Interaction of Extracellular *Amynthas gracilis* Hemoglobin (HbAg) With Anionic Surfactant SDS: Effects of Oligomeric Dissociation and Oxidation.

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INTRODUCTION: *Amynthas gracilis* (HbAg), a giant extracellular hemoglobin, has a molecular mass around 3.6 MDa. It belongs to a class of proteins, known as hexagonal bilayer, which are highly cooperative respiratory macromolecules found in annelids. ***OBJECTIVES***: The study focus on the effect of the SDS upon the stability HbAg, at pH values 5.0 and 7.0. ***MATERIALS AND METHODS***: Optical absorption, fluorescence emission and light scattering (LSI) spectroscopy techniques were used to evaluate the HbAg oligomeric dissociation, unfolding and the oxidation processes as a function SDS concentration. The HbAg solutions 0.1 mg/mL were exposed to different SDS concentrations in the range from 0 to 10 mM. ***DISCUSSION AND RESULTS***: Optical absorption spectra of HbAg, at pH 5.0, as a function of SDS show significant shifts of Soret band from 414 to around 400 nm is observed. This behavior is assigned to the oxidation of native oxy-HbAg into met-HbAg. Moreover, the LSI at intermediate SDS concentrations indicate that the SDS, at pH 5.0, promotes the formation of large aggregates. At high surfactant concentration, the LSI decrease, probably, due to the re-solubilization of aggregation species in the solution. The addition of SDS in the HbAg solutions promotes a significant increase in fluorescence emission around 20-fold. These results are consistent reduction of intrinsic quenching of tryptophan fluorescence by iron of the heme groups, due to the SDS promotes the dissociation of the oligomer. At pH 7.0, it was not observed the formation of protein aggregates /surfactant, however SDS at high concentrations the protein is completely oxidized and dissociated. Thus, the interaction of SDS with HbAg at pH 5.0 was clearly weaker as compared to the HbAg at pH 7.0. ***CONCLUSION***: Spectroscopic data are discussed and compared with the literature in order to improve the understanding of hemoglobin-surfactant interaction as well as the relevance of the isoelectric point of hemoglobins on their structure-activity relationship.

Keywords: hemoprotein; interaction protein and surfactant; spectroscopy