Glossoscolex paulistus hemoglobin: an extraordinary oxygen carrier

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Introduction: Extracellular hemoglobins are giant proteins, known as erythrocruorins, which are highly cooperative respiratory macromolecules found in mollusks and annelids. *Glossoscolex paulistus* hemoglobin (HbGp) has an oligomeric structure composed of heme-containing globin-like chains (144 subunits) and 36 additional polypeptide chains lacking a heme group, named linkers, and a total molecular mass of 3600 kDa. **Objectives:** The present work focuses on the study of oxy-HbGp oligomeric stability, as a function of storage time. **Material and methods:** This study was performed using several techniques, such as dynamic light scattering (DLS), analytical ultracentrifugation (AUC), optical absorption and size exclusion chromatography (SEC). **Results and discussions:** Our present data show that HbGp can be stored for one year at 4 °C and the oligomeric structure remains un-dissociated. The Soret and Q bands are centered, respectively, at 415, and 540 and 575 nm, being characteristic of the native form. A major contribution of subunits species and the blue-shift in the Soret band are observed for the older protein solutions. These results indicate that HbGp storage for longer periods of time than a year changes its capacity to carry oxygen. HbGp stocks ageing 4-6 years present typical absorption spectra of hemichrome species, where the iron is in the oxidized state, Fe^{3+}. AUC and SEC analyses show that the contribution of HbGp-subunits, such as dodecamer $(\boldsymbol{abcd})_3$, tretramer ***abcd***, trimer ***abc*** and monomer ***d***, increases with the protein aging due to the lower stability of the HbGp with the time. Despite the HbGp stability, as well as, oxygen carrying capacity, reduction with time, the stability of this protein is still larger than that from mammalian hemoglobins. **Conclusion:** Our results display that the extracellular hemoglobins are systems quite stable and resistant to the auto-oxidation. These properties make these proteins an interesting system that has been studied due their potential use as blood substitutes.

Keywords: *Glossoscolex paulistus* hemoglobin, auto-oxidation, oligomeric stability.
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