HEMOGLOBINS FROM ENDEMIC AND CASUAL-VISITING HYDROTHERMAL VENT FISH SPECIES

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Fish show unique adaptability in blood O_2 transporting properties in response to environmental stresses with regard to both intraspecific adaptations (that commonly occur rapidly and involve changes in the activity of intra-erythrocytic factors) and interspecific adaptations [that have occurred through evolution and commonly involve changes in the structure and intrinsic oxygenation properties of the hemoglobin (Hb)] (1). Deep-sea hydrothermal vents offer some of the harshest conditions for life associated with the chaotic mixing of hot (350°C), anoxic water that contain high levels of H_2S, carbon monoxide and heavy metals, and cold (2°C) O_2-laden bottom water at high hydrostatic pressures (2). Hydrothermal vent invertebrate animals (as exemplified in the giant vestimentiferan worm *Riftia*) correspondingly show striking differentiation in Hb structure and functions that include O_2 transport, H_2S-removal, and supplying these gases to metabolizing tissues and endosymbiotic chemolithoautothrophic bacteria that oxidize sulphide for nutritional needs. With no information available on hydrothermal-vent vertebrates we studied Hb function in the vent-endemic, zoarcid eelpout, *Thermarces cerberus* (captured at the East Pacific Rise) and in the benthopelagic anguillid, *Symenchelis parasitica* (captured at Mid Atlantic Ridge vents that it may visit for feeding). The preparation and functional characterization of the Hbs were carried out essentially as previously described (3).

Isoelectric focusing resolved *Symenchelis* Hb into three main components, cathodal HbI and anodal Hbs II and III. As found in other "Class II" teleost fish [that have cathodal as well as isoHbs (4)], the "stripped" (cofactor-free) cathodal

Hb (I) showed a higher O_2 affinity than corresponding anodal Hbs (Fig. 1), and a reversed Bohr effect (O_2 affinity increased with falling pH) that was obliterated in the presence of the erythrocytic effector ATP (3,5). In contrast to moderate sensitivities of HbII to both effectors, the cathodal HbI showed a strong ATP effect and an insignificant chloride effect. The functional heterogeneity indicates a division of labour, with the abundant cathodal Hb I functioning as O_2 reserve and carrier under anoxic/hypoxic conditions and activity- or environmentally-induced acidoses, where oxygenation of the anodal Hb is compromised by pronounced Bohr and Root effects (decreases in O_2 affinity and O_2 carrying capacity at low pH). Remarkably the O_2 affinities of *Symenchelis* isoHbs are considerably lower than those in shallow-water species from temperate and tropical habitats (*Hoplosternum* and *Anguilla*) (Fig. 1).

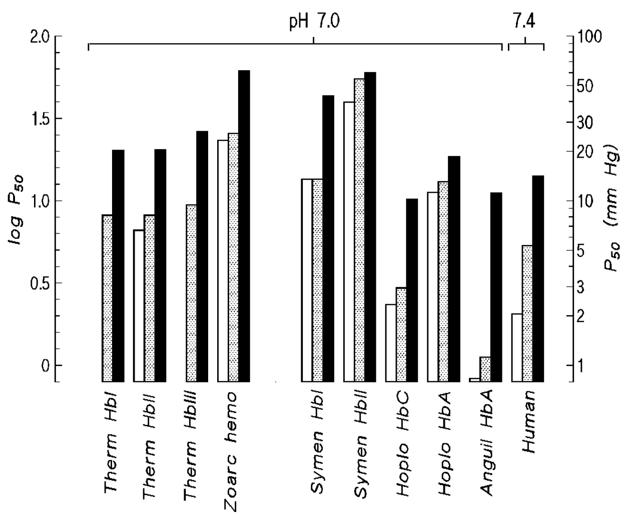

Figure 1. Half-saturation O_2 tensions (P_{50}) at 25°C of isoHbs from the vent-endemic eelpout *Thermarces cerberus* (Therm) and the deep-sea anguillid *Symenchelis parasitica* (Symen), compared to values for cathodal (HbC) and anodal (HbA) Hbs of the catfish *Hoplosternum littorale* (Hoplo) and the eel *Anguilla anguilla* (Anguil) and cofactor-free hemolysates from the coastal eelpout *Zoarces viviparous* and humans. *Open columns*, stripped Hb; *shaded columns*, stripped Hb in presence of 0.1 M chloride; *solid columns*, stripped Hbs in the presence of 0.1 M chloride and saturating ATP concentrations.

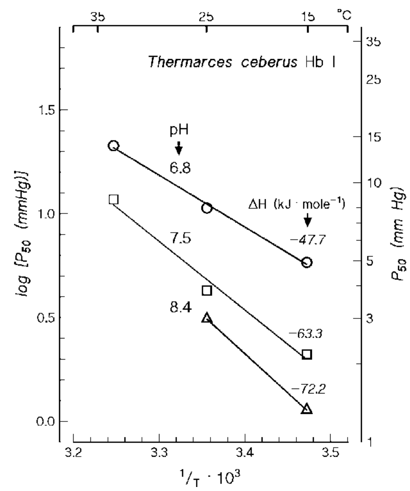

Figure 2. Van't Hoff plots relating P_{50} values of *Thermarces* HbI at 3 pH values to absolute temperature (T). *Italic numbers*, overall heats of oxygenation ()H).

Based on Hb multiplicity *Thermaces* belongs to "Class I", having only anodal Hbs that display similar O_2 affinities and similar (normal) Bohr effects (4). Significantly, the isoHbs tested (Hbs I, II and III) exhibit much higher O_2 affinities compared to stripped hemolysates from the shallow-water "control" eelpout, *Zoarces viviparous* (Fig. 1). All else equal, this translates to higher blood-O_2 affinity and O_2 loading potential in *Thermarces*.

Unless compensated by linked endothermic processes (like proton and anion dissociation), the exothermic nature of heme oxygenation dictates drastic variations in blood-O_2 affinity in animals living in thermally unstable habitats that would be maladaptive in species from hydrothermal-vent sites that periodically are subjected to hot, hypoxic water. While the high intrinsic heat of oxygenation (H = -72 $kJ.mole^{-1}$) observed in *Thermarces* Hb in the absence of organic phosphates and at high pH (8.4, where the Bohr effect is absent) reveals that the intrinsic temperature sensitivity is not reduced compared to other Hbs, the numerically lower values found at lower pH where the Bohr effect is operative (Fig 2) and in the presence of ATP (no shown), indicate markedly reduced *in vivo* temperature sensitivities.

The findings are discussed in relation to Hb function and metabolic requirements under hydrothermal vent conditions and the perspectives for future research.

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