

CRYSTALLOGRAPHIC AND SMALL ANGLE SCATTERING STUDIES OF HEMOGLOBIN CRYSTAL NUCLEATION AND CLUSTER FORMATION

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Hemoglobin is a key iron-containing oxygen-transport protein in the human red blood cells and in almost all vertebrates. The 3D structure of the hemoglobin molecule has been determined by X-ray crystallography since the early works of Max Perutz in 1959. In the context of COVID-19, it has been recently shown that lower hemoglobin levels increase the risk for severe respiratory failure [1]. We used X-ray diffraction (XRD) and small-angle neutron scattering (SANS) to investigate the cluster formation and the crystal nucleation of commercial protein hemoglobin in different crystallization media. The effects of pH variations and polyethylene glycol (PEG) concentrations in the nucleant solutions were examined in order to find suitable conditions for single protein crystal formation. The XRD results established the quality of the obtained hemoglobin crystals and the corresponding crystallographic resolution. The performed SANS and gel chromatography experiments revealed the existence of protein monomers and dimers as well as the formation of higher order clusters in the investigated nucleant media. The obtained crystals were either disk-like or wire-like, but never developed a complete 3D structure. Despite that this effect has been previously observed, it has not been systematically investigated and explained yet. Our results elucidate the structural diversity of the commercial hemoglobin nucleation and pave the road for obtaining higher resolution crystal structures of hemoglobin [2].

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