STUDIES OF GIANT EXTRACELLULAR HEMOGLOBIN Amynthas gracilis (HbAg) AS A PROTOTYPE BIOSENSOR FOR ENVIRONMENTAL CONTAMINATION.

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The hemoproteins are a class of proteins that play a vital role in the organisms. Within this class we can mention myoglobin, intracellular hemoglobin and cytochrome c, which are intensively studied. A strong motivation to study these giant extracellular hemoglobin is due to their high oligomeric stability, oxidation resistance, high cooperativity and high affinity to bind oxygen, in addition to their potential use in biotechnological applications such as biosensor for environmental contamination. In this work, studies were performed to determine the properties of HbAg in the absence and presence of copper sulfate (CuSO₄), to contribute to understanding how the copper effects the protein oligomeric structure and stability of HbAg, in the presence of an oxidation agent. Consequently evaluate their potential use as biosensor of environmental contamination. Optical absorption studies were performed for oxy-HbAg forms, at several copper concentration, at pH 7.0 and 25°C, in 0.1 mg/mL protein concentration. Optical absorption data show that in the low copper concentration range (0-10 µM), a significant decrease of absorption at the Soret band maximum (416 nm) and a shift to the blue are observed. Spectral changes for copper concentrations above 12 µM were observed: decrease absorption Q bands (500–600 nm); increase in absorbance at 700 nm and the ligand-to-metal charge transfer (LMCT) band (usually above 600 nm) were present. This change suggests the presence of significant light scattering for HbAg–Cu solutions at metal concentration above 10 µM. Thus, considering that 0.1 mg / ml protein correspondence to 10 µM HbAg heme concentration, the data suggest that a reason HbAg / Cu of 1:1 have a partial oxidation hemoglobin. These results suggest that HbAg is very sensitive to the presence of heavy metals.

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