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Sugar-appended polyamidoamine dendrimer conjugates with cyclodextrins as cell-specific non-viral vectors ☆

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ABSTRACT

The widespread use of various cyclodextrin (CyD)-appended polymers and polyrotaxanes as gene carriers has been reported. Among the various polyamidoamine dendrimer (dendrimer) conjugates with CyDs (CDE), the dendrimer (G3) conjugate with α-CyD having an average degree of substitution (DS) of 2.4 (α-CDE (G3, DS 2)) displayed remarkable properties as DNA carriers. In an attempt to develop cell-specific gene transfer carriers, we prepared some sugar-appended α-CDEs, e.g. mannosylated, galactosylated, and lactosylated α-CDEs. In addition, PEGylated Lac-α-CDEs (G3) were prepared and evaluated as a hepatocyte-selective and serum-resistant gene transfer carrier. Moreover, PEGylated-α-CDE/CyD polypseudorotaxane systems for novel sustained DNA release system have been developed. Interestingly, glucronylglucosyl-β-cyclodextrin (GUG-β-CyD) conjugates with dendrimer (G2) (GUG-β-CDE (G2)) had superior gene transfer activity to α-CDE (G2), expecting a development of new series of sugar-appended CDEs over α-CDEs (G2). Collectively, sugar-appended α-CDEs have the potential as novel cell-specific and safe carriers for DNA.

Contents

1. Introduction

Gene therapy is emerging as a potential strategy for the treatment of genetic diseases, cancers, cardiovascular diseases and infectious diseases [1]. Their use has proceeded rapidly from transfection of cell cultures to clinical gene therapy applications. Non-viral gene therapy is challenged by inefficient delivery at the level of intracellular processing. Several barriers have been described and studied, including failure to escape from vesicular structures, lysosomal degradation, enzymatic degradation in the cytosol, entrapment in the highly viscous and crowded cytosol, lack of transport towards the nucleus and uptake into the nucleus, and finally inefficiency of transcription and/or translation [2]. Among clinical trials employing approximately 1800 gene therapy protocols performed for various diseases up to 2012, the percentages of lipofection and naked DNA were 6.2% and 18.6%, respectively. However, the polyfection has never been officially applied to clinical gene therapy. Nonetheless, the widespread use of a variety of polymers for gene transfer has been made, because several advantages of the polyfection method are

Abbreviation: α-CyD, α-cyclodextrin; PAMAM, polyamidoamine; α-CDEs, dendrimer conjugates with α-CyD; Gal-α-CDE, galactose-appended α-CDE; pDNA, plasmid pDNA; ASGPR, asialoglycoprotein receptor; CyDs, cyclodextrins; β-CyD, β-cyclodextrin; CDP, β-CyD-containing polycations; PEI, polyethyleneimine; PLL, poly-L-lysine; LPS, lipopolysaccharide; MR, mannose receptor; FR, folate receptor; GlcNAc, N-acetyl-D-glucosamine; GalNAc, N-acetylgalactosamine; BSA, bovine serum albumin; TTR, transthyretin; ATTR, amyloidogenic transthyretin; Fuc-R, fucose receptor.

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known, *e.g.* multifunctions, biocompatibility, facile manufacturing, and robust and stable formulation [3]. Of polymers, cationic polymers include poly-L-lysine (PLL), polyethyleneimine (PEI), polyamidoamine starburst dendrimers (dendrimers), natural histones, synthetic polypeptides, 2-dimethylaminoethyl methacrylate, or carbohydrate-based polymers such as chitosan [4,5]. Since most of them are synthetic compounds, the molecular weight can be modified, and ligands and antibodies can be attached to them.

Dendrimer is a spherical, highly ordered, dendritic polymer with positively charged primary amino groups on the surface at physiological pH [6]. Dendrimers have been widely used for biomedical applications, including drug delivery, tissue engineering, gene transfer, and contrast enhancement for magnetic resonance imaging [7]. Gene transfer activity of dendrimers with high generation is acknowledged to be superior to those with a low generation. For example, gene transfer activity was shown by increasing the generation from G5 and G10, with a plateau in activity after G8 [8]. Additionally, maximal transfection efficiency was obtained using dendrimer (G6) rather than dendrimer with higher generation [9], possibly due to the rigid structure and cytotoxicity of dendrimers with more than dendrimer (G7). Thus, dendrimers with lower generations (generations 1 to 3) do not have efficient gene transfer activity, whereas dendrimers with higher generations exhibit cytotoxicity [8]. Wang et al. [10] demonstrated that dendrimers (G4) are a novel candidate of nanocarrier for gene delivery agents in breast cancer treatment. Nevertheless, a major concern with the use of dendrimers as vectors for gene delivery is their cytotoxicity, which may be due to the interaction between the positively charged dendrimer and the negatively charged cellular surface [11]. Actually, intravenous administration of dendrimers to mice was lethal, causing a disseminated intravascular coagulation-like condition through the reason why dendrimers (G7) activate platelets and dramatically alter their morphology and attenuate platelet-dependent thrombin generation [12]. Additionally, the anti-inflammatory effects of dendrimers have been reported [13]. Thus, we must pay attention to these unexpected issues for the use of dendrimers and the preparation of the new dendrimer conjugates and derivatives.

Cyclodextrins (CyDs) were first isolated approximately 100 years ago and were characterized as cyclic oligosaccharides of α-D-glucopyranose containing a hydrophobic central cavity and hydrophilic outer surface [14,15]. CyDs are known to form inclusion complexes with a variety of guest molecules in solution and in a solid state. The solubilization of lipophilic compounds by CyDs has many uses in the pharmaceutical fields [16]. In the cell biology fields, CyDs at higher concentration induce hemolysis and decrease the integrity of the mucosal epithelial cells and blood brain barrier (BBB) [17–19], and extract cholesterol, phospholipids and proteins by CyDs from biological membranes, which are useful for investigating the function of caveolae, lipid rafts, and cholesterol transporter [20,21]. Interestingly, 2-hydroxypropyl-β-CyD (HP-β-CyD) is used for the treatment of Niemann–Pick type C disease utilizing its cholesterol extraction ability [22,23]. Meanwhile, it is acknowledged that CyDs interact with DNA and native oligonucleotides only very slightly. Therefore, the potential of CyDs as carriers for DNA and oligonucleotides on the basis of their direct interaction would not be expected. Based on this idea, the alternative use of CyDs for carriers of DNA and oligonucleotides has been required.

The widespread use of various CyD-appended polymers and polyrotaxanes as gene carriers has been reported, *e.g.*, cationic star polymers consisting of α-CyD core and oligoethyleneimine arms [24], polypropyleneimine (PPI) dendrimer graft β-CyD [25], low molecular weight PEI cross-linked by HP-β-CyD or 2-hydroxypropyl-γ-CyD (HP-γ-CyD) [26], low molecular weight PEIs linked by β-CyD [27], linear PEI through γ-CyD and biocleavable polyrotaxane [28], cationic supramolecules consisting of oligoethyleneimine-grafted α-CyDs [29], chitosan/CyD nanoparticles for the airway epithelium [30], receptor-mediated, tumor-targeted gene delivery using folate-terminated polyrotaxanes [31], CY11 peptides, which have been proven to combine especially with fibroblast growth factor receptors on cell membranes, coupled low-molecular-weight (600 Da) PEI crosslinked to β-CyD [32], water-soluble chitosan-graft-(PEI-β-CyD) (CPC) cationic copolymers [33], and a transferrin-modified, CyD polymer-based gene delivery system [34]. It is noted that Davis and co-workers have reported a number of uses of β-CyD-containing polycations (CDP) with adamantine-polyethethylene glycol (PEG) or adamantine-PEG-transferrin for gene [35–41], DNAzyme [42] and siRNA [43–47] delivery. Prominently, the targeted, nanoparticle formulation of siRNA, denoted as CALAA-01, consists of a CDP, a PEG steric stabilization agent, and human transferrin as a targeting ligand for binding to transferrin receptors that are typically upregulated on cancer cells. The four-component formulation is self-assembled into nanoparticles in the pharmacy and administered intravenously to patients [43]. A CALAA-01 complex with siRNA to M2 subunit of ribonucleotide reductase (R2) is used in the first-in-human phase I clinical trial involving the systemic administration of siRNA to patients with solid cancers. It provides evidence of inducing an RNAi mechanism of action in a human from the delivered siRNA [48]. Excellent reviews and books on this subject have appeared in recent years [49–53]. The objective of this review is to focus on the potential use of the dendrimer conjugates with CyDs (CDEs) as high performance DNA carriers, especially sugar-appended CDEs. The siRNA delivery using CDEs has been reviewed [54].

2. Parent cyclodextrin/dendrimer conjugates (CDEs) as DNA carriers

Design criteria for non-viral vectors are protection of DNA packing of large DNA, easy administration, serum stability, targetability to specific cell types, ease of fabrication, inexpensive synthesis, facile purification, robust stability, internalization, endolysosomal escape, nuclear transport, efficient unpackaging, infection of non-dividing cells, safety, non-toxic, non-immunogenic, non-pathogenic and adequate pharmacokinetic properties [55]. Ordinarily, cationic polymers are lacking of a hydrophobic domain, and thereby unable to fuse/destabilize the endosome by direct interaction with the endosomal membrane, causing low gene transfer activity [56]. Actually, the first-generation cationic polymers, such as PLL, were quite inefficient regarding endosomal escape and gene transfer activity. Meanwhile, second generation cationic polymers, such as PEI and dendrimers, can enhance endosomal escape through the proton sponge effects. Thus, endosomal escaping ability of polymers is inevitable for high gene transfer activity.

Arima and colleagues have developed various CDEs, and the chemical structures are shown in Fig. 1. Firstly, Arima et al. [57] reported three CDEs (G2, G3, G4) with α-, β- and γ-CyD at a molar ratio of 1:1 (dendrimer:CyD). Of the CDEs, dendrimer (G2) conjugate with α-CyD (α-CDE (G2)) elicited luciferase gene transfer activity approximately 100 times higher than dendrimers (G2) or non-covalent mixtures of dendrimer (G2) and α-CyD, when pDNA encoding luciferase gene was used [57]. Herein, CyDs were used due to their prospective endosomal disrupting effects through the release of membrane components from endosomal membranes after endocytosis, cooperating with the proton sponge effect of dendrimer in the α-CDE (G2) molecule. This endosomal escaping ability of α-CDE (G2) was estimated from their hemolytic activity, liposomal membrane-disruptive effect and intracellular distribution [58,59]. Next, Kihara et al. [59,60] performed the optimization study of the chemical structure of CDEs, demonstrating that α-CDE (G3) with a degree of substitution (DS) of 2.4 (α-CDE (G3, DS 2)) was clarified to have best transfection efficiency with low cytotoxicity, *i.e.* gene transfer activity of α-CDE (G3, DS 2) was found to be superior to that of TransFast™ (TF) and Lipofectin™ (LF), commercially-available transfection regents. Also, α-CDE (G3, DS 2) exhibited a high efficient RNAi effect for a novel carrier for short hairpin RNA (shRNA) [61]. Moreover, Arima et al. [62] revealed that α-CDE (G3, DS 2) has the potential as a siRNA carrier against luciferase gene [63] and endogenous genes. The potential use of α-CDE (G3, DS 2)