International Journal of Pharmaceutics 523 (2017) 217–228

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Enhanced cellular uptake and gene silencing activity of siRNA using temperature-responsive polymer-modified liposome

Jian Wang, Eri Ayano, Yoshie Maitani, Hideko Kanazawa*

Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato, Tokyo 105-8512, Japan

ARTICLE INFO

Article history:
Received 6 January 2017
Received in revised form 6 March 2017
Accepted 18 March 2017
Available online 19 March 2017

Keywords:
siRNA delivery temperature-responsive liposome
NIPAAm
Gene silencing
Cationic liposome
Cellular uptake

ABSTRACT

Short interfering RNA (siRNA) delivery systems using nanoparticle carriers have been limited by inefficient intracellular delivery. One drawback is the poor cellular uptake of siRNA/particle complexes through the plasma membrane and release of the nucleic acids into the cytosol. In this study, to develop the temperature-responsive liposome as a novel carrier for siRNA delivery, we prepared lipoplexes and assessed cellular uptake of siRNA and gene silencing activity of target genes, compared with those of a commercial transfection reagent, Lipofectamine RNAiMAX, and non-modified or PEGylated liposomes. The temperature-responsive polymer, *N*-isopropylacrylamide-*co*-*N*,*N*'-dimethylaminopropylacrylamide [P(NIPAAm-*co*-DMAPAAm)]-modified liposome induced faster intracellular delivery because P(NIPAAm-*co*-DMAPAAm) exhibits a lower critical solution temperature (LCST) changing its nature from hydrophilic to hydrophobic above the LCST. The temperature-responsive liposomes showed significantly higher gene silencing activity than other carriers with less cytotoxicity. Furthermore, we showed that the temperature-responsive lipoplexes were internalized mainly via microtubule-dependent transport and also by the clathrin-mediated endocytosis pathway. This is the first report that temperature-responsive polymer-modified liposomes thermally enhanced silencing activity of siRNA. The dehydrated polymer on the liposomes, and its aggregation caused around the LCST, can probably be attributed to effective cellular uptake of the lipoplexes for gene silencing activity by interaction with the cell membrane.

1. Introduction

Gene-based therapies have been extensively investigated to treat diseases by delivering nucleic acids into cells to induce or silence specific gene expression (Fire et al., 1998). Cationic liposomes are the most common lipid-based nanocarriers used for non-viral delivery of nucleic acids (small interfering RNA (siRNA), plasmid DNA (pDNA), oligonucleotides, etc.) since the cationic charge can complex the anionic gene fragment and enhance cellular uptake. Surface coating with hydrophilic polymers, such as polyethylene glycol (PEG), to reduce excessive interaction seems very effective for stabilizing liposomes in the blood stream (Amoozgar and Yeo, 2012; Bai et al., 2013; Vllasaliu et al., 2014). The importance of PEGylated liposomes is growing for gene delivery in vivo. Yet, in many cases, PEGylated liposomes show lower transfection efficiency due to reduced contact between the lipid component and the cell membrane, or inhibition of endosomal release (Leus et al., 2014; Remaut et al., 2007; Santel et al., 2006).

To overcome these problems, conditionally cleavable PEG-modified liposomes have been designed that respond to changes of local pH and enzymes (Hatakeyama et al., 2007; Kirpotin et al., 1996). Another approach has focused on the use of alternative polymers, such as pH-sensitive and temperature-responsive polymers. The traditional temperature-responsive liposomes composed of temperature-responsive lipids Dipalmitoylphosphatidylcholine (DPPC), and the liposomes show the greatest permeation of the lipid membrane at its gel-to-liquid crystal phase transition temperature (Anyarambhatla and Needham, 1999). Among them, temperature-responsive polymer-modified liposomes have advantages for both cellular uptake and release of liposomes because of the reduced hydrated layer in response to temperature changes (Wang et al., 2017). Indeed, recently a temperature-responsive biopolymer, elastin-like polypeptide modified liposome, was reported to show enhanced cellular uptake following the dehydration of biopolymer molecules on the liposomal surface (Na et al., 2012).

* Corresponding author.
E-mail address: kanazawa-hd@pha.keio.ac.jp (H. Kanazawa).

http://dx.doi.org/10.1016/j.ijpharm.2017.03.035
0378-5173/

Poly(*N*-isopropylacrylamide) (PNIPAAm) is a well-known temperature-responsive polymer, and exhibits a lower critical solution temperature (LCST) at 32 °C in aqueous solution by thermotropic coil-to-globule conformational transition of the grafted NIPAAm chains at the LCST, as a result of breakage of hydrogen bonds between the copolymer segments and water molecules (Duan et al., 2006; Gil and Hudson, 2004). The LCST can be modulated by copolymerization with other monomers (Hiruta et al., 2015; Yamada et al., 2015). PNIPAAm-copolymer-modified liposomes have been mostly reported for triggered release of liposomal drugs (Kono et al., 1999). However, in regard to thermally triggered cellular uptake, a few studies including the polymer complexed with nucleic acids have been reported; NIPAAm and 2-(dimethylamino) ethyl methacrylate (DMAEMA)/pDNA complex (Hinrichs et al., 1999), NIPAAm-conjugated siRNA, quantum dots, cell-penetrating peptide system (Kim et al., 2010) and PNIPAAm-*b*-PAMPTMA (poly((3-acrylamidopropyl)trimethylammonium chloride)) diblock copolymer/siRNA complexes (Cardoso et al., 2014). To the best of our knowledge, no reported studies have explored gene delivery using PNIPAAm copolymer-modified liposomes.

We synthesized a copolymer, poly(NIPAAm–*co*-*N*,*N*'-dimethylaminopropylacrilamide), P(NIPAAm-*co*-DMAPAAm) with a lipid anchor, which exhibits LCST at approximately 40 °C in aqueous solution, and prepared its modified *N*-[1-(2,3-Dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methyl-sulfate (DOTAP)/L-α-phosphatidylethanolamine, dioleoyl (DOPE) liposomes as temperature-responsive liposomes (Fig. 1). Since temperature-responsive liposomes alone showed enhanced cellular uptake at 37 °C, the liposomes with temperature-triggered tunable surface properties had the strong potential for a gene delivery carrier.

In this study, to develop temperature-responsive liposomes as a novel carrier for siRNA delivery, we prepared lipoplexes and

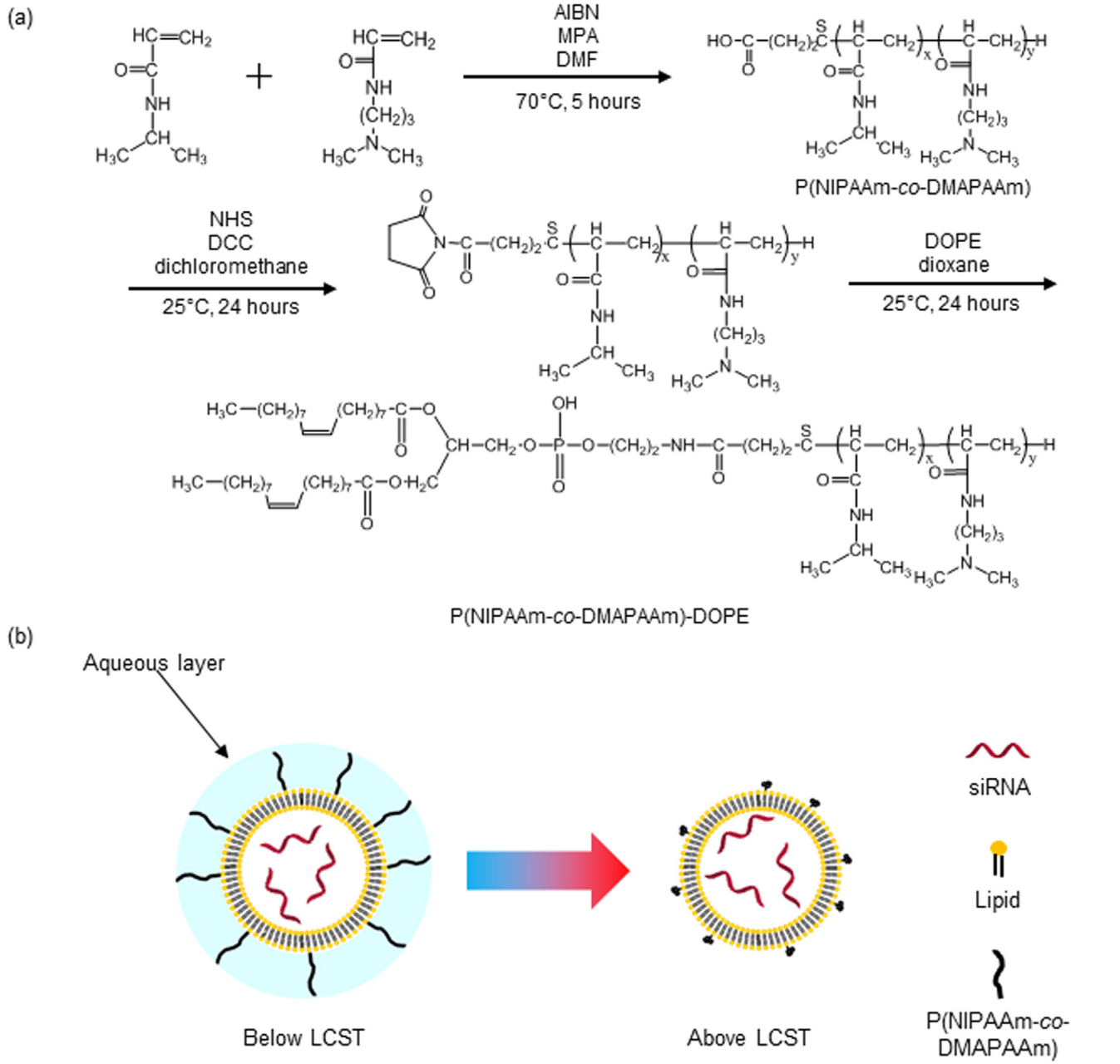

Fig. 1. The synthesize scheme of temperature-response polymer, P(NIPAAm-co-DMAPAAm) conjugation to DOPE (a), and temperature-induced property change of temperature-response liposomes across its LCST (b).