ENZYME COUPLED GOLD NANOPARTICLES: COMPARISON BETWEEN CARBODIIMIDE AND PHYSICAL ADSORPTION

Paula Ciaurriz[1], Edurne Teiletxea Malda[1,2], Luis Olangua[1], Aaron C. Asensio[1], Jose F. Moran[2]

[1]FideNa, Edificio Jerónimo de Ayanz, C/ Tajonar s/n, 31006 Pamplona, Navarra, Spain
[2]Instituto de Agrobiotecnología, Universidad Pública de Navarra-CSIC-Gobierno de Navarra, Campus de Arrosadia s/n, 31006, Pamplona, Navarra,
paula.ciaurriz@fidena.es

Conjugation of biomolecules with inorganic materials provides access to a variety of functional hybrid systems with applications in biotechnology, medicine and catalysis. Coupling gold nanoparticles (AuNPs) and protein/enzymes allow us to design multifunctional nanocarriers for new therapy and sensing technology [1]. In the case of enzymes, the effective conjugates require the retention of protein structure and function [2].

The aim of this work is to design multifunctional protein nanocarriers. For this purpose a superoxide dismutase (SOD) enzyme (VuFeSOD) is used as a model protein and 15nm AuNPs as a carrier.

SOD is a metallo-enzyme that catalyzes the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen. These enzymes have great physiological significance and therapeutic potential in the prevention of the oxidative damage from superoxide radicals. The superoxide radical and SOD have been implicated in many disease states including inflammatory diseases, diseases of ischemia and reperfusion, neurodegenerative diseases, and cancer, as well as more subtle roles in cell signaling and perhaps in immune function [3]. FeSOD, a plant superoxide dismutase, is a very stable biomolecule. It contains a Fe atom in a well characterized 3D protein structure [4], which may serve as model for conjugates studies, as well as for developing methods and strategies easily applicable to human SODs.

AuNPs are excellent candidates for protein bioconjugation due to their high surface volume ratio, biocompatibility, and the ability to form functionalized bioconjugates via simple chemistry [5-6].

In this work we present the comparison between covalent conjugation and physical adsorption of FeSOD on 15nm AuNPs. Our approach is based on the utilization of different concentration of mercaptoundecanoic acid (MUA) capped AuNPs for covalent linkage with the protein. The covalent conjugates were obtained by water soluble carbodiimide reactions [sulfo-NHS (N-hydroxysulfo-succinimide) and EDC (1-ethyl3(3-dimethylaminopropyl) carbodiimide hydrochloryde)]. We tested different reaction conditions and measured the biological activity of the conjugates. The results indicated that the enzyme activity is affected by the conjugation method, and the optimal protocol for the conjugation is achieved.

The formation of the conjugates, stability, and activity of the different steps were study by dynamic light scattering (DLS), ζ-potential measurement, UV-VIS spectroscopy, scanning electron microscopy and agarose gel electrophoresis.

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