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Generation of highly potent nonviral gene vectors by complexation of lipoplexes and transferrin-bearing fusogenic polymer-modified liposomes in aqueous glucose solution

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Abstract

We reported previously that complexation of lipoplexes containing 3,5-dipentadecyloxybenzamidine (TRX-20) and transferrin-bearing succinylated poly(glycidol) (SucPG)-modified liposome, which becomes fusogenic under weakly acidic conditions, might produce gene carriers with high transfection activity. For the present study, we prepared the lipoplex–SucPG-modified liposome complexes by mixing them either in phosphate-buffered saline or in an aqueous 5% glucose solution. The complexes prepared in phosphate-buffered saline have large particles of more than 800 nm, whereas the complexes prepared in the glucose solution were remarkably small: 200–300 nm. The small complexes were taken up more effectively by HeLa cells, and their transfection was induced more efficiently than the large complexes'. In addition, the small complexes achieved cellular transfection more efficiently in the presence of serum than in the absence of serum, without marked cytotoxicity. Considering that their affinity to the cell is based on ligand–receptor interaction, the small complexes are highly promising as a safe vector with high transfection activity and high target cell specificity.

Keywords: Nonviral vector; Liposome; Lipoplex; Gene therapy; Transferrin; Fusion

1. Introduction

Numerous efforts have been made to develop efficient vectors for gene therapy [1–3]. Currently, recombinant viruses are used for this purpose because of their high transfection activity. However, the use of nonviral vectors is still desired from the viewpoint of safety. Lipoplexes and polyplexes, which are respective complexes of cationic liposomes and cationic polymers with DNA, are currently viewed as promising nonviral gene vectors, although their activity must be improved. For that reason, development of a new strategy for the production of potent nonviral gene vectors is highly desired.

The key processes that control efficiency of gene transfection mediated by nonviral vectors are becoming apparent along with the features and functions that vectors should possess for increasing their transfection activity [4–6]. For example, binding of vector–gene complexes to cellular membranes is the first process during vector–gene complex-mediated transfection. Similarly to lipoplexes and polyplexes, most nonviral vector–gene complexes have positively charged properties, which exhibit strong affinity to cell surface and engender cellular transfection. However, for the same reason, these complexes present some disadvantages, such as difficulty for the cell-specific gene delivery, strong cytotoxicity, and lack of serum-resistance. Accordingly, a neutral or negatively charged surface and the use of a target cell-specific ligand might be preferable to solve these problems.

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In addition to the surface properties, possession of functions that control intracellular trafficking of genes is important for vectors to achieve efficient transfection. The major pathway through which vector–gene complexes are taken up by cells is considered to be endocytosis [4–7]. Therefore, after internalization into endosome, ideally vectors should escort the gene to its final destination, the nucleus. For this purpose, various capabilities have been given to vectors, such as those for endosome-to-cytoplasm transfer [8–10], localization at nucleus [11–13], and controlled release of gene in cytoplasm [14–16]. Among them, it is particularly important for the vectors to possess an ability to transfer from endosome into cytoplasm because otherwise vector–gene complexes are delivered to lysosome and degraded by lysosomal enzymes [4,5].

To date, various methods have been explored to enhance the endosomal escape of the entrapped gene. Incorporation of membrane-destabilizing molecules to gene vectors has been used frequently to destabilize endosomal membrane and improve their transfection activity [8–10,17,18]. In fact, membrane-disruptive peptides, such as viral fusion peptides, and synthetic polymers, were shown to enhance polyplex-mediated [19–21] and lipoplex-mediated [9,18,22,23] transfection of cells. However, incorporation of membrane-active molecules into vector–gene complexes does not always increase their transfection activity efficiently. Reportedly, membrane-active peptides derived from influenza virus hemagglutinin greatly increased lipospermine/DNA complexes with low transfection activity, but only slightly affected the activity of the complex with optimal composition [8]. Therefore, attachment of membrane-active molecules might not induce significant destabilization of endosome for the efficient transfer of huge vector–gene complexes.

The proton sponge effect induced by polymers containing secondary and tertiary amines has also been used to promote endosomal escape of genes [24,25]. Poly(ethylenimine) (PEI) and poly(amidoamine) (PAMAM) dendrimers achieve efficient transfection through the proton sponge effect. However, introduction of membrane-destabilizing molecules, such as melittin and cyclodextrins, to PEI [26,27] and PAMAM dendrimers [28,29] were reported to increase their transfection activity, which indicates that the proton sponge effect induced by these polymers is insufficient to cause efficient escape of genes from endosome [27,30].

For lipid-based gene vectors, induction of fusion with endosome is particularly important to promote transfer of gene into cytosol and increase transfection efficiency [31]. In fact, results of many studies have shown that the fusion process plays an important role in efficient transfection mediated by lipoplexes [32–35]. In addition, it is considered that dissociation of genes from lipoplexes might take place during fusion between the lipoplexes and the endosomal membranes by forming a charge-neutral ion pair between the cationic lipids and anionic lipids of the endosomal membrane [36–39]. Therefore, providing vectors with high fusion ability might develop efficient vectors.

For a previous study [40,41], we prepared complexes of lipoplexes and liposomes modified with transferrin-bearing succinylated poly(glycidol) (SucPG), which generate fusogenic activity at mildly acidic pH (Fig. 1) [42,43]. These complexes, which are termed SucPG complexes, were designed to achieve cell transfection through efficient internalization into cells through transferrin receptor-mediated endocytosis and subsequent release of DNA into the cytoplasm by fusion with endosomal membrane [40]. These complexes have a structure in which negatively charged SucPG-modified liposomes are associated with positively charged lipoplexes through electrostatic interaction. Transfection activity of 3β-(N-(N′,N′-dimethylaminoethane)carbamoyl)cholesterol (DC-chol) [40] and 3,5-dipentadecyloxybenzamidine (TRX-20) [41,48] lipoplexes was actually enhanced by complexation with SucPG-modified liposomes.

Because many types of proliferating malignant cells overexpress transferrin receptor, transferrin is considered to be a promising ligand for the delivery of genes to cancer cells [44,45]. Transferrin–transferrin receptor complexes are known to be routed into the endosomal compartment after endocytosis via clathrin-coated pits, which is the process for

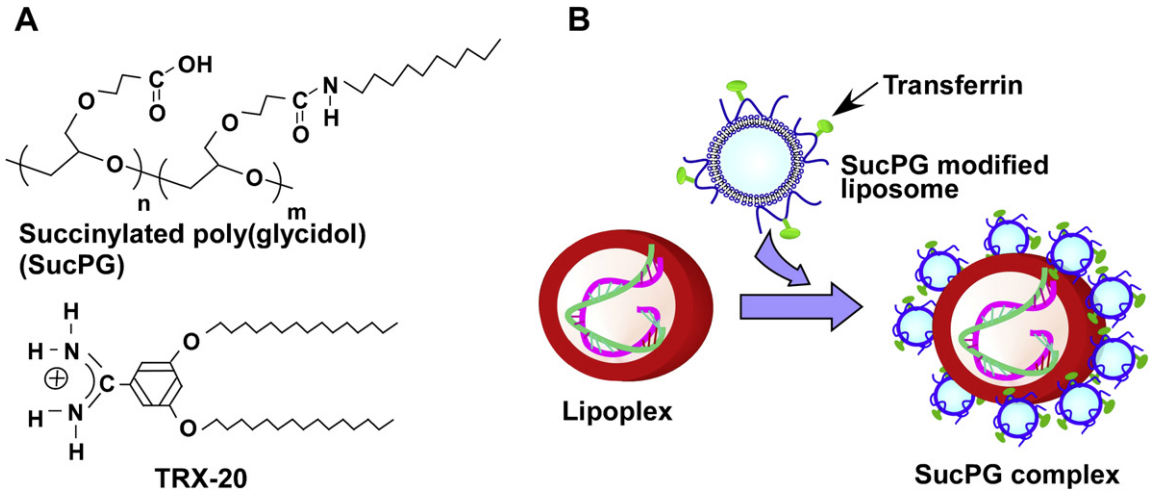

Fig. 1. (A) Structures of SucPG and TRX-20. (B) Preparation of SucPG complex bearing transferrin.