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Kinetic analysis of protein production after DNA transfection

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Abstract

The production of an exogenous protein by the transfection of a plasmid DNA encoding the protein was kinetically analyzed, to determine the efficiency of the transfection. Cultured NIH3T3 or HeLa cells, and the luciferase protein were used as a model system in this experiment. The findings indicate that at least a 8×10^4- and 4×10^3-fold molar amounts of luciferase protein was produced from one copy of the plasmid DNA molecule in NIH3T3 and HeLa cells, respectively. The rate of elimination of luciferase activity upon DNA transfection was smaller than that for the luciferase protein itself (k_{el} for DNA transfection $< k_{el}$ for the luciferase protein), suggesting that a decrease in intranuclear active DNA was the main determinant of the elimination rate in this case. A preliminary pharmacokinetic model is proposed, based on the results obtained.

Keywords: DNA transfection; AUC; Intranuclear disposition

1. Introduction

Gene delivery is a promising approach and considerable efforts have been made to improve the efficiency of protein production (Mahato et al., 1997; Rolland, 1998; Kamiya et al., 2001; Niidome and Huang, 2002). To achieve successful protein production, an efficient and targeted gene delivery system in which intracellular trafficking is also considered is necessary. In addition, protein production from exogenous genes is a transient phenomenon in most cases of non-viral vectors. We previously proposed that the 'controlled intranuclear disposition' of delivered genes would also be highly important for achieving practical gene therapy (Kamiya et al., 2003). Thus, it would be both interesting and important to evaluate protein production as the result of DNA transfection in a quantitative manner.

In this study, we report on the analysis of protein production in cultured mammalian cells by DNA transfection with cationic lipids, in an attempt to better understand the kinetic features that are involved. The luciferase protein was chosen as a model protein because its activity decreases relatively rapidly (Nguyen et al., 1989; Thompson et al., 1991) and

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this characteristic would be suitable for the analysis. Based on our kinetic analysis, it was estimated that huge amount of luciferase protein could be produced from one copy of the plasmid DNA molecule in mouse NIH3T3 cells. The rate of elimination of luciferase activity when DNA transfection was used was much smaller than that for the protein itself, suggesting that a decrease in intranuclear active DNA was a major factor in the rate of elimination of luciferase activity. Similar tendencies were obtained with HeLa cells. A preliminary pharmacokinetic model is proposed, based on the results obtained in this study.

2. Experimental

2.1. Materials

The firefly (*Photinus pyralis*) luciferase protein (molecular weight: 1.2×10^5) was obtained from Sigma–Aldrich (St. Louis, MO, USA). The pcDNA 3.1 (+)-luc 2 plasmid (7037 bp) was constructed by inserting the firefly luciferase gene (*Hin*dIII–*Xba*I fragment) of the pGL3-Control plasmid (Promega, Madison, WI, USA) into the pcDNA 3.1 (+) plasmid (Invitrogen, Groningen, the Netherlands) pretreated with the same restriction enzymes. The luciferase gene in the pcDNA 3.1 (+)-luc 2 plasmid is expressed under the control of the cytomegalovirus promoter. The pTriEx-3 Neo plasmid was obtained from Novagen (Madison, WI, USA). These plasmid DNAs were purified with a Qiagen (Hilden, Germany) Plasmid Mini Kit.

2.2. Protein and DNA deliveries

NIH3T3 cells (4×10^4 cells/well) were incubated in DMEM medium supplemented with 10% fetal calf serum under an atmosphere of 5% CO_2/air at 37 °C for 24 h. The luciferase protein (1 and 3 μg (8.3 and 25 pmol)) was mixed with 0.4 and 1.2 μg, respectively, of Chariot reagent (Active Motif, Carlsbad, CA, USA) and incubated at room temperature for 30 min. The cells were washed with phosphate-buffered saline (PBS), and the protein–Chariot mixture (total 100 μl) and DMEM medium without serum (100 μl) were added to the cells. After a 1 h incubation under 5% CO_2 at 37 °C, 1 ml of DMEM medium supplemented with 10% serum was added, and the cells were incubated at 37 °C. The cells were washed with PBS before the luciferase assay.

DNA transfection was carried out with the Lipofectamine Plus Reagent (Invitrogen) essentially according to the supplier's instructions. NIH3T3 cells (4×10^4 cells/well) were incubated in DMEM medium with 10% fetal calf serum under 5% CO_2/air at 37 °C for 24 h. 0.3 and 1 ng (0.08 and 0.23 fmol) of the pcDNA 3.1 (+)-luc 2 plasmids containing the luciferase gene were mixed with 'carrier DNA', the pTriEx-3 Neo plasmid, to give a total amount of 400 ng. The DNA was mixed with lipids and transfected into the cells. After a 1 h incubation under 5% CO_2 at 37 °C, 1 ml of DMEM medium containing 10% serum was added, and the cells were incubated at 37 °C. After a further 23 h, the lipid–DNA complex was removed and the cells were incubated in DMEM medium supplemented with 10% serum. The medium was changed at 24 h intervals. The cells were washed with PBS before the luciferase assay.

Introduction of the luciferase protein and DNA into HeLa was carried out using the same procedures.

Luciferase activity was measured with a Luciferase Assay System with a Reporter Lysis Buffer Kit (Promega). The activity is expressed as fmol/well based on known amounts of the luciferase protein as standards.

2.3. Quantitative evaluation of protein production after DNA transfection

We applied the simple one-compartment model to the amount (activity) of luciferase per well. The luciferase protein was assumed to be cleared by first-order kinetics. The rate constants for elimination and half-lives were calculated using data for the elimination phase. AUC (area under the luciferase amount–time curve) values, which are equal to $\int_0^t (\text{luciferase amount})\,dt$, were used to evaluate the amount of luciferase protein, considering the time factor. For a linear system, the AUC value is proportional to amount of protein (Eq. (1)).

$$\text{luciferase uptake} = k \cdot \text{AUC} \quad (1)$$

where k is a constant. Thus, the value of AUC/uptake is constant (and equal to $1/k$). This constant value was used to estimate the amount of luciferase protein produced after DNA transfection.