

ENHANCEMENT OF LIPID CATIONIC TRANSFECTIONS IN THE PRESENCE OF SERUM

FIELD OF THE INVENTION

This invention is in the field of genetic engineering, in particular methods for enhancing the efficiency of transformation of eukaryotic cells.

BACKGROUND OF THE INVENTION

Cationic lipid reagents are the most effective and simplest method for DNA transfection of eukaryotic cells. A number of such reagents are known to the art, e.g. as described in U.S. patent applications 08/090,290, abandoned, 08/195,866, 08/171,232. Lipofectin™ (Gibco/BRL:Life Technologies, Inc., Gaithersburg, Md.; Felgner, P. L., et al. (1987) Proc. Natl. Acad. Sci. USA 84:7413) and LipofectACE™ (Whitt, M. A., et al. (1991) Focus 13:8) reagents contain monocationic lipids and are highly effective at transfection in the presence or absence of serum (Brunette, E., et al. (1992) Nuc. Acids Res. 20:1151; Ciccarone, V., et al. (1993) Focus 15, 80.). LipofectAMINE™ reagent (U.S. Pat. No. 5,334,761; Gibco/BRL:Life Technologies, Inc., Gaithersburg, Md.) contains a polycationic lipid and is up to 30-fold more active in serum-free transfection than the monocationic reagents (Hawley-Nelson, P., et al. (1993) Focus 15:73). However LipofectAMINE™ transfection activity is decreased in the presence of serum and is roughly equal to that of the monocationic reagents.

U.S. Pat. No. 5,286,634 of Stadler et al. for "Synergistic Method for Host Cell Transformation" issued Feb. 15, 1994 discloses the use of a polycationic compound to treat a host cell for a period of time prior to treating with a DNA-liposome complex to improve transformation of the cell. The invention was exemplified using plant cells which do not require serum in culture media or in vivo. The method of said patent is not believed effective in enhancing transfection of mammalian cells because the preferred polycationic compound of said patent (Polybrene™), is toxic to mammalian cells in the absence of serum.

A problem with transfection of eukaryotic cells by means of liposomes is the fact that culturing such cells in vitro requires the use of serum in the medium for best results, and the use of serum in culture media is standard in the art. However, the use of serum in the culture medium substantially inhibits the efficiency of liposome transfection. Further, in the transfection of animal cells in vivo, serum is inherently present, again with an inhibiting effect on the efficiency of liposome transfection. Therefore a need exists for a method of eukaryotic transfection in the presence of serum which counteracts the inhibiting effects of the serum.

In addition, some liposomes are toxic to the cells being transformed. Thus, a method for counteracting the toxic effects of liposomes is needed to improve the efficiency of liposome transfection of eukaryotic cells.

All publications and patents referred to herein are specifically incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a bar graph showing Polybrene™ enhancement of β -gal activity in transfections of BHK-21 cells in serum and inhibition of protein levels in the presence of serum. Group A are cells transfected using 6 μ l LipofectAMINE™, no Polybrene™ and no fetal bovine serum (FBS); Group B are cells transfected using 6 μ l LipofectAMINE™, and no

Polybrene™, with 5% fetal bovine serum present; Group C are cells transfected using 18 μ l LipofectAMINE™, and 20 μ l Polybrene™, with 5% fetal bovine serum present.

FIG. 2 is a bar graph showing Polybrene™ enhancement of β -gal activity in transfection of COS-7 Cells in serum and inhibition of protein levels in the presence of serum. Group A are cells transfected using 5 μ l LipofectAMINE™, no Polybrene™ and no fetal bovine serum (FBS); Group B are cells transfected using 8 μ l LipofectAMINE™, and no Polybrene™, with 5% fetal bovine serum present; Group C are cells transfected using 12 μ l LipofectAMINE™, and 80 μ l Polybrene™, with 5% fetal bovine serum present.

SUMMARY OF THE INVENTION

A method is provided for enhancing transfection efficiency of eukaryotic cells comprising contacting said cells with a lipid aggregate comprising nucleic acid and a cationic lipid in the presence of a polycationic compound and serum. The polycationic compound is preferably Polybrene™.

The polycationic compound is present at a concentration of about 20 to about 80 μ g/ml, more preferably between about 20 and about 40 μ g/ml. The lipid aggregate, which is composed of liposomes of nucleic acid and cationic lipid, preferably comprises between about 5 and about 20 μ l per ml, more preferably between about 12 and about 15 μ l per ml.

Some lipid aggregates are toxic to cells, and the use of the polycationic in the method of this invention substantially decreases such toxicity as measured in vitro by proteins present in the medium as a result of cell lysis, or as measured by means known to the art.

The method of this invention is applicable to eukaryotic cells, preferably mammalian, and more preferably human cells, and may be performed in vivo, e.g. in gene therapy as known to the art, or in vitro in cell culture, also as known to the art.

In the preferred embodiment of this invention, the polycationic compound is Polybrene™ and the lipid aggregate comprises cationic liposomes of LipofectAMINE™ and DNA.

Transfection efficiency is "enhanced" when an improvement of at least about 5 percent, preferably about 10 percent, and more preferably about 20 percent in efficiency is shown using the protocols set forth in the examples hereof.

"Lipid Aggregate" is a generic term which includes liposomes of all types, both unilamellar and multilamellar, as well as micelles and more amorphous aggregates of cationic lipid or lipid mixed with amphipathic lipids such as phospholipids.

"Target Cell" refers to any cell to which a desired compound is delivered, using a lipid aggregate as carrier for the desired compound.

"Transfection" is used herein to mean the delivery of expressible nucleic acid to a target cell, such that the target cell is rendered capable of expressing said nucleic acid. It will be understood that the term "nucleic acid" includes both DNA and RNA without regard to molecular weight, and the term "expression" means any manifestation of the functional presence of the nucleic acid within the cell, including without limitation, both transient expression and stable expression. Transfection may be performed in vitro or in vivo, and in this invention is performed in the presence of serum.

"Delivery" is used to denote a process by which a desired compound is transferred to a target cell such that the desired