998 *J Biol Chem* 273: 13583-13592). This globin can be classified as representative of the group 1 of Vinogradov.

Hemoglobin - The hemoglobin of *B. glabrata* is a glycoprotein of 1.75 MDa (AP Almeida & AGA Neves 1974 *Biochim Biophys Acta* 371: 140-146) and was purified to homogeneity from the hemolymph of the animal. The dissociation products of the hemoglobin were analyzed by a 5-15% gradient SDS-PAGE and 3% agarose gel electrophoresis (SDS-AGE), giving a band of 360 kDa and a band of 180 kDa after reduction with β -mercaptoethanol. The hemoglobin was digested by four different proteases (thrombin, trypsin, chymotrypsin and subtilisin) showing several equivalent fragments with molecular weights multiples of its minimum molecular weight - 17.7 kDa (EA Figueiredo et al. 1973 *Comp Biochem Physiol* 44B: 481-491) suggesting the presence of several heme binding domains in the same polypeptide chain. The circular dichroism spectrum of the protein showed a characteristic high alfa-helices content which is compatible with the idea of heme domains containing the characteristic "globin fold". We propose that this hemoglobin is a pentamer of ~ 1.8 MDa composed of 360 kDa subunits, each formed by two 180 kDa chains linked in pairs by disulfide bridges, and each of these chains comprises ten heme binding domains of ~ 18 kDa (MHL Arndt & MM Santoro 1998 *Comp Biochem Physiol B* in press). The cooperativity and Bohr effect of this hemoglobin was observed in an approximation of natural conditions of pH and osmolality of the solution. The P_{50} and Hill coefficient obtained were 5.4 mmHg and 1.6, respectively. Nevertheless, these data were demonstrated to be dependent on the ionic strength and rupture of the disulfide bridges (MCS Nascimento et al. 1982 *Comp Biochem Physiol* 73B: 251-256). The high affinity ($P_{50} = 5.4$ mmHg) of this hemoglobin can be related to storage of oxygen that can be used during the diving of the snail.

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Within the mollusks, this kind of structure on hemoglobin (group 5 of Vinogradov) was reported in other Planorbidae snails (Gastropoda): *Helisoma trivolvis* (NB Terwilliger et al. 1976 *Biochim Biophys Acta* 453: 101-110), *Planorbis corneus* (EJ Wood & LJ Mosby 1975 *Biochem J* 149: 437-445), *Planorbella duryi* (TT Herskovits & MG Hamilton 1990 *Comp Biochem Physiol* 95B: 321-326), *Indoplanorbis exustus* (TO chiai et al. 1989 *Comp Biochem Physiol* 93B: 935-940), *B. tenagophyla*, *B. straminea*, *B. occidentalis*, *B. amazonica*, *B. obstructa* (MHL Arndt et al. 1996 XI International Conference on Supramolecular Organization and Functional Regulation of Invertebrate Dioxygen Binding Proteins, Padova, Itália, p. A2) and in two families of clams (Bivalvia), Astartidae and Carditidae (RC Terwilliger & NB Terwilliger 1985a *Comp Biochem Physiol* 81B: 255-261). This structure was also reported in the brine shrimp *Artemia* sp. (Crustacea) and one of the chains containing nine globin domains is the only of this kind fully sequenced (AM Manning et al. 1990 *Nature* 348: 653-656). In *B. glabrata* the presence of this kind of structure on hemoglobin was interpreted as a strong evidence of several gene

duplications from a myoglobin ancestor as an adaptation to increase the molecular weight of the protein. This increase of molecular weight appeared not only after the gene duplications but, additionally, after the appearance of cysteine residues (not present in myoglobin) with the formation of the disulfide bridges and after the aggregation of the subunits in an higher level assembly (Arndt & Santoro 1998 *loc. cit.*). This high molecular weight globin evolved probably to respond to the physiological need to control the osmotic pressure of the hemolymph and to avoid its loss after filtration by the renal system of the snail. Many important points are already known about this respiratory pigment. Nevertheless, there are several questions that remain unanswered: How many different chains exist within this Hb? How similar are the domains within a chain? Are they all functional? How long ago occurred the first duplication event? How similar are the domains compared to its own myoglobin and other invertebrate and vertebrate globins? How are the domains organized in the subunit and these subunits are disposed on the quaternary level? As one can see, many important questions concerning the structure, function and evolution of this protein remain to be elucidated.