Circular Dichroism studies on the temperature stability of the giant extracellular hemoglobin of *Glossoscolex paulistus* (HbGp)

Carvalho, J. W. P.; Santiago, P. S.; Tabak. M.

Instituto de Química de São Carlos – Universidade de São Paulo - Brazil

The giant extracellular hemoglobin of *Glossoscolex paulistus* has a complex oligomeric structure, composed of heme-containing globin-like subunits (trimers and monomers) and subunits lacking hemes, linkers, with a total molecular mass of 3.6 MDa. Recent studies by dynamic light scattering (DLS) of oxy-HbGp (P.S.Santiago, Biophys.J. 94, 2228 (2008)) have shown that the protein is very stable at pH 7.0 up to 52°C. At alkaline pH (above pH 7.5) oligomeric dissociation occurs at lower temperatures in the range 45-35°C. In this work, circular dichroism (CD) studies are performed for HbGp in oxy- and cyanomet- forms as a function of temperature and for several pH values. Experiments were performed in the near UV to monitor protein secondary structure (0.025 to 0.2 $mg.mL^{-1}$ protein) as well as in the Soret band spectral region (3.0 $mg.mL^{-1}$ protein) to monitor changes in the heme group. Analysis of CD data based on a two-state thermodynamic model for HbGp denaturation, allowed to obtain the fraction of denatured protein, the critical denaturation temperatures as a function of pH, the equilibrium constants and free energies. HbGp in cyanomet- form is significantly more stable as compared to oxy-HbGp presenting critical temperatures in the range 69-47°C, higher by 10-15°C. Differently from DLS, where the oligomeric dissociation is observed, CD data suggest that the protein denaturates as a whole, loosing its secondary structure simultaneously for all subunits. In agreement with DLS data, our results show that increasing the pH leads to a reduction of the critical denaturation temperature. Finally, HbGp is very stable at acidic pH 6.0. Support: FAPESP, CNPq. brazilian agencies.