Linkage of Metal Ions and pH on Hemopexin-Heme Complex Stability

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The 58 kDa glycoprotein Hemopexin (Hx) circulates in blood plasma as the apo- or heme-free form to scavenge potentially toxic free heme for transport to the liver and subsequent disposal. Recently, we developed a gentle protocol to isolate Hx from human blood plasma that is based on immobilized metal affinity chelate chromatography and that eliminates precipitation steps and drastic changes in pH to yield monomeric forms of the protein (Mauk et al. (2005) *Biochemistry 44*, 1864-1871). We also demonstrated that heme-free and heme-bound hemopexin bind different metal ions with different affinities, and that ions such as Cu^{2+} and Zn^{2+} affect other properties of the holo-protein such as the nature of heme binding. Here, we investigate how these metal ions influence the stability of Hx-heme to thermal denaturation, and we use this property to identify factors that are important for heme release *in vivo*. The melting temperature (T_m) of oxidized holo-Hx assessed by loss of absorbance in the Soret region is, respectively, 4.8 and 6.5 °C lower in the presence of Cu^{2+} and Zn^{2+} than it is in the presence of other or no metals ions under comparable conditions (T_m = 64.4 °C, A). Similarly, the enthalpy change associated with protein denaturation when these metal ions are present is lower by 14 and 34%. Chloride concentrations comparable to those found in hepatocyte endosomes (~60 mM, B) stabilize the protein diminishing to some degree the negative effect of Cu^{2+} and Zn^{2+}. By comparison, Hx is most susceptible to thermal denaturation under mildly acidic conditions (*i.e.*, pH 5; T_m = 46.8 °C, C) stressing the relevance of physiological conditions on the membrane receptor-mediated heme release from hemopexin. Efforts are in progress to crystallize human hemopexin and to express recombinant forms of the deglycosylated protein to help identify, among other things, sites of metal ion binding.