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Review

Development of analytical methods for functional analysis of intracellular protein using signal-responsive silica or organic nanoparticles

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ABSTRACT

Because proteins control cellular function, intracellular protein analysis is needed to gain a better understanding of life and disease. However, *in situ* protein analysis still faces many difficulties because proteins are heterogeneously located within the cell and the types and amount of proteins within the cell are ever changing. Recently, nanotechnology has received increasing attention and multiple protein-containing nanoparticles have been developed. Nanoparticles offer a promising tool for intracellular protein analysis because (1) they can permeate the cellular membrane after modification or changing composition, (2) the stability of various proteins is improved by encapsulation within nanoparticles, and (3) protein release and activity can be controlled. In this review, we discuss the development of analytical methods for intracellular functional protein analysis using signal-responsive silica and organic nanoparticles.

Contents

1. Introduction

Proteins are a major component of all living organisms and play numerous roles essential to life such as converting substrates into products, transporting biomolecules, and maintaining structural integrity. Interestingly, most proteins do not have one single function *in vivo*, but perform an array of activities within the cell, depending on their localization, timing of activation, quantity, and post-translational modification. For instance, caspases, which function in the apoptotic pathway, also play roles in cell migration, cell shaping, and late-onset neurodegeneration [1]. Therefore, determining the amount and type of protein at specific locations within the cell is essential to fully understand its function. However, assessing localized protein function is difficult given the limited sensitivity and resolution of currently available analytical methods based on HPLC and capillary electrophoresis [2,3]. One of the issues that need to be addressed is the small amount of protein within a specific area. To circumvent this, the protein concentration can be artificially altered and the subsequent cellular effects can be observed. Currently, several methods exist to decrease the protein concentration within the cell, such as gene knockout and gene silencing by short interfering RNA (siRNA) [4–6]. While these methods are very popular for studying protein function, they are indirect. Moreover, spatiotemporal- and dosage-control of protein activity by using these methods is difficult.

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In the field of nanotechnology, a nanomaterial is defined as having at least one dimension in the 1–100-nm size range. The use of nanomaterials has grown in recent years and they have become an important tool in a variety of fields within academia, industry, and health care [7,8]. Recently, nanoparticles have been used for delivering active protein to specific cells to allow the study of protein function within the cell [9]. In addition, signal-responsive nanoparticles that can release proteins in spatial-, temporal-, and dosage-controlled fashions have been developed. Specifically, protein-containing nanoparticles are internalized within the cell and the protein release is trigged by a particular stimulus. These novel technologies offer great promise for functional analysis of protein subcellular localization. In this review, the development of novel release methods using nanoparticles for assessing protein function within the cell is discussed. These methods are expected to have applications in the analysis of spatiotemporal and dosage-dependent protein activities within the cell.

2. Protein-containing nanoparticles

Many nanomaterials such as silica, organic molecules, gold, metal oxides, etc., have been used to either encapsulate a functional molecule or directly bind to a functional moiety [10–12]. For example, in the medical field, pharmaceutical-containing nanoparticles have been developed for targeted drug delivery within the body, which reduces the chance of harmful side effects [10–13]. For this purpose, nanoparticles that are considered safe and effective drug delivery systems (DDS) are used. Because protein is a very biocompatible compound, many attempts have been made at preparing nanoparticles from protein. For example, ferritin, a large iron-containing spherical protein, and self-assembling vault nanoparticles consisting of a protein shell with a lipophilic core have been used [14,15]. For effective delivery of paclitaxel, a mitotic inhibitor drug used for cancer treatment, the compound has been bound to 130-nm nanoparticles of human serum albumin [16].

In the field of DDS, many studies have been focused on functionalization of nanoparticles, which allows for targeted delivery, active cellular uptake, and release from the nanoparticle in response to a certain signal [10–13]. Various signals such as electric or light pulses, temperature, magnetic field, etc., and biological signals (enzymes, proteins) have been used for drug release [17–23]. Among these, light is one of the most promising signals because it is relatively safe and it is currently used in phototherapy. For the preparation of nanoparticles that release the drug in response to light irradiation, light-sensitive materials such as gold or carbon nanomaterials have been used [24]. One major disadvantage of light is its low penetration depth in the body, especially at short wavelengths. Therefore, nanoparticle-drug release based on light is mainly used for the treatment of skin diseases, and can be combined with UV/visible light phototherapy. However, the number of studies on light-triggered protein release for drug delivery is limited [24]. This review is a continuation of our previous review [25] and focuses on nanoparticles for application in intracellular protein function assessment, as many reviews of the application of protein nanoparticles as DDS have previously been published [26–29].

The use of proteins encapsulated in signal-responsive nanoparticles to assess protein function is novel in the sense that protein activity is masked until an external stimulus triggers its controlled release. Several previous studies have used nanoparticles prepared from silica and organic compounds to this end [30–50], and in each case, the chosen nanoparticles adhered to several requirements (illustrated in Fig. 1) that will be further discussed:

1) Preparation, storage, and degradation of nanoparticles should not negatively affect the encapsulated protein.
2) Encapsulated proteins must cross the cell membrane.
3) Nanoparticles should not be toxic to the cell.
4) Encapsulated proteins must be rapidly released when required.

2.1. Preparation, storage, and degradation of nanoparticles should not negatively affect the encapsulated protein

In general, proteins denature and are inactivated when heated or exposed to organic solvents. Therefore, the preparation of nanoparticles for protein encapsulation should be carried out under mild conditions. Retention of the protein within the nanoparticles requires various interactions between the nanoparticle and the protein. Three main interactions that are associated with protein immobilization within nanoparticles include chemical bonding, adsorption (hydrophobic or electrostatic interactions), and entrapment (Fig. 2). In addition, multiple of these interaction types can occur simultaneously. Chemical bonding, the strongest interaction, greatly reduces the risk of protein leaching from the nanoparticles. However, too strong a nanoparticle-protein interaction could negatively affect protein release. Moreover, many proteins are degraded or denatured by the chemical reaction required for formation of the nanoparticle-protein bond [51]. Although immobilization by adsorption is an easy technique, it can be applied to only a limited number of protein-nanoparticle combinations because an adequate interaction between the protein and nanoparticle is required. Entrapment, where the mesh-like structure of the nanoparticle encapsulates the protein of interest, is an alternative technique. This technique has been shown to be effective for immobilizing a variety of proteins, regardless of their size or structure [52–54]. In any case, when nanoparticles interact with a protein of interest, it is important to reduce protein leaching while ensuring that the protein-nanoparticle interaction is sufficiently labile for the protein to be released when acted upon by the appropriate stimulus.

The environment (water content, hydrophilicity, *etc.*) within the nanoparticle determines the stability of the encapsulated protein and the protein-containing nanoparticle. The most favorable environment would closely resemble the conditions *in vivo*. Nanoparticles prepared from biocompatible materials with high water content are preferable for protein storage within nanoparticles. Therefore, polyethylene glycol (PEG) and silica-based hydrogels have been widely used to improve protein stability [34,52–55].

2.2. Encapsulated proteins must cross the cell membrane

Although the cell membrane is impermeable to most proteins, encapsulation of proteins within membrane-permeable nanoparticles substantially improves their internalization. The internalization of nanoparticles occurs with the aid of their positive zeta potential or through the presence of cell-penetrating peptides (CPP) such as trans-activating transcriptional activator (TAT) encoded by human immunodeficiency virus (HIV)-1 and the octaarginine (R8) peptide (Fig. 2) [55,56]. The internalization occurs either directly by interacting with membrane-embedded receptors or indirectly by associating with the lipid bilayer [57,58]. Though CPPs have been reported not to be toxic below 100 μM, the cationic nanoparticles derived from cationic compounds such as polyethylenimine are associated with significant cytotoxic effects [59,60]. The use of low-molecular-weight cationic compounds reduces these concerns [61].

Once a nanoparticle is internalized via endocytosis, it is trapped within a spherical vesicle called an endosome. Typically, the contents of an endosome are degraded after fusion with a lysosome or excreted by the cell through exocytosis [62]. Thus, the nanoparticles must escape the action of the endosome to function as protein carriers. If the surface charge of the nanoparticles is positive, it