International Journal of Pharmaceutics 441 (2013) 146–150

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Enhanced transgene expression from chromatinized plasmid DNA in mouse liver

Hiroyuki Kamiya[a,b,c,d,*], Shiho Miyamoto[a], Hitomi Goto[b], Genki N. Kanda[a,b], Miwako Kobayashi[c], Ichiro Matsuoka[c], Hideyoshi Harashima[b,d]

[a] Graduate School of Science and Engineering, Ehime University, 2-5 Bunkyo-cho, Matsuyama 790-8577, Japan
[b] Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan
[c] College of Pharmaceutical Sciences, Matsuyama University, 4-2 Bunkyo-cho, Matsuyama 790-8578, Japan
[d] CREST, Japan Science and Technology, Japan

ARTICLE INFO

Article history:
Received 13 September 2012
Received in revised form 30 October 2012
Accepted 5 December 2012
Available online 12 December 2012

Keywords:
Exogenous DNA
Histone
Nucleosome
Intranuclear disposition
Expression efficiency

ABSTRACT

Plasmid DNA was chromatinized with core histones (H2A, H2B, H3, and H4) *in vitro* and was delivered into mouse liver by hydrodynamics-based administration. Transgene expression from the chromatinized plasmid DNA was more efficient than that from plasmid DNA delivered in the naked form. The use of acetylation-enriched histones isolated from cells treated with a histone deacetylase inhibitor (trichostatin A) seemed to be more effective. These results indicated that chromatinized plasmid DNA is useful for efficient transgene expression *in vivo*.

1. Introduction

Plasmid DNA delivered with nonviral vectors binds to histones, which are the principal protein components of chromatin, to form nucleosomes (Hebbar and Archer, 2008; Reeves et al., 1985). The nucleosome core particle consists of ~147 bp of DNA wrapped around a histone octamer, containing two molecules each of H2A, H2B, H3, and H4 (Luger et al., 1997). The binding of histones generally limits the access of transcription factors to their recognition sites. Thus, the regulation of histone binding to the exogenous DNA would be an important factor for transgene expression, and hence for the intranuclear disposition of the plasmid DNA (Kamiya et al., 2003). Indeed, transgene expression was influenced by the introduction of DNA sequences that modulate histone dynamics into plasmid DNAs (Fukunaga et al., 2012; Kamiya et al., 2007, 2009; Nishikawa et al., 2003; Sumida et al., 2006).

Recently, histones have been used as vehicles for plasmid DNAs (Kaouass et al., 2006 and references therein). Moreover, Jans and co-workers reported transfection with reconstituted chromatin, obtained by mixing a histone octamer and plasmid DNA (Wagstaff et al., 2008). These reports suggested that the complexes of histone(s) and plasmid DNA are useful delivery tools. However, we previously showed that simply mixing plasmid DNA with a histone (H3) suppresses transgene expression after nuclear entry (Kamiya et al., 2010). Thus, the report by Jans and co-workers raised the question of whether chromatin-mediated gene delivery is advantageous, from the viewpoint of transgene expression efficiency in the nucleus.

In addition to binding to mammalian chromosomal DNA, various histone modifications, such as acetylation, methylation, phosphorylation, ubiquitination, and ADP-ribosylation, are believed to play a pivotal role in the modulation of chromatin structure and function ("histone code" hypothesis) (Strahl and Allis, 2000). The acetylation of core histones is a well-characterized modification. Highly acetylated histones are regarded as a hallmark of nucleosomes at active genes, while those at repressed genes are hypoacetylated (Turner, 2000). The acetylation is considered to influence gene expression by modifying the chromatin conformation and/or the recruitment of regulatory factors.

It would be quite interesting to study transgene expression efficiency from plasmid DNA chromatinized *in vitro*, since nucleosome positioning is involved in gene transcription from genomic DNA (Bai and Morozov, 2010) and chromatinized plasmid DNA has been used as a delivery tool (Wagstaff et al., 2008). To examine the efficiency of transgene expression from the plasmid DNA, we delivered plasmid DNA chromatinized with histones into mouse liver. Moreover, plasmid DNA chromatinized with acetylation-enriched histones was also delivered. The results obtained in this study

Abbreviations: TSA, trichostatin A; Q-PCR, quantitative PCR; RLU, relative light units; NLS, nuclear localizing signal.

* Corresponding author. Tel.: +81 89 927 9609; fax: +81 89 927 9590.
E-mail address: hirokam@ehime-u.ac.jp (H. Kamiya).

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2012.12.004

indicated that chromatinized plasmid DNA is useful for efficient transgene expression *in vivo*.

2. Materials and methods

2.1. Materials

Oligodeoxyribonucleotides were purchased from Life Technologies Japan (Tokyo, Japan) in purified forms. The pYK-CMV-luc plasmid DNA (Ochiai et al., 2006), containing the luciferase gene, was purified with a Qiagen (Hilden, Germany) EndoFree Plasmid Mega kit.

2.2. Animals

All mice were maintained in an environment with a controlled temperature and photoperiod (23 °C, 12-h light and 12-h dark), with food and water provided *ad libitum*. All experimental procedures followed the Guideline for Animals Experimentation prepared by the Animal Care and Use Committee of Matsuyama University.

2.3. Extraction and purification of core histones

Core histones (H2A, H2B, H3, and H4) were extracted from mouse Hepa 1-6 cells and then purified with an Active Motif Histone Purification Mini Kit (Carlsbad, CA). Acetylated histones were obtained from Hepa 1-6 cells treated with trichostatin A (TSA, 200 ng/mL) for 18 h.

The acetylation of histone H3 was confirmed by dot blotting, using an anti-acetyl histone H3 antibody (catalog number 39139; Active Motif) and an anti-histone H3 antibody (catalog number 07-690; Upstate Biotechnology, Lake Placid, NY, USA), and an ECL Western Blotting Starter Kit (GE Healthcare Bio-Sciences, Piscataway, NJ, USA).

The pYK-CMV-luc plasmid DNA (2.5 μg) was chromatinized with the extracted core histones (2.7 μg), using recombinant nucleosome assembly protein-1 (NAP-1) and the ATP-utilizing chromatin assembly and remodeling factor (ACF) complex (Chromatin Assembly Kit, Active Motif), according to the supplier's recommendations.

2.4. Hydrodynamics-based injection of chromatinized DNA

The chromatinized plasmid DNA (1.25 μg as DNA) in 2 mL of saline was injected into the tail vein of six week-old female Balb/c mice within 5 s, by hydrodynamics-based administration (Liu et al., 1999; Zhang et al., 1999). The livers were harvested from the injected mice at 24 h after injection, and the luciferase activity and the amount of the exogenous DNA were measured, as described below.

2.5. Luciferase activity

Livers were minced with scissors and homogenized completely in lysis buffer (100 mM Tris–HCl, 2 mM EDTA, 0.1% Triton X-100, pH 7.8). After centrifugation at 16,400 × *g* for 10 min at 4 °C, the supernatant was examined for luciferase activity, using a Luciferase Assay System kit (Promega, Madison, WI, USA).

2.6. Isolation of nuclear DNA and quantitative PCR

Livers were homogenized in extraction buffer (3 mM Tris–HCl, 0.1 mM EDTA, 250 mM sucrose, pH 7.4). After centrifugation at 800 × *g* for 5 min at 4 °C, the pellet was resuspended in DNA lysis buffer (10 mM Tris-HCl, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% (w/v) IGEPAL-CA630, pH 7.4) (Tachibana et al., 2002). After centrifugation at 1400 × *g* for 5 min at 4 °C, the pellet was washed twice with DNA lysis buffer. The intranuclear DNA was extracted with the SepaGene reagent (EIDIA, Tokyo, Japan).

Quantitative PCR (Q-PCR) was performed using a Bio-Rad CFX96 Real Time PCR System (Hercules, CA, USA), and EvaGreen chemistry (Bio-Rad). A portion of the recovered DNA was analyzed by Q-PCR. The *luciferase* gene in the mouse liver was detected using the following primers: luciferase upper, 5′-dGGTCCTATGATTATGTCCGGTTATG-3′; luciferase lower, 5′-dATGTAGCCATCCATCCTTGTCAAT-3′. Data were expressed as the ratio to the *Gapdh* gene, which was determined using the following primers: Gapdh upper, 5′-dTTGTGATGGGTGTGAACCAC-3′; Gapdh lower, 5′-dGTTGTCATGGATGACCTTGG-3′.

2.7. Statistical analysis

Statistical significance was examined by the Student's *t*-test. Levels of $P < 0.05$ were considered to be significant.

3. Results

3.1. Purification of core histones and nucleosome formation

We first isolated the core histones (H2A, H2B, H3, and H4) that constitute the nucleosome core particle, together with 147-bp of DNA, from mouse Hepa 1-6 cells. Histone acetylation is regulated by two families of evolutionarily conserved enzymes, histone acetyltransferases and histone deacetylases (Khochbin et al., 2001; Yang and Seto, 2007). We isolated acetylated histones from the same cell line after an 18 h treatment with a histone deacetylase inhibitor, TSA, for the chromatinization reactions. These conditions were determined by the ratio of acetylated histone H3 to H3 (Fig. 1A) and the cell viability (data not shown). We confirmed that the core histones isolated from the TSA-treated cells contained acetylated histone H3 (Fig. 1B).

We chromatinized the luciferase-plasmid DNA (pYK-CMV-luc, 8454 bp) (Ochiai et al., 2006), using a commercially available kit. Nucleosome assembly protein-1 (NAP-1) (Nakagawa et al., 2001), a histone chaperone, was incubated with the isolated core histones. The plasmid DNA was chromatinized by mixing the histone-NAP-1 solution with ATP-utilizing chromatin assembly and remodeling factor (ACF) (Nakagawa et al., 2001). Successful chromatinization was confirmed by partial nuclease digestion, followed by agarose gel electrophoresis. DNA fragment ladders indicate formation of nucleosomes (Fig. 1C). No difference was observed for plasmid DNAs chromatinized with control and acetylated core histones (lanes 2 and 5).

3.2. Luciferase expression from chromatinized plasmid DNA

We delivered the chromatinized plasmid DNA (1.25 μg as DNA) to the livers of female six week-old Balb/c mice by hydrodynamics-based administration (Liu et al., 1999; Zhang et al., 1999). The livers were harvested at 24 h after injection, and the luciferase activities and the amounts of the exogenous DNAs were quantitated.

As shown in Fig. 2, the luciferase expression from the chromatinized plasmid DNA was higher than that from the plasmid DNA delivered in the naked form (3.3×10^7 relative light units (RLU)/mg protein *vs.* 1.5×10^7 RLU/mg protein) with statistical significance ($P < 0.05$). Meanwhile, the injection of a plasmid DNA solution containing the core histones without the chromatinization reactions did not affect the expression (data not shown). Therefore, the *in vitro* chromatinization of the plasmid DNA increased the transgene expression in the mouse liver.