

Spectroscopic studies on *Glossoscolex paulistus* hemoglobin labeled with fluorescein isothiocyanate: effects of urea.

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Introduction: *Glossoscolex paulistus* extracellular hemoglobin (HbGp) is characterized by a molecular mass of 3,600 kDa, a high oligomeric stability, resistance to oxidation and a high affinity to oxygen. **Objective:** The current study focuses the effects of urea on the protein stability, as monitored by fluorescein isothiocyanate (FITC) probe bound to oxy-HbGp. **Material and Methods:** Optical absorption, static fluorescence and time-correlated single photon counting (TCSPC) were used in our study. **Results and Discussion:** Absorptions of the probe at 492 nm, as well as, of the protein at 415 nm allowed to estimate the amount of bound FITC, giving a value of 0.13 FITC per heme group. Steady-state fluorescence showed a significant quenching of FITC emission upon binding to HbGp, with a quantum yield of 10% for the HbGp-FITC system. Time-resolved fluorescence data show that FITC in pure buffer has a two-exponential decay with 92% contribution of 3.9 ns and 9% of 1.2 ns components. Oxy-HbGp labeled with FITC presents multi-exponential decay with three-four lifetimes. Two lifetimes are in sub-ns range while a third one is close to 3.8 ns. Increase of urea concentration leads to an increase of the longer lifetime contribution. Up to 2.5 mol/L urea the changes are small. At larger urea concentration, in the range 3.5-6.0 mol/L, the main contribution is assigned to longer lifetimes. This behavior is due to a decrease of probe emission quenching and increase of exposure of fluorescein moiety to the solvent, both associated to the oligomeric dissociation of HbGp at higher urea concentration. Monitoring of tryptophan emission decays show a four-exponential behavior with predominance of sub-ns components, consistent with strong quenching due to the hemes. **Conclusions:** Time-resolved fluorescence data display that for HbGp-FITC the contribution of shorter lifetimes is reduced upon addition of denaturant. Both tryptophan and probe emissions are increased significantly in the presence of urea.

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