Preparation of Liposomes and Their Use in Studies with Paramecium

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Liposomes are synthetic lipid vesicles, first described in 1965 by Bangham, et al. (1, 6). The major interests in liposomes are as models of biological membranes and as exogenous delivery systems for targeting of encapsulated agents, such as deoxyribonucleic acid (DNA), drugs or enzymes to specific tissues. Methods for preparation of liposomes are varied (12): sonication of dried lipid films in presence of aqueous solution, reverse phase evaporation, extrusion through a small orifice (French press), ethanol injection into aqueous media, detergent dialysis (7, 9), gel filtration (4), and transient pH changes (2).

Here we report preparation of liposomes by simple autoclaving, the use of primulin for visualization of liposomes in a fluorescent microscope, and the use of CHAPS detergent for liposome preparation in the detergent dialysis method.

Materials and Methods

Chloroform and diethyl ether were reagent grade and were used without further purification. β-Octyl glucoside and tocopherol were from Sigma Chemical Company (St. Louis, MO). Primulin (Direct Yellow 59) was from Aldrich Chemical Company (Milwaukee, WI). 11-(9-anthroyloxy)-undecanoic acid, 2-(9-anthroyloxy)-palmitic acid, cholesteryl anthracene-9-carboxylate, and dansyldipalmitoyl-L-α-phosphatidyl ethanolamine (dansyl-DPPE) were from Molecular Probes, Inc. (Junction City, OR). CHAPS detergent (3-((3-Cholamidopropyl)-dimethylammonio)-1-propanesulfonate) was from PolySciences, Inc. (Warrenton, PA) or Calbiochem-Behring, Inc. (La-Jolla, CA). Dipalmitoylphosphocholine, a synthetic phospholipid was obtained from Molecular Probes, Inc. or Sigma. Dialysis tubing was Spectrapor, 1/4" diameter, molecular weight cutoff = 12,000 from Arthur H. Thomas Company (Philadelphis, PA).

Results

1. Preparation of Liposomes by Autoclaving

Methods for reverse phase evaporation of volatile organic solvents for liposome preparation involve dissolving the phospholipid in chloroform with a neutral lipid such as tocopherol and mixing this solution with the same amounts of lipids dissolved in diethyl ether before evaporation under a stream of nitrogen gas (5, 10). We have found that the chloroform and ether may be removed quickly by autocalving the mixture at 15 psi for 12 minutes. Many large liposomes are formed. If none of the components for liposome preparation are heat labile, autoclaving compares favorably to nitrogen-induced evaporation in size and numbers of liposomes produced. For example, 2.4 mg (3.33 μmol) DPPC and 1 drop of tocopherol are dissolved in 1 ml of chloroform and 1 ml of diethylether separately. One ml 50 mM KC1 is added to the chloroform solution and 5 ml 50 mM KC1 is added to the ether solution. Both solutions are shaken vigorously, mixed together and shaken again before autoclaving. The resulting liposomes are mainly in the 0.5 to 20 μ diameter range. The cylindrical-type autoclave used is from American Sterilizer Co. (Erie, PA) with an inner chamber volume of 6.95 1 (2.45 ft^3, diameter = 15 in, depth = 24 in) and electrical heat. We experienced no difficulties in autoclaving 1 ml of ether and 1 ml of chloroform in an autoclave

of this size and think that the procedure is not hazardous. However, potential hazards should be recognized.

We have prepared fluorescent liposomes by adding a fluorescent lipid to the DPPC-tocopherol in chloroform and ether solutions. 11-(9-anthroyloxy)-undecanoic acid, 2-(9-anthroyloxy)-palmitic acid, and cholesteryl anthracene-9-carboxylate all are incorporated into the synthetic membrane uniformly.

We also have prepared fluorescent liposomes from a fluorescent phopholipid, dansyl-DPPE, without DPPC. This shows that fluorescent liposomes can be prepared from fluorescent phospholipids as well as by intercalation of fluorescent fatty acid and cholesterol derivatives into the DPPC bilayer.

2. Use of Primulin to Visualize Liposomes

Primulin has been used as a spray reagent for detection of lipids on thin-layer chromatograms (11). Liposomes may be viewed in primulin solutions with fluorescent microscopy. Aqueous solutions of 0.1 to 0.001 percent primulin are useful. One drop of liposome preparation and one drop of primulin solution are mixed on a microscope slide and viewed with a filter system having an excitation range of 300-400 nm and a barrier filter above 420 nm. Addition of fluorescent microspheres of determined size allows estimation of liposome size by direct comparison.

3. Use of CHAPS Detergent for Liposome Preparation

β-Octyl glucoside and other detergents have been used to solubilize lipids before dialysis to remove the detergent and form liposomes (7, 8, 9). The synthesis of CHAPS detergent was reported in the literature in 1980 (3) and then became available commercially. When 60 mM CHAPS and β-octyl glucoside are compared by dissolving DPPC (7.9 mg in 5 ml) in each detergent in 50 mM KCl and dialyzing against 50 mM KCl at a temperature (41°C) above the transition point of DPPC, the liposomes produced are in greater numbers with greater uniformity in the case of CHAPS. We conclude that the use of CHAPS detergent is preferable to β-octyl glucoside, at least when synthetic DPPC is the phospholipid of choice.

Discussion

We have prepared these liposomes for the purpose of delivering water-soluble materials to the food vacuoles of paramecia and for the incorporation of fluorescent lipids into the vacuolar membranes of paramecia. The size of these liposomes is such that they are rapidly engulfed by *Paramecium multi-micronucleatum* and *Paramecium tetraaurelia*. The paramecia containing fluorescent and non-fluorescent liposomes in their food vacuoles may be photographed successfully with Kodak 400 Daylight film. Further experiments with uptake of liposomes by paramecia are in progress.

Summary

We make three points about liposome preparation:

1. Liposomes, including fluorescent liposomes, may be prepared through reverse-phase evaporation by autoclaving.
2. Liposomes prepared with synthetic DPPC may be visualized with primulin, a water-soluble dye with an affinity for lipids, and fluorescence microscopy.
3. CHAPS detergent is superior to β-octyl glucoside detergent in liposome

preparation by the detergent dialysis method with synthetic DPPC. Additionally, fluorescent and non-fluorescent liposomes prepared by these methods are engulfed rapidly by two species of paramecia.

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