587e Two Step Mechanism for the Nucleation Fibers of Sickle Cell Anemia Hemoglobin

Peter G. Vekilov, Oleg Galkin, and Weichun Pan

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. Nucleation is the only process in the sequence of phenomena that comprise the molecular biology, physical chemistry, and pathophysiology of the disease whose rate is an exponential function of the system parameters and as such is the easiest to suppress. In recent years, there have been significant theoretical and experimental advances in the field of nucleation of solid phases (crystals, polymers, aggregates, gels, etc.) of colloid and protein substances. One of the significant novel ideas is that the nucleation of any ordered solid phase—crystal, linear or planar periodic array, etc.—from solution, involves a precursor, a disordered fluid aggregate of mesoscopic size, akin to a droplet of the dense liquids, found for many proteins. We will present results of three types pf tests for the applicability of this mechanism to the nucleation of HbS polymers. 1. Using a direct technique 1, we determined the rates of homogeneous nucleation and the characteristic delay times for nucleation of HbS polymers, as well as growth rate of the polymers, as functions of temperature, at several HbS concentrations. We developed a statistical theory of two-step nucleation, which shows that with this mechanism, the nucleation delay time should be an exponential function of temperature. In the case of one-step nucleation, the delay time follows the temperature dependence of the growth rate. We found that while the growth rate of the polymers is practically independent of temperature (an intriguing fact on its own right) the nucleation delay time depends exponentially on temperature, i.e., these tests support the two-step mechanism. 2. We monitored the orientation of the HbS polymers in the electric field of polarized light. Because of the negligible energy of interaction of the filed with HbS molecules, and the low rotational diffusivity of macroscopic HbS polymer fibers, these determinations are a very sensitive probe of (a) the structure of small clusters of HbS, and (b) of their local environment. The results of these determinations indicate that the ordered HbS polymer nuclei are formed in an environment of high viscosity, i.e., they again support the two-step nucleation mechanism. 3. Time-dependent dynamic light scattering characterizations of the supersaturated and undersaturated HbS solutions shows the presence of HbS clusters of several hundred nanometers in size. Their evolution suggests that these are mesoscopic liquid clusters and the likely dense liquid 2 precursors to the ordered HbS nuclei, representing the first step of the two-step nucleation mechanism. The two-step mechanism may open new avenues for control of the HbS polymerization not by affecting the assembly of individual HbS molecules into polymers, as done before, but by controlling the kinetics of formation of the precursor. As shown for protein crystals, these latter kinetics are affected by concentrations 10-30-fold lower than the protein concentration.

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