CHAPTER TWENTY-TWO

Preparation of Fatty Acid Micelles

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Contents

Abstract

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.

1. 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

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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 (Berclaz et al., 2001; Hanczyc et al., 2003; Zhu and Szostak, 2009).

2. EQUIPMENT

Vortex mixer
pH meter
Single depression glass slides
1.5-ml microcentrifuge tubes

3. MATERIALS

Oleic acid
Myristoleic acid
Sodium hydroxide (NaOH)
Deionized water

3.1. Solutions & buffers

Step 1 NaOH solution

Component	Final concentration	Stock	Amount
NaOH	100 mM	10 M	1 ml

Add water to 100 ml

Oleic acid/NaOH solution

Component	Final Concentration	Stock	Amount
Oleic acid	100 mM	>99%	32 μl
NaOH	100 mM	100 mM	1 ml

Myristoleic acid/NaOH solution

Component	Final concentration	Stock	Amount
Myristoleic acid	100 mM	>99%	28 μl
NaOH	100 mM	100 mM	1 ml

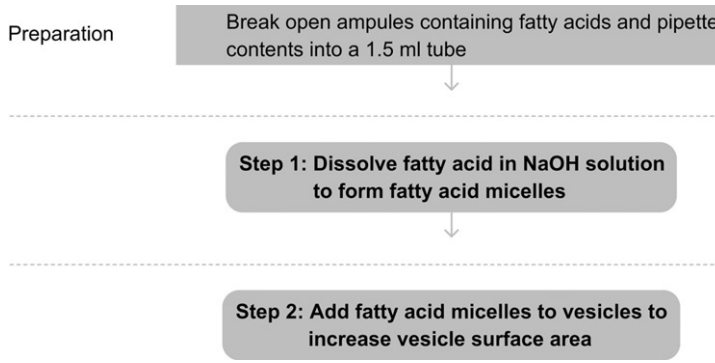

Figure 22.1 Flowchart of the complete protocol, including preparation.

4. PROTOCOL

4.1. Duration

Preparation	About 10 min
Protocol	About 1 h

4.2. Preparation

Fatty acids are often shipped in sealed glass ampules. Break the ampule and pipette the fatty acid into a 1.5-ml microcentrifuge tube for later use.

4.3. Caution

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

See Fig. 22.1 for the flowchart of the complete protocol.

5. STEP 1 DISSOLVE FATTY ACID IN NAOH SOLUTION

5.1. Overview

Dissolve the fatty acid in a NaOH solution at a 1:1 molar ratio.

5.2. 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 microcentrifuge tube. Add the

NaOH solution, so that the final molar amounts of the fatty acid and NaOH in the solution are equal.

1.2 Vortex the sample using a vortex mixer for 30 s to fully disperse the fatty acid in the NaOH solution (see Fig. 22.2). (Large visible air bubbles may form during the process, as shown in Figs. 22.2 & 22.3.)

1.3 Allow the sample to sit for 1 h until the air bubbles disappear. Fatty acid micelles should form during this incubation.

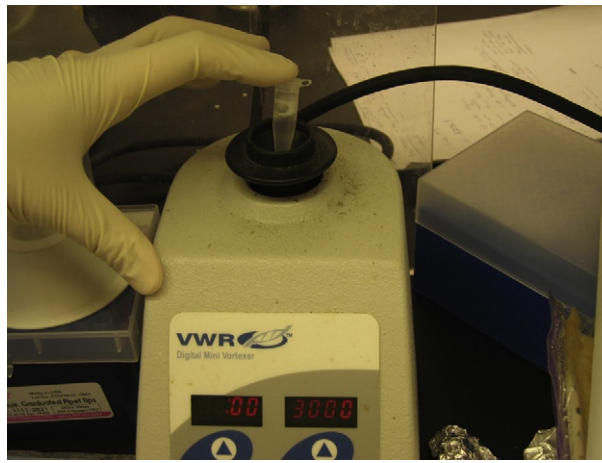

Figure 22.2 Vortex the sample using a vortex mixer for 30 s to fully disperse the fatty acid in the NaOH solution.

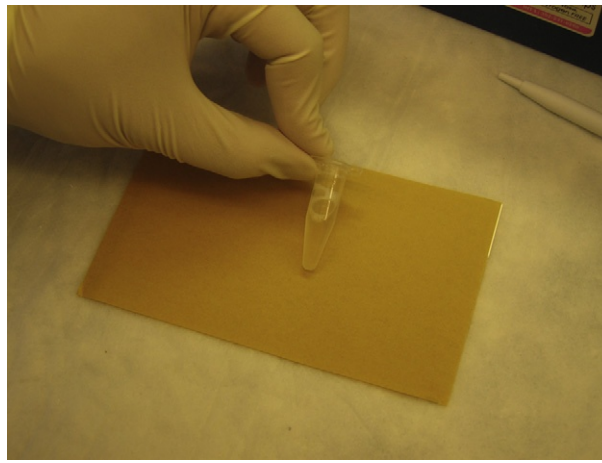

Figure 22.3 Large visible air bubbles appear after vortexing.

5.3. Tip

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

5.4. Tip

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

6. STEP 2 ADDITION OF FATTY ACID MICELLES TO VESICLES

6.1. Overview

Add fatty acid micelles to fatty acid vesicles or phospholipid vesicles for experiments on vesicle growth.

6.2. Duration

10 min

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

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.

6.3. Tip

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

REFERENCES

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