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GENE DELIVERY

Enhanced nuclear import and transfection efficiency of plasmid DNA using streptavidin-fused importin-β

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Abstract

In order to enhance the nuclear import of exogenous genes, novel plasmid DNA/importin-β conjugates, which consist of a biotinylated plasmid DNA and a recombinant streptavidin-fused importin-β, were prepared. The spacer length between plasmid DNA and biotin and the number of introduced biotin were adjusted. The microinjection of plasmid DNA/importin-β conjugates into the cytoplasm of NIH3T3 cells resulted in the nuclear localization of conjugates and the higher expression efficiency, compared to intact plasmid DNA alone. These results indicate that plasmid DNA/importin-β conjugates would be an important tool to enhance the nuclear localization of exogenous DNA in non-viral gene delivery system.

Keywords: Importin; Gene delivery; Non-viral vector; Nuclear import; Transfection

1. Introduction

Current gene therapy strategies are depending on gene transfer by either viral vectors or non-viral vectors. Viral vectors, often constructed from retrovirus or adenovirus, are highly efficient in the transfer of genes and their subsequent expression of these genes by utilizing viral infection mechanism. In fact, use of viral vectors as a vehicle for

Abbreviations: PEG, poly(ethylene glycol); FBS, fetal bovine serum; PBS, phosphate-buffered saline; aa, amino acids; LB medium, Luria–Bertani medium; GFP, green fluorescent protein; GST, glutathione *S*-transferase; NPC, nuclear pore complex; NLS, nuclear localization signal; WGA, wheat germ agglutinin; RLU, relative light unit.

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gene transfer has already been widely applied in humans (reviewed in Refs. [1–4]). However, serious accidents involving viral gene therapies have been reported, such as one incident in the United States (1999) [5] and another in France (2002) [6]. Therefore, a great deal of effort has been directed towards the development of effective non-viral vectors, which are expected to be safer than viral vectors [7,8]. Since the transfection efficiency of non-viral vectors is much lower than that of viral vectors, often due to the limited nuclear import of the transgene, many studies have focused on overcoming this problem [9–13].

Protein transfer between the cytoplasm and the nucleus is facilitated by the nuclear pore complex (NPC) of the nuclear envelope [14–18]. While proteins with sizes up to about 40–60 kDa can freely diffuse through the nuclear pores, larger proteins require a nuclear localization signal (NLS) which is recognized by nuclear transport protein (e.g., importin-α and -β). To improve nuclear import efficiency, linear DNA or plasmid DNA have been modified with NLS-conjugates [19–22]. This strategy has resulted in highly efficient expression of linear DNA-NLS constructs, but not for plasmid DNA-NLS one [23,24]. While use of the NLS to improve transfection efficiency is promising, additional studies are required to validate its usability in non-viral gene delivery.

Our previous report has suggested that the charge interaction between anionic DNA and cationic NLS renders the NLS inaccessible by burying it in the DNA [23]. Furthermore, importin-β has frequently been shown to play an essential role in nuclear import by interacting with the NPC, and by delivering the bioactive compounds into the nucleus [25–27]. We therefore hypothesized that the direct conjugation of importin-β to the plasmid DNA may significantly increase its nuclear import efficiency. To test this hypothesis, we prepared plasmid DNA/importin-β conjugates consisting of biotinylated plasmid DNA and recombinant streptavidin-fused importin-β. In addition, we adjusted the spacer length between plasmid DNA and biotin and the number of biotin molecules introduced. We show that this novel conjugate could significantly contribute to the development of a highly efficient non-viral gene delivery system.

2. Materials and methods

2.1. Cell culture

NIH3T3 cells (mouse fibroblast) were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum at 37 °C with 5% CO_2 atmosphere. Cultured cells were plated on marked coverslips 24 h before use for nuclear import assays and protein expression.

2.2. Purification of recombinant proteins

The expression vector of the green fluorescent protein (GFP) chimeras of recombinant mouse importin-β, pGEX-2T-GFP-importin-β, was generated as described previously [16]. To construct the expression vectors of recombinant mouse importin-β (1–643 aa)-streptavidin (1–170 aa), the streptavidin (1–170 aa) fragment was obtained by cutting out the *Hin*dIII–*Eco*RI fragment from pGSH [28], purified with QIAquick Gel Extraction Kit (QIAGEN), and inserted into *Hin*dIII–*Eco*RI sites of pGEX-2T-GFP-importin-β.

Expression and purification of recombinant mouse importin-β (1–643 aa)-streptavidin (1–170 aa) mutant were performed as described previously [15,16]. Briefly, the expression vector of recombinant mouse importin-β fused with streptavidin was transformed into *Escherichia coli* strain BL21. The *E. coli* were grown in LB medium with 100 μg/ml ampicillin at 37 °C to a density of 1.0–1.5 (OD_{550}). The expression of recombinant streptavidin-fused importin-β was induced by 0.5 mM isopropyl-β-D-thiogalactopyranoside for 14 h at 20 °C and was purified with Glutathione Sepharose™ 4B and PD-10 column (Amersham Pharmacia Biotech).

2.3. Preparation of biotinylated and rhodamine-labeled plasmid DNA

Biotinylated plasmid DNAs of pGL3-control and pBESTluc (Promega) and pDsRed-2-Nuc (Clonetech) were prepared by diazo-coupling method as shown in Scheme 1 and purified with ultrafiltration (Ultrafree-MC; norminal molecular weight limit, 10 kDa; Millipore) as reported previously [23]. The short spacer was introduced to plasmid DNA using the *Label* IT™