Poster

BSA nanoparticles as innovative heterogeneous nucleants for protein crystallization

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Understanding the three-dimensional structure of biological macromolecules, like proteins, has significantly advanced our comprehension of fundamental cellular processes.

The first step is the crystallization, that remains the most challenging aspect of structural determination [1].

However, it's well-known the potential of nanomaterials [2] to induce protein nucleation, and in this context, our idea is to explore nanomaterials that expose protein portions as a new type of nucleant.

Bovine Serum Albumin nanoparticles (BSA-NPs) are recognized to have different applications especially in nanobiotechnology and nanomedicine due to the easy availability, water solubility, biodegradability, and noncytotoxic [3].

To achieve our aim, we synthesized stable BSA-NPs of varying sizes (140 nm and 240 nm), using desolvation techniques, and efficiently purified by dialysis and ultracentrifuge.

BSA itself and other model proteins, such as lysozyme and thaumatin were used as targets to test the effect of nanoparticles. In addition, it's also been tested the enzyme NAD(P)H quinone reductase from the plant Arabidopsis thaliana [4] (AtNQR), whose structure has been recently determined in our research group.

The role of nanoparticles in the nucleation process was observed for all proteins tested, albeit with different effects from protein to protein.

In detail, in the case of BSA, most of the crystals grew from the surface of the nanoparticle precipitate, indicating an interaction between the BSA molecules in solution and those on the surface of the nanoparticles that induced a local increase in the concentration protein to the over-saturated conditions necessary for nucleation. The tests of lysozyme crystallisation showed a clear difference between the nanoparticles of different sizes. In particular, the larger ones induced the formation of a greater number of nuclei up to massive nucleation, suggesting a relationship between the protein surface area exposed and ability to induce nucleation. These promising preliminary findings pave the way for future endeavors, including expanding the number of model proteins tested, conducting X-ray diffraction analysis to validate crystal parameters, and investigating BSA-NPs' nucleation induction potential in yet-to-be-crystallized proteins.

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