Urea And Guanidine Hydrochloride Inhibit The Thermal Aggregation Of *Glossoscolex paulistus* Hemoglobin (HbGp)?

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Introduction: *Glossoscolex paulistus* hemoglobin (HbGp) has a molecular mass of 3600 kDa. It belongs to a class of proteins, known as hexagonal bilayer, which are highly cooperative respiratory macromolecules found in mollusks and annelids. The oligomeric structure is composed of heme-containing globin-like chains (144 subunits) and 36 additional polypeptide chains lacking a heme group, and named linkers. **Material and Methods:** The present work focuses on the study of the thermal stability of oxy-HbGp in the presence of urea and guanidine hydrochloride (GuHCl) by Dynamic light scattering (DLS) and circular dichroism (CD). **Results and Discussion:** DLS data show that, the increase in urea concentration promotes the HbGp oligomeric dissociation followed by unfolding process at high temperatures. At 4.0 mol/L of urea, at 60 °C, the size of aggregates presents hydrodynamic diameter (Dh) around 40 nm, while in the absence of the denaturant agent the Dh value of 160 nm was observed. In contrast, the presence of GuHCl induces a significant 11-fold increase in the Dh values, due to the formation of super-aggregates in the solution. On the other hand, the CD data show that the unfolding critical temperature (Tc) of oxy-HbGp decreases sharply from 57 °C, at 0.0 mol/L of denaturant, to 45 °C, in the presence of 3.5 mol/L of urea. This shift in the Tc values shows that the addition of urea induces a loss of HbGp oligomeric stability. **Conclusions:** In summary, our results show that urea inhibits the thermal aggregation, while guanidine hydrochloride promotes the formation of super-aggregates. Besides, HbGp is quite stable in the presence of urea as compared to the guanidine effect.

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