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Mechanisms of Homogeneous Nucleation of Polymers of Sickle Cell Anemia Hemoglobin in Deoxy State

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Abstract

The primary pathogenic event of sickle cell anemia is the polymerization of the mutant hemoglobin (Hb) S within the red blood cells, occurring when HbS is in deoxy state in the venous circulation. Polymerization is known to start with nucleation of individual polymer fibers, followed by growth and branching *via* secondary nucleation, yet the mechanisms of nucleation of the primary fibers have never been subjected to dedicated tests. We implement a technique for direct determination of rates and induction times of primary nucleation of HbS fibers, based on detection of emerging HbS polymers using optical differential interference contrast microscopy after laser photolysis of CO-HbS. We show that: (i) nucleation throughout these determinations occurs homogeneously and not on foreign substrates; (ii) individual nucleation events are independent of each other; (iii) the nucleation rates are of the order of 10^6–10^8 cm^{-3} s^{-1}; (iv) nucleation induction times agree with an *a priori* prediction based on Zeldovich's theory; (v) in the probed parameter space, the nucleus contains 11 or 12 molecules. The nucleation rate values are comparable to those leading to erythrocyte sickling *in vivo* and suggest that the mechanisms deduced from *in vitro* experiments might provide physiologically relevant insights. While the statistics and dynamics of nucleation suggest mechanisms akin to those for small-molecule and protein crystals, the nucleation rate values are nine to ten orders of magnitude higher than those known for protein crystals. These high values cannot be rationalized within the current understanding of the nucleation processes.
Author Keywords: sickle cell anemia; hemoglobin S polymerization; fiber nucleation; homogeneous nucleation rate; nucleus size
Abbreviations: Hb, hemoglobin; DIC, differential interference contrast