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Nanoparticles-Based Hybrid Systems: Nanotechnology and Protein Engineering

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Abstract

The use of nanoparticles has been extensively exploited in biotechnology, materials, nanoelectronics, cancer therapy and imaging, environment and biofuel industry areas. The unique properties of the nanoparticles depends on the core of nanomaterial, the coating and functionalization agents, which play a key role in biocompatibility and targeting abilities of the nanomaterial. The functionalization can be achieved by small molecules and/or using macromolecules. Proteins are the principal macromolecules in the development of reliable and biomimetic hybrid materials for practical technological applications. Thus, in this work, protein engineering aspects involved in hybrid nanomaterials achievement are discussed.

Keywords

Nanoparticles; Biomimetics; Protein engineering; Hybrid systems

Introduction

Nanoparticles (NPs) have been extensively exploited because of its unique properties, depending on their size, shape, physical chemical and biological properties, which allow the most diverse applications. The benefits of the nanometric structures reaches biotechnology, materials, nanoelectronics, cancer therapy and imaging, environment and biomass conversion areas [1,2].

The manipulation of material at the nanoscopic level allows the exploration and production of existing materials with unprecedented properties. An electrically conductive material at the macroscopic and microscopic scales may become an electrical insulator at the nanoscale [1]. Using modern strategies, such as controlled radical polymerizations, supramolecular polymerizations or stepwise synthesis, polymers with precisely controlled molecular structures can be synthesized. Moreover, such tailored polymers can be folded or self-assembled into defined nanoscale morphologies [3].

The manipulation of the core properties of nanomaterials can be conjugated with diverse compounds, which provides a coating layer and/or a functionalization agent yielding a special targeting ability to that nanoparticle. For most applications of nanomaterials, the construction of a specific coating layer on the surfaces of the particle core allows their further surface functionalization, which is crucial for directing nanoparticles to desirable target. Layer coating formation can be achieved by using various chemical compounds/macromolecules/NPs such as small molecules, polymers and inorganic materials. Suitable coating and functionalization agents may improve the biocompatibility of the nanoparticle [2].

Nanoparticles functionalization and proteins attachment

The functionalization compounds include small molecules like carboxylic acids (nitrilotriacetic acid (NTA), aminobutyl-NTA, isothiocyanobenzyl-EDTA) [4-6], but also includes macromolecules, that can be attached to NPs by carboxylic acids or by biotin-avidin complexes, for instance. The binding of macromolecules like enzymes and antibodies to NPs has many applications, such as protein purification and immobilization, cancer therapy and imaging. Frequently the suitable attachment of protein to the NPs ligands requires previous protein engineering strategies. The binding of a 6 histidine tag to the carboxy- or amino-terminal end of the polypeptide is the most simple and original example of a successful protein engineering strategy to design a generalized purification scheme applied to virtually any protein [7].

The protein engineering strategies for attachment to NPs surface also finds applications as targeted delivery agents, labeling of cells and further biotechnological applications. Targeting specific cells is useful for cancer therapy and imaging, for instance. Antibody-conjugated Fe_3O_4 NPs has selectively bound the human glioblastoma multiforme (GBM) cells expressing the epidermal growth factor receptor (EGFR) deletion mutant (EGFRVIII) [8].

Protein engineering and the molecular biomimetics

Combining the most beneficial targeting properties of inorganic/organic based nanostructures and protein

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engineering, very effective hybrid compounds with peculiar properties can be achieved. Therefore, by merging recent advances in molecular biology with state-of-the-art engineering and physical sciences, the term "molecular biomimetics" emerges to refer to engineering materials at the molecular level using molecular biological routes. Biomimetics can thus be considered a particularly promising path for Nano and bio nanotechnology [9].

The approach of molecular biomimetics and protein engineering expands the design and use of multifunctional molecular systems in a wide range of applications, from disease diagnosis, therapeutics to areas of nanotechnology where the integration of inorganic, organic and biological materials is required [9].

The assembly of hybrid materials can be accomplished at the molecular scale using the protein specific recognition properties. Proteins are then the principal molecules in the development of reliable and biomimetic hybrid materials systems for practical technological applications [10]. Thus, the condition for the construction of these nanostructures are proteins designed for the molecular recognition of inorganic surfaces. That is performed by protein engineering.

Protein engineering has for decades been a powerful tool in biotechnology to produce a vast number of proteins/enzymes with vast potential in industrial applications [11]. This field of knowledge has expanded not only in the industrial area, but also in the medical field, due to its therapeutic potential in biosensors development, cellular imaging and immunoassays [9,11].

Considering industrial applications, protein engineering has been used to improve the performance of enzymes involved in lignocellulose degradation, as well as the synthesis of biofuels [12]. In the field of lignocellulosic materials degradation, strategic mixtures of enzymes can be co-located, mimetizing a cellulosome inspired by the interactions between specific proteins domains. Bayer and co-workers designed cellulossomes for biotechnological applications based on interactions between specific pairs of cohesin–dockerin [13]. In this sense, NPs core play key role by putting together different catalytic domains though a suitable attachment strategy. Carneiro and Ward developed a paramagnetic nanocomposite coated with chitosan and N-(5-Amino-1-carboxy-pentyl) iminodiacetic acid able to bind virtually any protein or set of proteins containing his-tag [6].

Protein engineering strategies point out the rational design and construction of nanoscale protein cages as a feasible and reachable strategy through conventional recombinant DNA technologies and microbial protein overexpression system. In therapeutic applications field, naturally formed nanostructures (such as viruses, flagella or protein oligomers) can be manipulated to acquire specific characteristics of interest. For example, by the insertion of cell targeting agents it is possible to control biodistribution allowing specific delivery of associated drugs [14].

Although the protein engineering is a valuable tool for construction of hybrid systems, there are some drawbacks related to the loss of catalytic activities in enzymes whose structure has been modified. Many of the proteins involved in key metabolic chains are oligomeric. These proteins have interfaces formed by a chain of weak interactions, which are crucial for the maintenance of the quaternary structure. In addition, the maintenance of these interfaces is often essential for catalysis, since residues from a polypeptide chain may participate in the catalytic center of the adjacent chain. This makes rational protein design a meticulous procedure prior to protein engineering itself. Consequently, the achievement of enzymatic multifunctional complexes through the engineering of proteins may be a complex and time-consuming path.

Conclusion and Expectations

At the interface of protein engineering and molecular biomimetics in which integration between inorganic, organic and protein components materials is critical for the constitution of nanostructured systems, the concept of enzymatic immobilization reappears not only as a mechanism to improve the biochemical characteristics of enzymes (such as thermostability), but also as a powerful tool allowing the achievement of multifunctional nanocomplexes applicable in diagnosis and therapy of diseases and optimization of industrial processes.

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