Molecular Masses Of Extracellular Hemoglobin Of *Glossoscolex Paulistus* Upon Oligomeric Dissociation: Analytical Ultracentrifugation Studies.

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The molecular mass (M) of the giant extracellular hemoglobin of *Glossoscolex paulistus* (HbGp) has been re-examined (Anal. Biochem. 385 (2009) 257-263) by analytical ultracentrifugation (AUC), giving a value of M of 3600±100 kDa, at pH 7.0. The sedimentation coefficient was found as 58-59 S. In this work, sedimentation velocity AUC experiments were performed on HbGp exposed to alkaline pH 10 and beta-mercaptoethanol, conditions promoting oligomeric dissociation. Pure monomer was also monitored at pH 7.0 and 10.0. SEDFIT gave V_{bar} =0.755±0.004 mL/mg for the whole protein and V_{bar} = 0.736±0.004 mL/mg for the monomer ***d***. The *s** (experimental sedimentation coefficient) was obtained from SEDFIT based on these V_{bar} values, buffer viscosity and density estimated by Sednterp. $s^o_{20,w}$ obtained for the pure monomer ***d*** was 1.92±0.04 S for both pH values 7.0 and 10.0. C(S) distributions for pure monomer indicated that a small contribution of dimer of monomers, $\boldsymbol{d}_2$, was also present in the solutions with $s^o_{20,w}$ of 3.2 S and a percentage contribution of 5 %. For the whole HbGp at pH 10.0 no contribution at 58-59 S was observed, suggesting complete oligomeric dissociation. C(S) distribution showed two additional peaks as compared to pure monomer: a peak at 4.1-4.2 S, probably due to the trimer, ***abc***; a second peak at 5.8-6.0 S, that could be associated to the tetramer, ***abcd***. Addition of beta-mercaptoethanol leads to the disappearance of the peak at 4.2 S, consistent with the reduction of the trimer ***abc*** disulfide bridges. Our AUC data are consistent with mass spectrometric data obtained previously.

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