Photodegradable Nanoparticles for Functional Analysis of Intracellular Protein

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There are two major shortcomings when using proteins for biotechnological applications: (1) low protein stability and (2) difficulty in controlling protein function. A possible solution to these problems may be in the use of nanoparticles to encapsulate proteins. Protein encapsulation can increase protein stability by hindering interactions between proteins and the external environment. Moreover, protein function will be suppressed when proteins are encapsulated, and function can be restored when the encapsulated proteins are released by nanoparticle degradation. This attribute of protein-containing nanoparticles allows for the spatiotemporal control of protein function, which is useful in basic research, as well as in various industrial and medical fields. In this review, we will introduce our recent achievements in development of protein-containing nanoparticles that releasing protein by light signal.

Keywords: Photodegradable nanoparticle, Intracellular protein, Light signal

1. Introduction

Proteins are a major component of all living things, and play numerous essential roles in vivo. Proteins possess such varied functions as converting substrates into products, transporting other biomolecules, and maintaining the structural integrity of living things. Given these facts, proteins are indispensable compounds for the maintenance of life. Interestingly, most proteins do not have just a single function in vivo, but can have an array of functions within the cell depending on their localization, timing of activation, quantity, and post-translational modification. For instance, caspases, which are known canonically to 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, not only in the entire cell, but also in a localized area of the cell is essential for understanding protein function. However, assessing localized protein function is difficult given the sensitivity and resolution needed by current analytical methods [2]. One specific issue that needs to be addressed is the fact that researchers are dealing with a small amount of protein within a localized area. To circumvent this issue, the protein concentration can be artificially altered and the subsequent cellular effects can be observed. Currently several methods exist to decrease protein concentration within the cell, such as gene knockouts and the silencing effects of short interfering RNA (siRNA) [3]. While these methods are very popular for studying protein function, they do so indirectly by silencing gene expression. Moreover, the spatiotemporal control over of protein function is difficult using these methods.

Recently, nanoparticles have been used for the direct control of protein function within the cell [4]. Specifically, protein-containing nanoparticles are internalized within the cell, with the eventual release of the protein occurring in response to a particular stimulus. These nanoparticles allow for the spatiotemporal control of protein release, which is promising for the analysis of localized protein function within the cell. In this review, the development of protein-containing nanoparticles
that releasing the protein by the external light signal for assessing protein function within the cell is discussed.

2. Protein-containing nanoparticles

In the field of nanotechnology, a nanomaterial is defined as having at least one dimension in the 1 to 100 nm size range. The use and prevalence of nanomaterials has grown in recent years, and has become an important tool in a variety of fields [5, 6]. Many nanomaterials have been developed that encapsulate protein [7]. For example, in the medical field, nanoparticles containing pharmacological drugs have been developed for delivery of these drugs to specific targets within the body. Targeted delivery of drugs by nanoparticles reduces the chance of harmful side effects [8]. Thus, nanoparticles are used as safe and effective drug delivery systems (DDS). It should be noted that this review does not examine these protein nanoparticles, because these nanoparticles have not been applied to assessing intracellular protein function, and many reviews of its application have already been published [9, 10].

The use of nanoparticle-encapsulated proteins to assess protein function is novel in that protein function is masked by nanoparticle encapsulation until an external light stimuli triggers its release. There are several studies that use nanoparticles in this way [11-21], and in each case, the chosen nanoparticles adhered to several requirements (listed below and illustrated in Fig. 1):

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 be not or less toxic to the cell.
4) Encapsulated proteins must be rapidly released when required.

3. Nanoparticles for application in intracellular protein analysis

We developed new four-arm PEG monomers (Fig. 2a) that consisted of four-arm PEG with photocleavable nitrobenzyl groups and connecting acrylic or methacrylic groups at the end of each arm [11]. These photo-responsive nanoparticles were prepared by vortexing a mixture of the four-arm PEG monomer, protein, ammonium persulfate (APS), and tetramethylthelylenediamine (TEMED) for 20 min. The size of the nanoparticles was approximately 150 nm, as determined by dynamic light scattering (DLS) analysis (Fig. 2b). The size of the nanoparticles could be adjusted (between 20 – 200 nm) by altering the concentration of the PEG monomer as well as the time for which the mixture was left to sit following vortexing [12].

The effect of nanoparticle irradiation on
enzyme activity was assessed using nanoparticle-encapsulated trypsin. Although almost no activity was observed before irradiation, substantial enzyme activity was observed after light exposure [11]. Theoretically, this makes sense because protein function is restricted when the protein is encapsulated within nanoparticles, effectively cutting the enzyme off from interacting with its substrates. Irradiation restores enzyme activity when the protein is released from the nanoparticle because it is now free to interact with its substrate(s) (Fig. 2b). In this way, protein activity can be controlled using nanoparticles.

An interesting technique for controlling protein function is termed as Protein Activation and Release from Cage by External Light (PARCEL) [11]. In this technique, a mesh structure is prepared by a polymerization reaction, which uses four-arm monomers in the presence of the protein of interest. This technique ensures the encapsulation of the protein within the mesh, and this has been successfully accomplished for a variety of proteins, including caspases, trypsin, ferritin, and BODIPY-casein. We recently found that these nanoparticles were also applicable for the controlled release of small molecules (e.g. rhodamine B) and nucleic acids (e.g. siRNA) [13-20]. A correlation was observed between the space size of the mesh structure of the nanoparticles and the size of the encapsulated protein by wet experiments and a computer simulation study [21, 22].
Irradiation-induced release of proteins from nanoparticles can be used to selectively study protein function within a given area of the cell. For instance, when nanoparticles are internalized into cells by using the microinjection technique, the nanoparticles are distributed throughout the cytoplasm of the entire cell. Subsequent irradiation of nanoparticles in a particular subcellular location will cause the selective release of encapsulated proteins in that area, thereby allowing assessment of local protein function [13]. This phenomenon was observed with encapsulated caspase proteins. Following internalization and distribution throughout the cell, irradiation-induced release of caspase proteins was restricted to a portion of the cell near the nucleus (Fig. 2c). Although the lamellipodia at the cell edge (distant from the irradiation point) did not change, the lamellipodia at the irradiated edge retracted approximately 2 min after light exposure (Fig. 2c). Four minutes after irradiation, the cell started to expand at the irradiated edge while the lamellipodia at the opposite edge maintained its original shape. The irradiation point coincided with the point at which the change in lamellipodia was initiated, suggesting that the change was initiated by the local activation of caspases. After approximately 10 min, the cell ruptured and died. Cell death was not observed when the same experiment was performed with nanoparticles that did not contain caspases. This experiment indicates that nanoparticles are applicable for assessing localized protein function within a cell.

4. Conclusion

Many new protein-containing nanoparticles have been developed, and applied to the spatiotemporal release of proteins within cells. This technique should enable the quantitative analysis of a localized protein, which was difficult by any other method.

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