Structural Characterization of the Oxidation Pathway of Antarctic Fish Hemoglobins

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Antarctic fish hemoglobins (AF-Hbs) exhibit a peculiar oxidation process. Our previous crystallographic and spectroscopic investigations have demonstrated that, upon oxidation, these proteins show a remarkable propensity to evolve toward the formation of low-spin hexa-coordinated species [1,2]. The crystal structures of the fully oxidized forms of AF-Hbs, isolated from *Trematomus newnesi* and *Trematomus bernacchii,* have also shown that α and β chains follow different oxidation pathways. Interestingly, the quaternary structures of these forms are intermediate between the physiological R and T hemoglobin states [1,2]. In order to obtain additional information on the structural features of the intermediate species along the oxidation pathway, we are currently characterizing AF-Hbs exposed to air for different time periods. Preliminary data reveal the presence of novel forms with unexpected structural properties. In particular, we detected (1) the presence of partially liganded forms with structures that are intermediate between the R and the T state, (2) the existence of hybrid α(aquomet)-β(penta-coordinated Fe^{3+}) forms, and (3) the occurrence of novel subunit-subunit interactions at the $\beta^1\beta^2$ interface.

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