BSA nanoparticles as a nano-bio heterogeneous nucleant for protein crystallization.

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Proteins are the most important biological macromolecules and are involved in almost every aspect of life. The elucidation of the three-dimensional structure of biological macromolecules by X-ray analysis has provided an important contribution to our current understanding of many basic mechanisms involved in life processes. However, obtaining crystals of proteins that diffract to high resolution, remains a major obstacle in structure determination. Therefore, the search for materials that can effectively induce the nucleation of crystals is a very active and promising endeavor.
In this context, our idea is to explore the ability of nanomaterials exposing protein portions on the surface in inducing protein nucleation. To this purpose, stable Bovine Serum Albumin nanoparticles (BSA-NPs) of two different sizes (140 nm and 240 nm) were synthetized using the desolvation methods, and efficiently purified by dialysis and ultracentrifuge. Dimension and morphological characterization have shown that BSA-NPs have a globular morphology, with a sphere-like shape, but composed by a several smaller spheres. Furthermore, CD analysis confirm the retention of the secondary structure of the albumin in nanoparticle form. Finally, the ability of BSA-NPs to act as nucleating agents for macromolecule nucleation was investigated using three model proteins (BSA, thaumatin, and lysozyme). The effect on inducing nucleation is visible for both sizes of BSA-NPs, but is more pronounced for larger ones, indicating a relationship between exposed protein surface area and nucleating power. The involvement of nanoparticles in nucleation is particularly evident in BSA and lysozyme crystallization. In fact, in the first case, the majority of BSA crystals grows around NPs precipitate and, in the second case, the presence of BSA-NPs causes a massive nucleation with respect the control. Thanks to these promising preliminary studies, future plans include expanding the number of model proteins tested, performing X-ray diffraction analysis on the obtained crystals to check the crystalline cell parameters, and testing BSA-NPs ability to induce nucleation in proteins that have not yet been crystallized.