Endocytosis, intracellular transport, and exocytosis of lanthanide-doped upconverting nanoparticles in single living cells

Yun Mi Bae a,b,1, Yong Il Park c,1, Sang Hwan Nam a,1, Jeong Hyun Kim c, Kyunghee Lee a, Hyung Min Kim a,2, Byeongjun Yoo c, Joon Sig Choib, Kang Taek Lee a,*, Taeghwan Hyeon c,*, Yung Doug Suh a,***

* Laboratory for Advanced Molecular Probing (LAMP), Research Center for Convergence Nanotechnology, Korea Research Institute of Chemical Technology, Daejeon 305-600, Republic of Korea
** World Class University (WCU) Program of Chemical Convergence for Energy & Environment (C2E2), School of Chemical and Biological Engineering, Seoul National University, Seoul 136-702, Republic of Korea
*** Laboratory for Advanced Molecular Probing (LAMP), Research Center for Convergence Nanotechnology, Korea Research Institute of Chemical Technology, Daejeon 305-600, Republic of Korea

A R T I C L E   I N F O

Article history:
Received 31 July 2012
Accepted 17 August 2012
Available online 13 September 2012

Keywords:
Nanoparticle
Image analysis
Fluorescence
Molecular imaging
Cytotoxicity

A B S T R A C T

Lanthanide-doped upconverting nanoparticles (UCNPs) have recently attracted enormous attention in the field of biological imaging owing to their unique optical properties (near-infrared excitation followed by photoluminescence in the visible spectral range). For biological applications, it is critical to understand the interaction between these nanoparticles and biological systems at the cellular level. In this paper, using epi-fluorescence microscopy with 980-nm excitation, a full intracellular pathway composed of endocytosis, active transport, and exocytosis was clearly visualized for PEG-phospholipid-coated UCNPs in single HeLa cells, which was experimentally feasible mostly thanks to the excellent photostability and low cytotoxicity thereof. Each step in the pathway was characterized and identified by various chemical inhibition studies and spectroscopic measurements.

1. Introduction

The biological applications of photonic nanomaterials have been the major research subjects in the area of nanotechnology and biomedicine [1]. In particular, thanks to the advances in the synthesis and surface conjugation technology, various types of nanomaterials with excellent optical properties and biological functions have been developed and are widely employed as imaging probes and diagnostic/therapeutic agents [2,3]. At the cellular level, one is mainly interested in observing the interaction of nanomaterials with the cellular components such as plasma membrane and various organelles in the cytoplasm. In order to understand such interactions in detail, it is crucial to first obtain the microscopic information on the mechanisms of uptake and intracellular pathways of nanomaterials, which strongly depends on the size, shape, charge, and surface composition of nanomaterials [4,5]. There have been a myriad of live-cell imaging studies employing various types of nanomaterials, semiconductor nanocrystals (quantum dots) being the most popular systems [6–8]. Recently, lanthanide ion-doped upconverting nanoparticles (UCNPs), which emit in the visible spectral range upon absorption of near-infrared (NIR) photons, have attracted great interest owing to their unique optical properties and potential applications such as biological imaging probes, sensors, and diagnostic/therapeutic agents [9–13]. In particular, UCNPs were proven to be ideal nanoprobes for live-cell imaging since (1) they do not exhibit photobleaching and photodamage [14,15], (2) the cytotoxicity is very low [14,16], and (3) NIR employed for excitation hardly induces cellular autofluorescence and photo-damage [14,17]. In our previous study, we demonstrated the real-time tracking of UCNPs (hexagonal NaYF4:Yb3+,Er3+) in living HeLa cells at the single-vesicle level [17]. Thanks to the photostability of UCNPs and noninvasiveness of NIR excitation, we could acquire real-time movies as long as 6 h at the frame rate of 20 fps, in which the dynamics of UCNPs transports by intracellular motor proteins was clearly visualized. In the current study, we followed the spatiotemporal distribution or intracellular pathways of endocytosed UCNPs in single living HeLa cells on