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Preparation of fatty acid micelles

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Purpose

Here we describe a method for preparing fatty acid micelles. The method for adding micelles to a buffered solution containing fatty acid or phospholipid vesicles is also discussed.

Theory

When the carboxylate head groups of fatty acid amphiphiles are deprotonated, they aggregate into monolayer spherical structures with the hydrophilic head groups pointing outwards and the hydrophobic hydrocarbon chains pointing inwards. These fatty acid micelles, when added to fatty acid or phospholipid vesicles, can be incorporated into the vesicle membranes, leading to an increase of vesicle surface area [1,2,3].

Equipment

Bench top vortex machine
Single depression glass slides
pH meter
1.5 ml Eppendorf tubes

Materials

Oleic acid
Myristoleic acid
NaOH
Deionized water

Solutions & buffers

Preparation—NaOH solution (100 mM)

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Component	Stock	Amount
NaOH solution	10 M	1 ml

Add water to 100 ml

Step 1—oleic acid/NaOH solution (100 mM each)

Component	Stock	Amount
oleic acid (pure)	>99%	32 ul
NaOH	100 mM	1 ml

myristoleic acid/NaOH solution (100 mM each)

Component	Stock	Amount
myristoleic acid (pure)	>99%	28 ul
NaOH	100 mM	1 ml

Protocol

Duration

Preparation	about 10 minutes
Protocol	about 1 hour

Preparation

Fatty acids are often shipped in sealed glass ampoules. Break the ampoule and pipette the fatty acid into an Eppendorf tube for later use.

Caution

Use paper towels or clean gloves to wrap the ampoule, to avoid injuries from broken glass pieces. All lipids should be stored at -20°C .

Step 1 Dissolving fatty acid in a NaOH solution

Overview

Dissolving fatty acid in a NaOH solution, to 1:1 molar concentration ratio

Duration

20 min

- 1.1 Pipette an appropriate amount (see the Solutions & Buffers section) of fatty acid into the bottom of a 1.5 ml Eppendorf tube. Add NaOH solution, so that the final molar amounts of fatty acid and NaOH are equal in the solution.

Tip

The sample should be maintained above the melting temperature of the fatty acid(s) used.

- 1.2 Vortex the sample using a bench top vortex machine for 30 seconds to fully disperse the fatty acid in the NaOH solution (see Fig. 1). (Large, visible air bubbles may form during the process, as shown in Fig. 1 and Fig. 2).

Tip

Avoid light by wrapping aluminum foil around the sample. Avoid oxygen by flushing the container with argon or nitrogen gas.

- 1.3 Allow the sample to sit for 1 hr, until the air bubbles disappear, and fatty acid micelles should have formed.

Step 2 Addition of fatty acid micelles to vesicles**Overview**

Addition of fatty acid micelles to fatty acid vesicles or phospholipid vesicles, for experiments on vesicle growth [1,2,3].

Duration

10 min

- 2.1 To ensure rapid mixing of fatty acid micelles and vesicles, pipette an appropriate amount of micelles into a 1.5 ml Eppendorf tube first, and add a volume of vesicle suspension. Use the pipette tip to stir the solution for rapid mixing.

Tip

The vesicle suspension should be buffered to avoid pH changes upon the addition of fatty acid micelles that contain NaOH.

- 2.2 For imaging, it is necessary to keep the vesicles in the field of view upon the addition of fatty acid micelles. A single depression glass slide can be used: pipette a drop of micelles onto the middle of the single depression glass slide, add vesicles onto the slide, and briefly stir the sample with the pipette tip. Optical imaging of vesicle growth can be performed by an inverted epifluorescence microscope with extra long working distance (ELWD) objective lenses of 20X or 40X magnification.

Source article(s) used to create this protocol

1. Chen IA, Roberts RW, Szostak JW. The emergence of competition between model protocells. *Science*. 2004 Sep 3; 305(5689):1474–6. [PubMed: 15353806]

2. Hanczyc MM, Fujikawa SM, Szostak JW. Experimental models of primitive cellular compartments: encapsulation, growth, and division. *Science*. 2003 Oct 24; 302(5645):618–22. [PubMed: 14576428]
3. Zhu TF, Szostak JW. Coupled Growth and Division of Model protocell membranes. *J Am Chem Soc*. 2009 Mar 26; 131(15):5705–13. [PubMed: 19323552]

Referenced literature

1. Berclaz N, Muller M, Walde P, Luisi PL. Growth and transformation of vesicles studied by ferritin labeling and cryotransmission electron microscopy. *J Phys Chem B*. 2001; 105:1056–64.
2. Hanczyc MM, Fujikawa SM, Szostak JW. Experimental models of primitive cellular compartments: encapsulation, growth, and division. *Science*. 2003 Oct 24; 302(5645):618–22. [PubMed: 14576428]
3. Zhu TF, Szostak JW. Coupled Growth and Division of Model protocell membranes. *J Am Chem Soc*. 2009 Mar 26; 131(15):5705–13. [PubMed: 19323552]

Fig. 1.
Vortex the sample using a bench top vortex machine for 30 seconds to fully disperse the fatty acid in the NaOH solution.

Fig. 2.
Large, visible air bubbles appear after vortex.