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Intracellular trafficking of particles inside endosomal vesicles is regulated by particle size

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ABSTRACT

Little comparative information is available on the detailed intracellular dynamics (diffusion, active movement, and distribution mechanisms) of nanoparticles (≤ 100 nm) and sub-micron particles (> 100 nm). Here, we quantitatively examined the intracellular movements of different-sized particles and of the endosomal vesicles containing those particles. We showed that silica nanoparticles of various sizes (30 to 100 nm) had greater motility than sub-micron particles in A549 cells. Although particles of different sizes localized in the early endosomes, late endosomes, and lysosomes in different proportions, their motilities did not vary, regardless of the vesicles in which they were localized. However, surprisingly, endosomal vesicles containing silica nanoparticles moved faster than those containing sub-micron particles. These results suggest that nanoparticles included within endosomal vesicles do not suppress the motility of the vesicles, whereas sub-micron particles perturb endosomal vesicle transport. Our data support a new hypothesis that differences in particle size influence membrane trafficking of endosomal vesicles.

1. Introduction

With the recent development of nanotechnology, nanoparticles have been used in a variety of fields, such as the food, cosmetics, and industries [1–3]. Recent studies in rodents have revealed that nanoparticles show greater tissue-penetration ability and internalization ability than conventional materials in various tissues (liver, spleen, and lung) [4,5]. Therefore, nanoparticles such as mesoporous silica nanoparticles, gold nanoparticles, and fullerene are expected to be particularly useful as novel drug-delivery carriers and contrast agents in the medical field [6–8]. In addition, the cellular uptake and intracellular localization of nanoparticles change depending on the properties of these particles (size, charge, chemical composition, and surface modification) [9–11]. For example, some reports show that after nanoparticles enter the cell they are localized not only near the cell membrane but also at the perinuclear side of the cytoplasm and in organelles such as the nucleus; by carrying drugs to the perinuclear side of the cell they can thus improve therapeutic effects [12,13]. These data suggest that nanoparticles have unique and therapeutically attractive behaviors in vivo and in vitro that is barely accomplished by conventional materials; their specific *in vivo* kinetics and intracellular dynamics give them potential as novel non-viral drug-delivery carriers. However, although assessment of the detailed intracellular dynamics of these particles—including distribution speed, spatiotemporal localization, intracellular processing, and excretion—is important if we are to understand the mechanisms of nanoparticle-specific intracellular dynamics, these mechanisms are still poorly understood.

Particle tracking by using real-time imaging is one of the most

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powerful methods of studying in detail the intracellular dynamics of particles such as protein aggregates, DNA aggregates, organelles, and viruses [14,15]. Particle tracking is expected to reveal those intracellular movements that are difficult to unravel by using conventional methods such as localization analysis in fixed cells [16]. However, only limited numbers of studies have reported the trajectories of nanoparticles and the mean square displacement (MSD) of their intracellular movements [17,18]. For this reason, the relationship between the properties and detailed intracellular dynamics of particles, including their motility (diffusion, velocity, and their mechanisms) has not yet been determined. Even the differences in intracellular motility between nanoparticles and sub-micron particles remain unclear.

Here, we investigated the intracellular trajectories and motilities of silica particles with diameters of 30, 50, 70, 100, 300, and 1000 nm inside the cell by using real-time imaging. We showed that silica nanoparticles (with diameters ≤ 100 nm) moved faster than sub-micron particles (with diameters > 100 nm). The silica particles were included within endosomal vesicles, which were then transported along the microtubules. Although silica nanoparticles and sub-micron particles were localized in early endosomes (EE), late endosomes (LE), and lysosomes (Ly) in different proportions, the differences in particle motility did not result from these differences in preferential localization. Surprisingly, regardless of the type of endosomal vesicle, those containing silica nanoparticles moved faster than those containing sub-micron particles. These results suggest that silica nanoparticles do not suppress the motility of endosomal vesicles, whereas sub-micron particles have suppressive effects on endosomal vesicle transport. The difference in endosomal motility between silica nanoparticles and sub-micron particles likely results from a difference in perturbation effects on endosomal vesicle transport. Our results reveal part of the mechanism of nanoparticle-specific intracellular dynamics and support a new hypothesis that differences in particle size influence the membrane trafficking of endosomal vesicles. These findings should help unravel the mechanisms of nanoparticle-specific dynamics, along with part of the cellular physiology of membrane trafficking of endosomal vesicles.

2. Materials and methods

2.1. Nanoparticles and sub-micron particles

Fluorescence-labeled silica particles were purchased from Micromod Partikeltechnologie (Rostock/Warnemünde, Germany). Silica particles that had diameters of 70, 300, and 1000 nm (nSP70, SP300, and SP1000; catalog numbers 40-00-701, 40-00-302, and 40-00-103, respectively) and were labeled with orange fluorescence (Rhodamine B: excitation and emission wavelengths, 569 and 585 nm, respectively) were used. The silica particles were sonicated for 5 min and vortexed for 1 min before use.

2.2. Cell line, antibodies, plasmids, and reagents

A549 cells (human lung carcinoma) were purchased from the American Type Culture Collection (Manassas, VA, USA). A549 cells were maintained at 37 °C in 5% CO_2 with culture medium [Dulbecco's Modified Eagle's Medium (DMEM; Wako Pure Chemical Industries, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS; Biosera, Kansas City, MO, USA) and 1% antibiotic-antimycotic-mix stock solution (Ab; Gibco, Carlsbad, CA, USA)]. Mouse monoclonal anti-early endosome antigen 1 (EEA1) antibody (clone name: 14/EEA1) was purchased from BD Transduction Laboratories (BD Biosciences, San Jose, CA, USA). Rabbit polyclonal anti-rab7 antibody (clone name: H-50) and mouse monoclonal anti-lysosome-associated membrane protein 1 (LAMP1) antibody (clone name: H4A3) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Alexa488-conjugated goat anti-mouse immunoglobulin G (IgG) secondary antibody and Alexa488-conjugated goat anti-rabbit IgG secondary antibody were purchased from Invitrogen (Carlsbad, CA, USA). Plasmids encoding the green fluorescence protein (GFP)-fusion endosome/lysosome marker proteins GFP-EEA1 wt (#42307), GFP-rab7 WT (#12605), and LAMP1-mGFP (GFP with an N-terminal palmitoylation to bind to the cell membrane; #34831) were purchased from Addgene (Cambridge, MA, USA). Nocodazole was purchased from Wako Pure Chemical Industries.

2.3. Microscopy

Fluorescence images (single-color live-cell images and immunofluorescence images) were obtained by using a custom-built objective-type total internal reflection fluorescence (TIRF) microscope, based on an inverted microscope (Ti-E; Nikon Co., Tokyo, Japan), with a 100 ×/1.49 numerical aperture Apo TIRF oil-immersion objective lens (Nikon Co.), a 4 × or 2.5 × intermediate variable magnification lens (VM lens C-4 ×, VM lens C-2.5 ×; Nikon Co.), and a scientific complementary metal oxide semiconductor camera (C11440-22CU, Hamamatsu Photonics, Shizuoka, Japan). Fluorescent probes were excited by a 488-nm or 561-nm laser (Spectra-Physics, Tokyo, Japan). Live-cell images were observed through a 525-nm or 609-nm emission filter (FF01-525/45-25 and FF01-609/54-25, Semrock, Lake Forest, IL, USA). Dual-color fluorescence live-cell imaging was performed by using the same optical system as for single-color live-cell imaging and dual-view optics (A8509; Hamamatsu Photonics). Each emission was split into two parts at a wavelength of 550 nm by dichroic mirrors (DM550LP and DM550SP; Hamamatsu Photonics).

2.4. Live-cell imaging of movements of silica particles

A549 cells were seeded on a 35-mm glass-base dish (Iwaki, Shizuoka, Japan) at a density of 2.5×10^5 cells/2.5 mL/dish. The dishes were sonicated with 1/10 N potassium hydroxide solution (Wako Pure Chemical Industries), ultrapure water, 99.5% ethanol (Wako Pure Chemical Industries), and ultrapure water in turn for 15 min each and then sterilized by ultraviolet irradiation for 2 h before being seeded with the cells. Cells were incubated for 24 h at 37 °C in 5% CO_2 before exposure to a silica particle suspension. Silica particle dispersions were prepared by diluting silica particle stock to a concentration of 25 μg/mL in culture medium just before addition to the cells. The cells were pulsed for 3 h at 37 °C with silica-particle-containing medium at 2.5 mL/dish. The particle-containing medium was then removed, and the cells were washed three times with phosphate-buffered saline (PBS). Fresh, particle-free medium was then added to the cells, which were chased for an additional 3 h. The medium was replaced with observation medium [10% FBS and 1% Ab in phenol-red-free DMEM (Wako Pure Chemical Industries)] just before observation. Cells were observed by using inclined illumination fluorescence microscopy. Images were captured at 100-ms intervals for 30 s.

2.5. Data analysis

Live-cell images were processed by using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The backgrounds of the images were subtracted by using ImageJ, and particle movements in the images were tracked by using the custom-written ImageJ plugin "PTA" (https://github.com/arayoshipta/projectPTAj) developed by Yoshiyuki Arai. The plugin determined the x–y position of each particle by two-dimensional Gaussian fitting with the Levenberg-Marquardt method and performed particle tracking by using a nearest-neighbor algorithm. The x–y position information (i.e., the trajectory of movement) and the MSD against time [$\rho(\Delta t)$], which is a convenient quantitative measure of stochastic movement, were given by the plugin. We extracted those trajectories that we were able to track for 3 s (30 frames). To analyze the movement of the silica particles, we calculated the α-coefficients of the MSD curves until $\Delta t = 2.5$ s (25 frames) by fitting to a power law, $\log [\rho(\Delta t)] = \alpha \log [(\Delta t)] + \zeta$, where α is the α-coefficient and ζ is a