

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-C 14. FIG. 46. However, a more recent

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 of preventing particle aggregation of lipid:nucleic acid complex particles, said method comprising the step of incorporating an non-cationic lipid into lipid:nucleic

acid complex particles comprising a cationic lipid and a nucleic acid polymer, wherein said additional lipid is a polyethylene glycol-based polymer.

2. The method of claim 1, wherein the polyethylene glycol-based polymer is selected from the group consisting of polyethylene glycol lipids, polyethylene glycol ceramides and ganglioside-modified lipids.

3. The method of claim 1, wherein the amount of additional lipid is from 1 to 15% of the particles.

4. The method of claim 1, wherein said nucleic acid is selected from the group consisting of DNA and RNA.

5. The method of claim 1, wherein said nucleic acid is DNA.

6. The method of claim 1, wherein said lipid:nucleic acid complex is lyophilized.

7. The method of claim 1, wherein the nucleic acid is linked to an expression vector to facilitate gene expression after entry into a cell.

8. A method of preparing a lipid:nucleic acid complex, comprising the steps of:

(a) combining a nucleic acid with a cationic lipid to produce a lipid:nucleic acid complex; and

(b) mixing the lipid:nucleic acid complex with a non-cationic lipid which is a polyethylene glycol-based polymer, wherein the polyethylene glycol-based lipid reduces the tendency of the lipid:nucleic acid complex to aggregate.

9. The method of claim 8, wherein the polyethylene glycol-based polymer is selected from the group consisting of polyethylene glycol lipids, polyethylene glycol ceramides and ganglioside-modified lipids.

10. The method of claim 8, wherein the amount of non-cationic lipid is from 1 to 15%.

11. The method of claim 8, wherein said nucleic acid is selected from the group consisting of DNA and RNA.

12. The method of claim 8, wherein said nucleic acid is DNA.

13. The method of claim 8, wherein said lipid:nucleic acid complex is lyophilized.

14. The method of claim 8, wherein the nucleic acid is linked to an expression vector to facilitate gene expression after entry into a cell.

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