

DNA in lipid-nucleic acid particles containing DODAC/DOPE is partially accessible to TO-PRO-1 at a lipid/DNA charge ratio (+/-) of 4:1, however, at 8:1 DNA is completely protected by the lipid component. This result suggests that the nucleic acid (DNA) is partially condensed at the lower charge ratio and fully condensed at the higher ratio (FIG. 11).

#### EXAMPLE 10

This example demonstrates the stability of lipid-nucleic acid particles in phosphate-buffered saline and in serum containing media.

A lipid-nucleic acid particle formulation was prepared according to the procedure described in Example 8. Portions of the formulation (using either ESM or DOPE as the neutral lipid) were combined with PBS (140 mM NaCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>) or serum-containing medium and incubated for two hours at 37° C. The resulting complexes were isolated and examined for any changes in QELS size results or transfection efficiency. No difference was found for any of the formulations, indicating that the complexes were not disrupted by either sodium or serum components. One portion which was incubated with PBS for 10 days still showed very good transfection efficiency.

#### EXAMPLE 11

This example illustrates the protection of DNA against DNase I which is afforded by the lipid-nucleic acid particles.

A lipid-nucleic acid particle formulation of 10 µg DNA, 160 nmoles DODAC and 160 nmoles ESM in 1 mL total volume was prepared according to the method described in Example 8. The susceptibility of the DNA in this formulation to degradation by DNase I was evaluated by mixing the formulation with DNase I in the presence of OGP (1:1 charge ratio). The level of DNase I was equivalent to that which degrades uncomplexed DNA within 10 minutes at 37° C. The reactions were stopped after 10 min by the addition of 25 mM EDTA. DNA was extracted using the Bligh and Dyer extraction procedure in the presence of 150 mM NaCl. Under these conditions the cationic lipid/DNA complex dissociates and the resulting DNA can be efficiently recovered from the aqueous fraction. This DNA was precipitated with 1/10th volume of 3M sodium acetate (pH 5.2) and 2.5 volumes of 95% ethanol and recovered by centrifugation at 14,000 g for 30 min at 4° C. The DNA pellet was resuspended in sterile distilled water and subjected to electrophoresis on a 0.8% agarose gel (Gibco, BRL). The results are shown in FIG. 12. As FIG. 12 indicates, complexes containing ESM provide protection of DNA from DNase I degradation.

#### EXAMPLE 12

This example illustrates the in vitro transfection of CHO or B16 cell lines using lipid-nucleic acid particles prepared by the method of Example 8.

In vitro transfection was performed using a 96-well cell culture plate (Costar, Cambridge, Mass., USA) containing 50% confluent growth of either Chinese Hamster Ovary

(CHO) or murine melanoma (B16) cell lines. Appropriate amounts (about 6–50 µL) of the lipid-nucleic acid particle formulation (10 µg DNA/mL) were premixed with medium containing 10% serum to a final volume of 150 µL. The medium surrounding the cells was removed using a needle syringe and replaced with the lipid-nucleic acid particles in 10% serum-containing medium. The cells and complex were incubated for a further 48 hours at 37° C. The transfection efficiency was evaluated using β-gal stain or an enzyme activity assay. Results are presented in FIG. 13.

The transfection study showed excellent transfection efficiency with ESM-containing complexes and with DOPE-containing complexes (not shown). A cationic lipid to DNA charge ratio of 3:1 to 4:1 gave the best in vitro transfection results.

#### VII. Conclusion

As discussed above, the present invention provides novel charge-neutralized lipid-nucleic acid complexes. These hydrophobic DNA intermediates can be isolated and the DNA exists in a non-condensed form as measured by dye binding and DNase I sensitivity. These complexes can be used in the preparation of other lipid-nucleic acid particles which are effective for delivering nucleic acids to target cells.

The methods described for the preparation and uses of the various nucleic acid particles can be used with essentially any nucleic acid which can exist in a lipophilic state when complexed with an appropriate cationic lipid. Examples of some constructs include those encoding adenine deaminase, the low density lipoprotein receptor for familial hypercholesterolemia, the CFTR gene for cystic fibrosis, galactocerebrosidase for Gaucher's disease, and dystrophin or utrophin into muscle cells for Duchenne's muscular dystrophy.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

What is claimed is:

1. A hydrophobic lipid-nucleic acid complex consisting essentially of cationic lipids and nucleic acids, which complex binds to TO-PRO-1, and is charge neutralized and soluble in organic solvents.

2. A complex in accordance with claim 1, wherein said nucleic acid is a plasmid.

3. A complex in accordance with claim 1, wherein said cationic lipids are members selected from the group consisting of DODAC, DDAB, DOTMA, DOSPA, DMRIE, DOGS and combinations thereof.

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