

Protein aggregation characterized by nanotechnology

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Abstract

Proteins can be found in crowded solutions, at high concentrations, or fixed on surfaces inside cells and still function. Proteins, on the other hand, tend to agglomerate and crash out in highly concentrated bulk solutions. For example, myoglobin and lysozyme, two tiny proteins whose activity in the cytoplasm of a cell is controlled by their association with a chaperonin for appropriate folding and unfolding, are examples of this. Siefker et al. have now demonstrated that myoglobin and lysozyme aggregation is also inhibited inside the pores of two mesoporous silica materials, SBA-15 and KIT-6.

The researchers use small-angle neutron scattering studies on mesoporous silica that has been loaded with either protein at escalating quantities. The scattering signals are indistinguishable from those of unloaded silica at low loading. The presence of a modest amount of protein cannot be consistently separated from the roughness of the pore walls due to low signal contrast. However, at high loading, close to the proteins' maximal packing density, a broad pattern in the scattering spectrum emerges that is diagnostic of species with intense, non-sterically inhibited intermolecular interactions but no aggregation. This liquid-like behaviour is caused by increased interactions between proteins and between proteins and pore walls, which encourage stability rather than aggregation, as seen in the cytosol.

In this paper, we use a nanopore device in conjunction with AFM and DLS to investigate the process of protein aggregation. γ -lactoglobulin variant A (γ LGa) and a neuronal Tau protein were employed as model proteins. A nanopore device's basic component is a nanometer-sized pore with a diameter of 5 to 20 nm created in a free-standing silicon nitride membrane supported by a silicon substrate that separates two PDMA chambers containing salt solution. A stable ionic current is created by applying a biased voltage across the nanopore membrane to a pair of silver chloride (AgCl) electrodes. When a charged protein molecule or a protein aggregate passes through a nanopore, a protein aggregate with a larger volume than a single protein molecule will block more current or generate a larger current drop amplitude, allowing a nanopore device to characterise protein aggregation in salt solution at the single molecule level. The volume of translocating protein molecules or aggregates are estimated using a calibrated nanopore by a standard that has known geometry such as a dsDNA molecule. We show that the solid state nanopore approach can measure protein aggregation number and aggregation number distribution in settings similar to their natural aqueous solution environment by utilising a reference dsDNA molecule. The nanopore studies were carried out at varying pH, temperature, and salt concentrations with applied voltages ranging from 60 to 210 mV. We present results on self-association and aggregation of γ LGa and Tau as determined by nanopore technique, AFM, and DLS.

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