SDS effects upon the oligomeric stability of Glossoscolex paulistus hemoglobin by analytical ultracentrifugation

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The extracellular hemoglobin of annelid Glossoscolex paulistus (HbGp) has a molecular mass of 3600 kDa and an oligomeric structure composed of heme-containing globin-like chains (Hb subunits) and 36 additional polypeptide chains lacking a heme group, and named linker. This work focuses on the characterization of dissociated species in equilibrium in the SDS-HbGp system, monitored by AUC. SDS induces HbGp oligomeric dissociation above 0.1 mmol/L of surfactant. At 0.1 mmol/L of SDS, in the presence of 290 and 300 μg/mL of oxy-HbGp, a single species is observed, characterized by sedimentation coefficient s20,w of 58.4 ± 0.5 S and molecular mass (MM) of 3,600 ± 80 kDa, assigned to the un-dissociated protein [1]. However, at 100 μg/mL of oxy-HbGp, the presence of added tetramer in the solution is noticed, suggesting that the oligomeric dissociation is dependent on the protein concentration. The increase of SDS concentration promotes the full oxy-HbGp oligomeric dissociation into smaller subunits, such as, monomer r, trimers a, b, c, tetramer a,b,b, and tetramer a,b,b,c. At 0.4 mmol/L of SDS, s20,w values of 2.18 ± 0.07 S, 3.45 ± 0.07 S, and 5.6 ± 0.5 S are assigned, respectively, to the monomer r, trimers a,b, b,b, and tetramers a,b,b,c species, with several SDS molecules bound to their structure (Fig. 1). The SDS effect upon the HbGp oligomeric stability is quite similar to that reported for the denaturant urea, and alkaline pH [2,3]. Thus, the extent of oxy-HbGp dissociation, in the presence of SDS, is given by dodecamer (abde)3, followed by tetramer a,b,b,c, and monomer r. Our results are consistent with literature reports for HbGp in different conditions, as monitored by AUC and MALDI-TOF-MS [3,4].

Continuous sedimentation coefficient distribution (S) curves for oxy-HbGp, in the presence of 0.4 mmol/L of SDS, at pH 7.0. The letters a, b, and c are associated to the monomer r, trimer a,b, and tetramer a,b,b,c species, respectively.

Acknowledgements. The authors thank FAPESP and CNPq for financial support.


Fig. 1. Sedimentation equilibrium profiles of octamers of oxy-HbGp in the absence (---) and presence (----) of 0.4 mmol/L of SDS.

Fig. 2. Dependence of ψ-potential of complexes of PAA with CTAB (1) and DMDODAX (2) on the surfactant’s concentration (n)

Fig. 3. Viscosimetric titration of PAA with CTAB at 293 K (1) 313 K (2) and 333 K (3).