Spectroscopic investigations on the *Glossoscolex paulistus* hemoglobin (HbGp) in the presence of 8-anilino-1-naphtalene-sulfonic acid (ANS) probe.

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Introduction: *Glossoscolex paulistus* extracellular hemoglobin (HbGp) has a molecular mass of 3.6 MDa and is extracted from *Glossoscolex paulistus* annelid. It belongs to a class of proteins, with a high resistance to oxidation and oligomeric stability. The present work focuses on the study of oxy-HbGp oligomeric stability at different pH values, in the presence of 8-anilino-1-naphtalene-sulfonic acid (ANS) probe and the characterization of its interactions sites with oxy-HbGp and DTAB micelles. **Material and Methods:** The present studies were performed using several techniques, such as, optical absorption UV-VIS, fluorescence emission and time resolved florescence. **Results and Discussion:** HbGp Soret band optical absorption in the pH range from 5.0 to 8.0 remains constant, while in acidic and alkaline media a significant decrease is observed. This finding implies that drastic pH values changes induce the iron oxidation. Moreover, fluorescence data, at pH 4.0, display an abrupt 31-fold increase in the tryptophan emission, and an 8-fold growth in the ANS emission, as compared to the value at pH 7.0. On the other hand, at alkaline medium the increase is smaller than that at pH 4.0, and probably can be associated with oligomeric dissociation, while the high intensity of ANS emission, at acidic medium, is due to aggregation process. The time resolved fluorescence data show that in presence of DTAB, at pH 7.0, the ANS probe has a single lifetime around 3.9 ± 0.3 ns. However, the interaction of the probe with HbGp results in different lifetimes of 1.2 ± 0.2 ns and 7.3 ± 0.6 ns. **Conclusions:** The ANS probe interacts strongly with HbGp at acidic medium as compared to the alkaline pH values. Moreover, ANS probe, in the presence of HbGp, binds less effectively to DTAB micelles as compared to pure DTAB micelles.

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