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Short communication

Intracellular delivery of recombinant proteins via gold nanoparticle–DNA aptamer composites is independent of the protein physicochemical properties and cell type

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ABSTRACT

Here, we report that the gold nanoparticle–DNA aptamer (AuNP–Apt) conjugate-based system can efficiently deliver recombinant proteins into mammalian cells in a manner independent of their size, isoelectric point, and cellular localization. Additionally, AuNP–Apt system-assisted protein delivery can be effective on primary and stem cells, indicating that its use is not limited to fast-dividing cells. We further show that the intravenously administered AuNP–Apt system can deliver proteins into rat organs. Our findings show that this system can serve as a simple, efficient, and versatile platform for the delivery of recombinant proteins into mammalian living systems.

Introduction

Compared with small-molecular drugs, protein therapy has several advantages, including higher specificity, greater activity, and less toxicity [1,2]. However, the use of many therapeutic proteins has been limited by their poor membrane permeability, susceptibility to endosomal internalization, instability, and immunogenicity [1,3–5]. One strategy for overcoming the poor membrane permeability of target proteins is to synthetically attach genetically fused proteins with cell-penetrating peptides, such as TAT-derived peptides, arginine-rich peptides, and amphiphilic peptides, to improve intracellular protein delivery [6]. Despite the improvement in their membrane permeability, however, these protein drugs linked to cell-permeable sequences have raised a problem in their clinical development because their unique cell-permeable properties can cause toxicity, especially with chronic use [7]. To achieve high therapeutic performance, several delivery vehicles for proteins have been developed based on different nanomaterials, such as polymeric, lipid-based, and inorganic nanoparticles (for a recent review, see Ref. [2]). However, these systems require a complex process of linking the target protein to a nanocarrier, which may alter the structure and function of the protein [8–11]. Furthermore, not only do most of these nanocarrier-based systems cause cytotoxicity in vivo but they can be used only for the delivery of a subset of proteins with certain properties [1,4,5]. It has also been suggested that these nanocarriers are effective on rapidly growing mammalian cells, the membrane of which is more susceptible to penetration by nanocarriers [12].

For these reasons, we previously developed a protein delivery system based on gold nanoparticles (AuNPs) functionalized with a DNA aptamer (AuNP–Apt) [13], by combining the properties of aptamers (e.g., high binding affinity, low immunogenicity, and long-term stability) [14] with the unusually efficient cellular uptake that results from conjugating AuNPs with a DNA oligonucleotide [15,16]. These properties of AuNP and RNA aptamer also have been utilized for the development of targeted delivery of a drug [17]. In this study, we investigated the delivery efficiency of this system in relation to the intrinsic properties of the

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delivered proteins, such as their size, isoelectric point (pI), and cellular localization. We also tested the ability of this system to deliver proteins into mammalian primary and stem cells that are known to be difficult to transfect with biomaterials, as well as into organs of rat.

Materials and methods

Synthesis of AuNP–Apt conjugates and preparation of AuNP–Apt–protein complexes

The Histidine aptamer-conjugated AuNP (AuNP–Apt^{His}) and AuNP–Apt^{His}–protein complex were prepared according to previously described procedures [13]. The procedure for preparation of recombinant proteins has been previously described and is shown in Table 1. C-terminally hexahistidine-tagged HPA3P peptide was synthesized and purified as described previously [18]. The size and ζ potential of nanoparticles were measured as described previously [13].

Visualization of protein uptake by AuNP–Apt conjugates

Visualization of recombinant proteins in cultured cells was carried out as previously described [13]. In brief, to detect the delivery of the proteins into cells, cells grown on 10 mm lysine-coated coverslips were incubated with the AuNP–Apt^{His}–protein complex for 1 h before being fixed with 4% paraformaldehyde (Sigma, USA). The fluorescence emitted by the Alexa 488 (495 nm excitation, 519 nm emission)-labeled secondary antibody was detected by laser scanning confocal microscopy (Carl Zeiss ZEN 2011, Germany). The relative fluorescence intensities were measured using ImageJ software (NIH, USA).

Animal experiments

Animal experiments were carried out as described previously [13]. In brief, the complex of AuNP and B-cell lymphoma 2 interacting mediator of cell death (BIM) protein complex was injected intravenously into four-week-old female Sprague–Dawley rats at a dose of 1 mg/kg AuNP–Apt^{His} and 10 μg BIM.

Visualization of BIM protein uptake by AuNP–Apt conjugates in rat organs

Visualization of the BIM protein in rat organs was carried ou t as previously described [13]. In brief, effective delivery of Alexa 488-labeled BIM by AuNP–Apt^{His} conjugates into the organs was detected as a green signal in the sectioned organs by laser scanning confocal microscopy (Carl Zeiss ZEN 2011, Germany). The relative fluorescence intensities were measured using ImageJ software (NIH, USA).

Transmission electron microscopy

Transmission electron microscopy (TEM) was performed as previously described [13]. In brief, liver sections were fixed in Karnovsky's glutaraldehyde–paraformaldehyde mixture in 0.2 M cacodylate buffer (pH 7.4) for approximately 3 h at room temperature. The liver sections were washed with cacodylate buffer (pH 7.4) to remove the fixatives, dehydrated in an alcohol series, embedded in Spurr's resin, and sliced to a thickness of 70 nm. TEM images were taken with a JEOL model JEM-1010 system operated at 80 kV accelerating voltage, with magnification at 10,000× and 25,000×.

Results and discussion

Effects of intrinsic properties of proteins on their intracellular delivery by AuNP–Apt^{His}

Every protein has its unique physicochemical characteristics, such as molecular weight (MW), pI, and cellular localization, which can affect its transfection efficiency into mammalian cells. Although AuNPs conjugated with a His-tag DNA aptamer (AuNP–Apt^{His}) have been successfully used in transporting several proteins into mammalian cells in vitro and in vivo [13,19], it still remained uncertain whether the system could deliver any proteins with various physicochemical characteristics. For this reason, we wished to test the effects of the MW, pI, and intracellular localization of proteins on their intracellular delivery by AuNP–Apt^{His} conjugates. To do this, we used 11 recombinant proteins with a wide range of molecular weight (MW) and isoelectric point (pI) values, which are listed in Table 1. These proteins have a hexahistidine tag at either the N- or C-terminus, and five of them have been shown to be effectively delivered into mammalian cells by AuNP–Apt^{His}. When AuNP–Apt^{His} conjugates were loaded with proteins, in general, the anionicity of particles was decreased, whereas the size of particles was increased (Table 1). These changes did not appear to be associated with molecular weight or pI of proteins. Each protein (1 μM) was mixed with AuNP–Apt^{His} (1 nM) and the complexes were applied to HeLa cells. After 1 h incubation, the

Table 1
Physicochemical properties of recombinant proteins used in this study.

Protein	Molecular weight (kDa)	pI	Size (nm)[a]	ζ-Potential (mV)[b]	Localization	Reference
HPA3P	3.2	11.41	n. d.	n. d.	n.d.	[18]
TM-JM 1/2	14.1	6.79	638.3 ± 120.9	2.9	Cytoplasmic membrane	[19]
Lamin 406-567	18.7	7.16	791.8 ± 223.7	−11.79	Nucleus	[24]
RraAV1	19.4	4.08	491.9 ± 103.5	−18.03	n.d.	[25]
BIM	23	8.24	436.3 ± 76.6	−11.94	Mitochondria	[13]
RNase III	26.4	7.19	718.3 ± 191.0	−6.74	n.d.	[26]
BCL-xL	26.9	5.56	734.7 ± 174.3	−18.73	Mitochondria	[13]
Lamin 406-665	28.1	7.87	866.2 ± 208.0	−10.94	Nucleus	[24]
FOXL2	39.6	9.16	260.1 ± 210.5	−14.84	Nucleus	[27]
AcrA	42.9	8.22	1096.7 ± 265.6	−7.8	n.d.	[13]
Enolase	46.5	6.04	141.6 ± 48.9	−10.19	n.d.	[28]

n.d.: not determined.
[a] The size of AuNP–Apt^{His}–protein complex was measured.
[b] The ζ-potential of AuNP–Apt^{His}–protein complex was measured.