Protein ordering of extracellular Giant Hemoglobin studied by Polarized Resonance Raman Scattering and Dynamic Light Scattering

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The extracellular giant hexagonal bilayer hemoglobin (Hbl Hb) of lugworms (Arenicola Marina) contains 144 heme groups (Fe-protoporphyrin IX, with D$_{4h}$ symmetry), which are the active sites for binding of O$_2$. The 144 oxygen binding subunits are assembled into 12 identical dodecameric subunits, each dodecamer having a local 3 fold axis of symmetry. The overall symmetry of the protein is D$_6$ [1, 2]. In mammals, hemoglobin (Hb) contains 4 oxygen binding sites and is, as opposed to Hbl Hb, organized in red blood cells (RBCs).

The polarization properties of Resonance Raman Scattering (RRS), defined by the depolarization ratio (DPR), have successfully been applied in structural investigations of metallo-porphyrins, oxy (HbO$_2$) and deoxy (Hb) hemoglobin [3 - 5] as well as to study the conformation and aggregation of HbO$_2$ within the RBCs [6]. Dynamic Light Scattering (DLS) has previously been applied in the determination of the size distribution and z-average hydrodynamic diameter of the earthworm Glossoscolex paulistus [7].

In the present research we have initiated an experimental and theoretical study on the ordering properties of the Hbl Hb using the combination of RRS and DLS. Polarized RRS has been performed on isolated HbO$_2$, Hb molecules and RBCs in buffer solution as well as on buffer solutions of Hbl Hb with different pH. The DLS measurements have been performed on the Hbl Hb solutions.

Table 1 show as an example the DPR values obtained for the anomalous polarized $a_{2g}$ band near 1550 ($\nu_{19}$) and the depolarized band near 1600 cm$^{-1}$ ($\nu_{10}$) for the deoxy form of Hb, RBC and Hbl Hb at pH=5 (single macro protein) and 10 (fragmented protein parts). The polarized RRS spectra were obtained with 532 nm excitation.

From the DLS data, the average hydrodynamic diameter ($d_h$) was calculated using Non Negative Least Squares (NNLS).

The DPR (deoxy) data show that the intra-molecular couplings in free Hb, perturbing the heme from D$_{4h}$ symmetry, are larger than the inter-molecular couplings in the RBCs, giving rise to aggregation of Hb. The DLS result for Hbl Hb (pH = 5) shows the presence of intact Hbl Hb molecules, whereas the DLS result for pH10 indicates that parts of the fragmented
proteins have aggregated. This is supported by the decrease of the DPR value for the a2g–mode, since a decrease is also found when the Hb molecules aggregate in the RBCs. The results obtained in our work indicate that the O2 affinity of Hb survives, when these are organized in the macromolecule. In addition, the visible absorption spectra for RBC, Hbl Hb (pH=5) and Hbl Hb (pH=10) are very similar to the visible absorption spectrum of free Hb.

At the conference the results will be further discussed together with a modeling of the polarized Raman intensities based on intra- and inter-molecular couplings and symmetry arguments.

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