LIGHT SCATTERING STUDY OF THE ALKALINE DISSOCIATION OF THE EXTRACELLULAR HEMOGLOBIN OF *GLOSSOSCOLEX PAULISTUS*

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Extracellular hemoglobin of *Glossoscolex paulistus* (HbGp) is constituted by a number of subunits containing heme groups, monomer and trimer, and non-heme structures, named linkers. Whole protein has a minimum molecular mass near 3.1×10^6 Da. HbGp samples (oxy-form) at 0.5 mg/mL were studied in this work by dynamic (DLS) and static light scattering (SLS), using a non-invasive back scatter (NIBS) equipment. At pH values in the range 6.0 to 8.0, HbGp presented a stable structure and a monodisperse size distribution with a z-average hydrodynamic diameter (D_h) of 27 nm. Transition to more alkaline pH induced an irreversible dissociation process, resulting in smaller particles (D_h = 10 nm) with a broader size distribution. D_h decreases from 27 nm to 10 nm, suggesting the complete hemoglobin dissociation, into monomers, tetramers, trimers and linkers. Dissociation presents a slow kinetics at pH 9.0, taking approximately 24 hours to complete the process. The dissociation rate constant progressively increases at higher pH values, becoming, at pH 10.5, too fast to be followed by DLS. Protein temperature stability ("melting point"), evaluated by DLS, was also pH dependent. Melting curves for HbGp showed oligomeric dissociation and protein denaturation as a function of pH.

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