Gold nanoparticle-sod enzyme conjugates for therapeutic applications

Edurne Teiletxea Malda[1], Luis Olangua[1], Aaron C. Asensio[2], Gurutze Arzamendi[3], Luis M. Gandía[3], Jose F. Moran[2]

[1]FideNa, Centro I+D en Electrónica y Comunicaciones 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, Spain

[3]Departamento de Química Aplicada, Universidad Pública de Navarra, Campus de Arrosadia s/n, 31006, Pamplona, Navarra, Spain

edurne.teiletxea@fidena.es

Design and development of devices based on enzyme immobilization onto gold nanoparticles is of great current interest in the field of bio-nanotechnology, mainly due to its potential applications in the fields of biosensors, disease diagnosis, and catalysis [1].

Gold nanoparticles (GNPs) are of specific importance due to their size-dependent catalytic, electrical and optical properties [2]. Thus, the very high surface-to-volume ratio leads to dramatic changes in their properties [3]. All nanoparticles syntheses involve the use of a size stabilizing agent, which associates with the surface of the particle providing charge, biocompatibility or solubility properties to keep the nanoparticles suspended, and thereby preventing their aggregation [4]. The physical properties strongly depend on the particle size, shape, interparticle distance and nature of the protecting organic shell [5].

Superoxide dismutase (SOD) is a metallo-enzyme that catalyzes the dismutation of superoxide radicals into hydrogen peroxide and oxygen [6]. Reactive oxygen species, such as superoxide radicals, are thought to underlie the pathogenesis of several diseases, such as familial amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, Down's syndrome, cataract, cardiac myocytes and several neurological disorders. SOD enzymes have great physiological significance and therapeutic potential in the prevention of the oxidative damage from superoxide radicals [7].

In this work we describe the synthesis of gold nanoparticles, the production and purification of recombinant protein FeSOD, and the immobilization of the purified enzymes on the gold nanoparticles.

Monodispersed GNPs with an average diameter of 20 nm were synthesized by a wet reduction method. An aqueous solution of the gold precursor hydrogen tetrachloroauric acid ($HAuCl_4$) was boiled during 1 h under vigorous stirring. Reductant solution, containing trisodium citrate and the stabilizer tannic acid was quickly added to the boiling solution of $HAuCl_4$ and heating is maintained for 15 min. Then the colloidal suspension of GNPs was cooled to room temperature, centrifuged and filtered [8]. The resulting nanoparticles were spherical and very uniform, with a narrow size distribution. The size distributions of the particles in the colloids were measured by Dynamic Light Scattering (DLS) and their shape was observed by Field-Emission Scanning Electron Microscopy (FE-SEM).

Recombinant FeSOD from cowpea (*Vigna unguiculata*) was over-expressed in *Escherichia coli* using self-induction system [9] **[**10**]**. Self-induction of the bacteria may take between 16-24 h with optimal results, after which period cells are harvest by centrifugation and either used or stored at – 80º C. FeSOD is over-expressed using plasmid pET28a(+), and the gene has been cloned at the *NdeI* site of this vector, which implies the synthesis of the protein with a 6(Hys)-tag [6].Up to 50 mg of protein can be affinity purified from 1L of bacteria culture in a single chromatography step with a 5 mL NTA-Ni column (Amersham-Pharmacia).

GNPs were incubated at pH 7.5 and at room temperature with the lowest amount of SOD enzyme that maintain stable the GNPs. The excess of protein was eliminated by centrifugation, followed by washing in phosphate saline buffer [11]. Once the GNPs-protein conjugate was achieved, the GNP-enzyme conjugates were characterized in more detail using DLS. The difference between the bare GNPs and conjugate GNPs corresponded to a monolayer of adsorbed FeSOD. Secondly, the absorption spectral measurements have showed that the peak attributed to the Surface Plasmon Band of GNPs shifted to higher wavelengths corresponding to the adsorption of protein. And finally, gel electrophoreses has been used to confirm the conjugation of GNPs-FeSOD. The mobility of the conjugate differs from both the free protein and free GNP; the conjugates became more mobile in the electric field than the GNP itself. Those data indicated that superoxide dismutase protein binds to gold colloids nanoparticles.

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