

preparations obtained with the different cationic lipids. As examples the elution profile for preparations containing DODAC FIG. 42(a); DOTMA, FIG. 42(b), and DSDAC, FIG. 42(c) are given after incubation in mouse serum for 30 min at 37° C.

EXAMPLE 28

This example demonstrates the encapsulation of plasmid DNA with the ionizable lipid AL-1 ($pK_a=6.6$) by the dialysis method (as described in EXAMPLE 1). AL-1 is positively charged at acidic pH and neutral at pH >7. Different concentrations of AL-1 were used in the lipid formulations at pH 4.8 and 7.5 respectively. The amount of encapsulated DNA was determined using the PicoGreen assay. Non-encapsulated DNA was removed first by anion exchange chromatography and the entrapped DNA determined with PicoGreen after solubilization of the lipid vesicles in detergent. Encapsulation of plasmid DNA using DODAC is shown as comparison. At pH 4.8 maximal encapsulation of approximately 75% of plasmid DNA was achieved with 8% AL-1 similar to the DODAC formulation at pH 7.5. However, no DNA entrapment was obtained with AL-1 at pH 7.5. FIG. 43. This clearly demonstrates the requirement of positively charged lipids for DNA entrapment.

EXAMPLE 29

This example shows the stability of the plasmid containing vesicles formed with AL-1 at pH 4.8 and the protection of the entrapped DNA from degradation by serum nucleases at pH 7.5. 3H -DNA and ^{14}C -CHE (cholesteryl hexadecyl ether) were used to follow the DNA and lipid respectively. The vesicles formed with AL-1 at Ph 4.8 were incubated in mouse serum for 1.5 hr at 37° C. at pH 7.5. The non-encapsulated DNA was not removed in the preparations used for serum incubation. After incubation in serum the vesicles were separated on a Sepharose CL6B column. Lipid and DNA were detected by radioactivity in the different fractions. FIG. 44. Approximately 60% of the DNA was protected from serum nucleases. When vesicles formed with AL-1 at pH 7.5 were incubated in serum virtually all the DNA was degraded and eluted as fragments separated from lipids. FIG. 45.

EXAMPLE 30

Example 30 demonstrates the effect of the PEG-ceramide concentration on the encapsulation efficiency by the dialysis method with 7.5% DODAC and DOPE. The non entrapped DNA in the various formulations with different PEG-C14 concentrations was separated by DEAE Sepharose CL6B chromatography. DNA and lipid recovered are shown as a function of %PEG-C14. Best entrapment was obtained with 10 mol% PEG-C14. FIG. 46. However, a more recent experiment showed optimum entrapment in the range of 10 to 15 mol% (data not shown).

VII. Conclusion

As discussed above, the present invention comprises novel lipid-nucleic acid complexes and methods of making them. In a number of embodiments, 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.

In further embodiments, the invention provides methods for preparing serum-stable nucleic acid-lipid particles which are useful for the transfection of cells, both in vitro and in vivo.

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 adenosine 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 for all purposes 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 method for the preparation of lipid-nucleic acid particles, comprising:
 - (a) contacting nucleic acids with a solution comprising non-cationic lipids and a detergent to form a nucleic acid-lipid mixture;
 - (b) contacting cationic lipids with said nucleic acid-lipid mixture to neutralize the negative charge of said nucleic acids and form a charge-neutralized mixture comprising detergent, nucleic acids and lipids; and
 - (c) removing said detergent from said charge-neutralized mixture to provide said lipid-nucleic acid particles in which said nucleic acids are protected from degradation.
2. The method in accordance with claim 1, wherein said solution of step (a) further comprises an organic solvent.
3. The method 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.
4. The method in accordance with claim 1, wherein said non-cationic lipids are selected from the group consisting of ESM, DOPE, polyethylene glycol-based polymers and combinations thereof.
5. The method in accordance with claim 1, wherein said detergent is octyl- β -D-glucopyranoside, said cationic lipid is DODAC, said non-cationic lipid is ESM, and said detergent is removed by dialysis.
6. The method in accordance with claim 5, wherein said non-cationic lipids are combinations of ESM and PEG-Ceramide.
7. The lipid-nucleic acid particle prepared according to claim 1.
8. A method for the preparation of lipid-nucleic acid particles, comprising:
 - (a) contacting an amount of cationic lipids with nucleic acids in a solution; said solution comprising of from about 15–35% water and about 65–85% organic solvent and said amount of cationic lipids being sufficient to produce a +/- charge ratio of from about 0.85 to about 2.0, to provide a hydrophobic, charge-neutralized lipid-nucleic acid complex;
 - (b) contacting said hydrophobic lipid-nucleic acid complex in solution with non-cationic lipids, to provide a lipid-nucleic acid mixture; and