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Photo-synthesis of protein-based nanoparticles and the application in drug delivery

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ABSTRACT

Recently, protein-based nanoparticles as drug delivery systems have attracted great interests due to the excellent behavior of high biocompatibility and biodegradability, and low toxicity. However, the synthesis techniques are generally costly, chemical reagents introduced, and especially present difficulties in producing homogeneous monodispersed nanoparticles. Here, we introduce a novel physical method to synthesize protein nanoparticles which can be accomplished under physiological condition only through ultraviolet (UV) illumination. By accurately adjusting the intensity and illumination time of UV light, disulfide bonds in proteins can be selectively reduced and the subsequent self-assembly process can be well controlled. Importantly, the co-assembly can also be dominated when the proteins mixed with either anti-cancer drugs, siRNA, or active targeting molecules. Both *in vitro* and *in vivo* experiments indicate that our synthesized protein–drug nanoparticles (drug-loading content and encapsulation efficiency being ca. 8.2% and 70%, respectively) not only possess the capability of traditional drug delivery systems (DDS), but also have a greater drug delivery efficiency to the tumor sites and a better inhibition of tumor growth (only 35% of volume comparing to the natural growing state), indicating it being a novel drug delivery system in tumor therapy.

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1. Introduction

Proteins require correctly folded conformations to perform their particular biological functions [1–4]. For lots of proteins, the folding and structural stability as well as their biological functions require the correct formation of disulfide bonds [5–7]. Normally, disulfide bonds are buried deeply in the proteins and very stable, which can be reduced only under the harsh conditions with both of the denaturing and reducing agents [8–12]. Recently, it was found that disulfide bonds could be reduced via a photochemical process under physiological condition without involving any denaturing or reducing agents [13–18]. This is because the aromatic residues (Trp, Tyr and Phe) excite under UV light (∼250–298 nm) and eject electrons from their side chains [19–22]. These electrons can be captured by nearby disulfide bonds which form transient disulfide electron adducts, resulting in the breakage of disulfide bonds. Subsequently, the photo-reduced protein undergoes an obvious conformation change to expose its buried hydrophobic residues and trigger further intermolecular self-assembly. We found that the unfolding and self-assembly of UV-illuminated proteins can be well controlled by adjusting the illumination time and intensity of UV light. Under different illumination conditions, the diversity of self-assembled nanoparticles can be easily realized from several nanometers to several micrometers, and even the formation of amyloid-like fibrils [23–25]. In fact, the sizes of these globular nanoparticles are quite dependent with the exposure of hydrophobic amino acids of illuminated proteins and their stability is dominated by the newly formed intermolecular disulfide bond. Thus, the co-assembly of proteins and anti-cancer drugs [26] or siRNA delivery (unpublished data) can also be well dominated. Furthermore, due to the existence of many free thiols exposed on the surface of these photo-synthesized protein–drug nanoparticles, a lot of functional targeting molecules can be easily modified on these nanoparticles [27]. In addition, our method for producing photo-synthesis nanoparticles has many potential applications in molecular interactions and mapping of biotechnology. The photo-reduction of disulfide bonds buried in proteins provides a novel method to investigate not only protein folding/unfolding and intermolecular self-assembly, but also the immobilization, molecular printing and drug delivery system [27–29]. Based on these studies, the photo-synthesis of protein nanoparticles is showing more and more potential applications in the future.

2. The principle of photo-reduction of disulfide bonds

Recently, it was found that disulfide bonds buried in proteins can be reduced by UV illumination, which dramatically affects the protein conformations as well as their biological functions [15–18,30,31]. The absorption of UV light by Trp residues was responsible for the reductive splitting of the nearby disulfide bonds and the production of new free thiols in a lot of proteins such as Fusarium solani pisi cutinase [15], goat α-lactalbumin [16] and hen egg white lysozyme [32]. In detail, the photo-reduction mechanism can be described as follows:

$$\mathrm{TrpH} \xrightarrow{h\nu} \mathrm{TrpH}^{\cdot+} + \mathrm{e}^{-}$$

$$\mathrm{RSSR} + \mathrm{e}^{-} \rightarrow (\mathrm{RSSR})^{\cdot-}$$

$$(\mathrm{RSSR})^{\cdot-} \rightarrow \mathrm{RS}^{\cdot} + \mathrm{RS}^{-}.$$

Under UV illumination, the Trp residues inside proteins can absorb photonics and subsequently transform into an excitation-state. The photo-ionization electrons from Trp residues are transferred to the nearby disulfide bonds, resulting in the breakage of disulfide bonds and the formation of free thiols. The electron transfer rate and the reduction rate of disulfide bonds are quite dependent on the distance between Trp residues and the nearly disulfide bonds [16,30,31]. The newly formed free thiol groups can easily form a new intra/intermolecular disulfide bonds even under the weak oxidative condition of dissolved oxygen in protein solution. The fluorescence emission spectra of Trp residues inside proteins will be largely altered due to the changing of neighing polarity conditions and the quenching during UV illumination process, which has been termed as an important character of photo-reduction phenomenon.