Chapter 2

The Use of Liposomes in the Study of Drug Metabolism: A Method to Incorporate the Enzymes of the Cytochrome P450 Monooxygenase System into Phospholipid, Bilayer Vesicles

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Abstract

Although lipids are essential for the optimal activity of the cytochromes P450 monooxygenase system, relatively little is known about the membrane environment in which these enzymes function. One approach used to mimic the structural arrangement of lipids and enzymes within the endoplasmic reticulum is to physically incorporate the cytochromes P450 and their redox partners in a vesicle bilayer of phospholipids. Several methods have been devised for this purpose. This chapter describes a method in which the P450 monooxygenase system is incorporated by first, solubilizing the enzymes and lipid with sodium glycocholate. After the protein and lipid aggregates are dispersed, the detergent is removed by adsorption using BioBeads SM-2 resin which leads to the formation of bilayer vesicles of phospholipid containing incorporated cytochrome P450 and NADPH cytochrome P450 reductase. This procedure requires relatively a short preparation time, provides concentrated reconstituted systems that can be used in a wide range of applications, allows for several enzyme samples to be prepared simultaneously so that different conditions can be compared, and results in minimal loss of active enzyme.

Key words: Phospholipid vesicles, Cytochromes P450, Reconstituted systems, Drug metabolism

1. Introduction

The cytochromes P450 (P450) represent a ubiquitous gene superfamily comprising a diversity of isoforms that are expressed virtually in every organism in a species-specific pattern and are responsible for most xenobiotic metabolism in vivo (1). Thus the cytochromes P450 play a key role in the oxidation and clearance of most drugs and, in some instances, bioactivate toxins and promutagens to reactive intermediates that bind to cellular macromolecules

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which, in turn, may lead to toxicity and/or the initiation of carcinogenesis (2–5). Because of these attributes, metabolism by the cytochromes P450 is relevant to a range of interests, including those of pharmacologists, toxicologists, and cancer researchers.

The monooxygenase reactions catalyzed by these enzymes require electrons that can be delivered by various redox partners (6). The NADPH-cytochrome P450 reductase (reductase) is the primary redox partner in vivo and is capable of delivering both electrons needed in the catalytic cycle of the P450. Thus, the minimum enzyme assemblage needed to study monooxygenase reactions by the P450 system includes the reductase and the P450 isoform of interest. Much of the early information regarding the substrate specificity of the individual isoforms has come from studies in which the metabolites are identified after incubating the compound of interest with the active P450 monooxygenase enzyme assemblage and a source of NADPH (7–9).

The P450 enzymes involved in xenobiotic metabolism and their redox partners are embedded in the membrane of the endoplasmic reticulum. As discussed in more depth later on, the membrane environment is essential for the functioning of the P450 monooxygenase system. Early attempts, over 30 years ago, to purify and characterize the various isoforms were limited by the dependence of enzyme activity on an unknown, heat-stable factor that was lost during the purification of the proteins. It was later found that the heat-stable factor was microsomal lipid (8, 10). Thus, it was determined that a lipid milieu was essential for the reconstitution of the catalytic activity of the purified enzymes. Subsequent studies have proposed that the lipid serves both as a "scaffold" to properly orient the P450 enzyme and the reductase for functional interaction (11, 12) and an effector that influences catalysis by the P450 enzyme (13). The effector role of phospholipid is evidenced by the modulation of P450 enzymatic activity at lipid:P450 enzyme ratios that are too low to facilitate the formation of liposomes. The "scaffold function" of lipid is ascribed to an additional level of modulation of enzyme activity observed at lipid concentrations at which liposomes form.

Most enzymatic studies with the purified P450 enzymes use a short-chain (C-12), non-physiologic lipid, dilaurylphosphatidylcholine (DLPC). The reasons for this choice are the following: (1) the ease of preparation of the reconstituted systems with this lipid and (2) the lipid stimulates metabolism with most of the commonly studied P450 enzymes (14). However, studies have shown that the enzyme-lipid assemblages in the reconstituted systems with DLPC bear little structural similarity to the monolamellar, bilayer arrangement of lipid in the endoplasmic reticulum (15–17). Thus, it is clear that in order to truly appreciate the significance of the "scaffold" effect of the lipid on P450 metabolism, the enzymes must be physically incorporated in a vesicular bilayer of lipid.

Several methods used to generate these P450 vesicular reconstituted systems (VRS) have been published (18–20). Unfortunately, the methods require detergent, and this makes the procedures considerably more difficult and time-consuming than those using DLPC. However, the methods have been applied extensively and have generated interesting findings regarding the scaffold effect of the lipid bilayer on P450 metabolism (21–25). The approach common to all the methods for the preparation of VRS involves the following sequence of steps: (a) drying of the lipid in order to remove organic solvents that would inactivate or denature the enzymes, (b) detergent treatment to solubilize and disperse the enzymes and the lipids, and (c) a detergent removal procedure which causes the lipids to coalesce and form lipid bilayer vesicles, and in the process, the enzymes are incorporated in the vesicles.

The thermodynamics associated with the detergent solubilization of a membrane complicate the ability of the VRS preparation methods to study the relationship between P450 activity and lipid concentrations. As mentioned above, this is an important aspect when evaluating the scaffold effect of lipids on enzymatic activity. The complexity of membrane solubilization has been reviewed in detail previously (26). At low concentrations, detergent binds to lipid and partitions between the lipid and aqueous phases (27, 28). Thus, in the simplest terms at low concentrations, two populations of detergent can be assigned – that bound to lipid and that partitioned into solution as monomers. The ratio of detergent concentrations in the two forms is dependent on the partition coefficient of the detergent for the aqueous and lipid phases.

The initial stage of lipid solubilization occurs when the detergent concentration is increased to a point at which the bilayers containing a mixture of detergent and lipid are lysed into mixed micelles (26). Furthermore, it has been shown that this process occurs when the monomeric detergent concentration, in equilibrium with that bound to lipid, approaches or reaches the critical micelle concentration (CMC) of the pure detergent in aqueous solution (26, 27). As the detergent concentration is increased above the threshold required for membrane lysis, the excess detergent binds primarily to the mixed micelles of lipid and detergent, and the monomeric detergent concentration remains relatively constant at the CMC of the detergent. In the process of increasing the detergent concentration above the lysis threshold, the sizes of these micelles are reduced. Upon complete solubilization of the lipid/enzyme assemblage, each component lipid and enzyme is contained in an individual micelle of detergent. Thus, the total concentration of detergent needed to lyse the membrane will depend on both the concentration of the lipid and the extent to which the detergent partitions to the lipid phase.

In applying the VRS methods at different concentrations of lipid, it is necessary to first identify the “effective” detergent to

lipid ratio (which is the ratio of the *lipid-bound* detergent concentration to the total lipid concentration) under a given set of optimized conditions that result in a desired degree of lipid/enzyme solubilization. The effective detergent to lipid ratio can then be used to calculate the detergent concentration needed to achieve comparable degrees of solubilization at different lipid concentrations. If the *total* detergent concentration (and not the *lipid-bound* detergent concentration) is adjusted in proportion to the lipid concentration in the methods for VRS preparation, the final VRS will have an excess detergent concentration (because *both* the monomeric and the lipid bound detergent concentrations will be scaled up). This in turn, likely will result in a high proportion of inactive enzyme because of the destructive effects of the excess detergent. Alternatively, if the detergent concentration is not adjusted when the lipid concentration is increased dramatically, the lipid will not be sufficiently solubilized to allow for the vesicular incorporation of the enzymes.

We have tested two of the published methods, cholate gel filtration and cholate dialysis (17) and have found the active P450 enzyme tends to be extremely labile to the detergent, whereas the reductase tends not to incorporate into the vesicular fraction of the enzyme/lipid assemblage. Furthermore, preparations made using gel filtration are diluted and can be prepared only one at a time, limiting the opportunity to compare different experimental conditions, whereas, the dialysis procedure used to remove detergent is labor intensive and extremely time-consuming (3×12 h incubations against 3 L of dialysis buffer).

Because of the limitations associated with these common methods for VRS preparation, we developed an improved technique for the preparation of P450 VRS (29). In the course of this work, we found a detergent (sodium glycocholate) that was less destructive to the P450 enzyme and utilized a more rapid way to remove the detergent by adsorption to BioBeads SM2 resin. We found this method to be superior to the two most commonly applied methods because of the following characteristics: (1) samples prepared using this method were also found to contain a higher proportion of the vesicular-incorporated reductase, (2) the detergent removal step could be carried out much more rapidly than the dialysis (2 h vs. 3 days), (3) the generated VRS samples could be easily prepared at relatively high enzyme concentrations (≥ 5 μM), and (4) several samples could be prepared simultaneously thus allowing for the comparison of different conditions used in the reconstitution of the enzymes. We found that the VRS prepared under these conditions had very high catalytic activity relative to the reconstituted systems made using sonicated DLPC (17). The later sections of this chapter describe in detail the VRS preparation method. In addition, this chapter shows an approach that can be used to estimate the

effective detergent to lipid ratio in order to obtain VRS preparations with comparable levels of enzyme incorporation and activity over a range of lipid concentrations.

This VRS method can be used to address general questions about the scaffold effect of the lipid. However, this type of reconstituted system also provides a more favorable structural framework with which to study the interaction of P450 enzymes with the various redox partners. Furthermore, the potential for future interest in the methods to prepare VRS with reductase and P450 is tremendous given the fact that lipids are known to separate into functional domains identified as "rafts" in studies with the plasma membrane (30). Evidence is just starting to accumulate that functional, lipid microdomains may also be present in the endoplasmic reticulum (31) and thus, may be significant in regulating P450 metabolism.

2. Materials

1. 0.5 M Hepes (pH 7.5)
2. 1 M $MgCl_2$
3. 10% (w/v) aqueous sodium glycocholate (Calbiochem La Jolla, CA); membrane phospholipid of choice in chloroform at a concentration of 10 mg/mL. We have routinely used phosphatidylcholine from bovine liver (Avanti Polar Lipids Alabaster, AL). The lipid is both light and air sensitive with a tendency to oxidation of unsaturated acyl chains. The chloroform solutions are stored at –20°C.
4. Concentrated enzyme stock solutions (>10°μM reductase or P450 enzyme, respectively) in 100°mM potassium phosphate (pH 7.4) with 20 % glycerol (enzyme stock solutions are frozen at –80°C).
5. BioBeads SM-2 (Bio-Rad Hercules, CA).
6. 5 μm syringe filter (GE Osmonics, Minnetonka, MN).

3. Methods

The general method characterized and described previously (29) results in a 0.5 mL solution of VRS containing 5 μM each of reductase and P450 enzyme and a 500:1 ratio of lipid:P450 (see Note 1). An adaptation is described below the general method which allows for adjustments in the lipid:P450 ratio of the VRS.

3.1. Preparation of the BioBeads SM-2 for Detergent Removal

This needs to be done at least two days before preparing the VRS. The use of BioBeads SM-2 to remove detergent from biological preparations has been described in depth previously (32).

1. Add 200 mL methanol to 30 g of BioBeads SM-2.
2. Stir for 15 min and collect beads by filtration with a Buchner funnel fitted with standard filter paper.
3. Immediately wash beads with another 500 mL of methanol; then repeat filtration.
4. Immediately wash beads with 1000 mL of water and repeat filtration.
5. Transfer beads to a chromatographic column and slowly wash beads with 2000 mL of water.
6. The moist beads are stored in ultrapure water at 4°C until required. When used, the beads are filtered as described above and added to the preparation containing detergent, lipid, and enzyme (supernatant derived from Step 5 of Subheading 3.3). Beads have been stored up to 3 months, periodically changing the water without noticeable problems in preparing the VRS.

3.2. General Method for Preparation of VRS: Drying the Lipid

1. Dry 1 mg of phospholipid (from a 10 mg/mL solution in chloroform) in a 1.5 mL microfuge tube overnight in a lyophilizer (see Note 2).
2. Release the vacuum on the lyophilizer by filling the chamber with N_2 (see Note 3).
3. Add 50 µL of 0.5 M of Hepes (pH 7.5) and 50 µL of 10% sodium glycocholate to the tube containing dried lipid (see Notes 4 and 5).

3.3. Detergent Solubilization of Lipid and enzyme

1. Blow nitrogen over the tube opening of the solution from step 3.2.3 before capping, then bath-sonicate, and periodically vortex the tube until the solution is clear (usually 5 min).
2. While the lipid is being solubilized (step 3.3.1.), add P450 enzyme and reductase (2.5 nmol of each) to a second 1.5 mL microfuge tube and dilute to 482.5 µL with ultrapure water.
3. Add 7.5 µL of 1 M $MgCl_2$ to the tube containing the P450 enzyme and the reductase.
4. Add the solution derived from step B.1 in approximate aliquots of 25 µL (approximately 1/4th the total volume of the solution) to the mixture of the P450 enzyme and the reductase (tube from step 3.3.3.). (See Note 6). Nitrogen is blown over the tube after the addition of each aliquot, and then the tube is capped and gently inverted to mix the enzymes with the detergent/lipid.
5. After the final addition of the solution from step B.1, blow nitrogen over the tube opening, cap the tube, invert several times, and incubate at 4°C for 1 h.

3.4. Removal of Detergent

1. Add 0.25 g of BioBeads SM-2 to the solution from step 5 of Subheading 3.3, blow nitrogen over the tube opening, cap the tube, and rock at 4°C for 2 h.
2. Draw off the solution from the Bio-Beads with a 26.5 gauge needle and filter through a 5 μm syringe filter.
3. The beads are rinsed twice with 0.2 mL aliquots of 50 mM Hepes (pH 7.4) and 15 mM $MgCl_2$. These bead washes are filtered and added to the original bead filtrate. The final filtrate (containing the original filtrate in addition to the two bead washes) is typically around 0.65 mL in volume.
4. The sample is ready for use. An aliquot (0.1 mL) of the solution is routinely taken to determine the recovery of active P450 by determining the amount of enzyme capable of forming a ferrous CO-complex (33). A second 0.1–0.2 mL aliquot is run through a Superose 6 size exclusion column (MWCO 5,000 kDa) to determine the efficiency of enzyme incorporation. In this chromatographic step, it is assumed that the protein and lipid eluting in the void volume of the column represent the components that are incorporated into the bilayer, lipid vesicles. Reductase incorporation and activity is determined in the final preparation and in the fractions from the column by measuring the rate of the reductase-mediated reduction of cytochrome *c* (34). In addition, the concentration of phospholipid can also be determined in each fraction (35) in order to more accurately determine the lipid:protein ratio of the vesicular fraction.

3.5. Calculation Used to Adjust the Detergent Concentration Needed for Preparation of VRS with Different Lipid Concentrations

In adjusting our VRS method to changing concentrations of lipid, we assume that at the optimized conditions, the lipid bilayer is sufficiently solubilized to allow for the physical incorporation of the enzymes into the PC bilayer vesicles that form as the detergent is removed. If the *monomeric* concentration of sodium glycocholate in our optimized VRS preparation is equal to the CMC (as predicted by the studies that have examined the solubilization of membranes by detergents (discussed in the Introduction)) we can approximate the concentration of lipid-bound detergent from the total concentration used in the optimized conditions (those described in the protocol). More specifically, with a molecular weight of 488 g/mol, a concentration of 1% sodium glycocholate solution corresponds to 20.5 mM. The CMC of this detergent is 7.1 mM (36). Thus, we assume that the concentration of lipid-bound detergent in the mixed detergent-lipid micelles is 13.4 mM (20.5 mM–7.1 mM). This value can be used to calculate the effective detergent to lipid ratio, and at any concentration of lipid, the concentration of detergent needed to achieve the same level of solubilization is then determined by first using the effective detergent to lipid ratio to calculate the amount of lipid-bound detergent and adding this amount to the CMC.

An example is shown to better explain the calculation involved:

Amount of lipid in the defined, optimized condition (those described in the protocol) = 1.25 μmol
Amount of lipid-bound detergent = 6.7 μmol (13,400 μM * 0.0005 L)
Thus, the effective detergent:lipid ratio = 5.36 mole of detergent/mole of PC
Amount of monomeric detergent in the reconstitution = 3.6 μmol (7,100 μM * 0.0005 L)

If one wants to make a reconstituted system with five times the lipid concentration as that in the defined, optimized condition, the calculation is as follows:

Amount of PC = 6.25 μmol
Amount of bound detergent = 5.36 detergent/PC × 6.25 μmol = 33.5 μmol detergent
Amount of monomeric detergent = CMC*volume = 3.6 μmol
Sum of monomeric and bound detergent in the 0.5 mL reconstitution = 37.1 μmol
Detergent concentration = 74.2 mM = 3.62%. Thus, one should add 3.62% (w/v) sodium glycocholate to solubilize the lipid in a VRS containing 6.25 μmol of phospholipid (*see* Note 7).

4. Notes

1. We have found that when the lipid concentration is ≤1250 μM, the bilayer vesicles of lipid do not readily form. Thus, in order to make vesicles with P450: lipid ratios ≤250:1, the P450 concentration should be lowered (and not the concentration of lipid) from those stated in the general method.
2. We have also prepared the VRS by drying the lipid solution under a stream of N_2. The lipid must be dried slowly and for a minimum of 2 h. If the flow of the nitrogen stream is too high, residual chloroform may be "trapped" under a film of lipid. This is apparent if the detergent-solubilized lipid (Step B.1) is cloudy. If this is observed, the enzymes will not incorporate properly in the lipid vesicles of the final preparation. In general, it has been found that the reductase does not incorporate into the vesicle bilayer as readily if the lipid is dried under N_2 as compared to the results obtained with lyophilized lipid.
3. Our lab flushes the lyophilizer with anaerobic nitrogen that has run through a heated column of BASF palladium catalyst (BASF RO-20).
4. Solutions are bubbled with N_2 (or preferably Argon) for 2–5 min before adding to the lipid.

5. We have also prepared the VRS using 0.5 M potassium phosphate (pH 7.25) without the $MgCl_2$ and have not observed any significant differences in the quality of the final preparations.
6. Initially, we would add the enzymes from the concentrated stock solutions to the sonicated lipid and detergent solution after the latter was diluted with water. However, it was found that this method resulted in a much higher loss of the active P450. It seems the incremental addition of the detergent is less harmful to the P450.
7. The amount of BioBeads should not be adjusted to remove the excess detergent. The adsorptive capacity of BioBeads has been studied extensively (37), and it has been shown that the beads will also adsorb lipids. Thus, when the beads are scaled up with detergent, the excess lipid is more readily adsorbed and the desired increase in lipid concentration in the VRS is not attained.

References

1. Porter TD, Coon MJ (1991) Cytochrome P-450. Multiplicity of isoforms, substrates, and catalytic and regulatory mechanisms. J Biol Chem 266:13469–13472
2. Guengerich FP (2001) Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. Chem Res Toxicol 14:611–650
3. Weng Y, Fang C, Turesky RJ, Behr M, Kaminsky LS, Ding X (2007) Determination of the role of target tissue metabolism in lung carcinogenesis using conditional cytochrome P450 reductase-null mice. Cancer Res 67:7825–7832
4. Iyanagi T (2007) Molecular mechanism of phase I and phase II drug-metabolizing enzymes: implications for detoxification. Int Rev Cytol 260:35–112
5. Rooney PH, Telfer C, McFadyen MC, Melvin WT, Murray GI (2004) The role of cytochrome P450 in cytotoxic bioactivation: future therapeutic directions. Curr Cancer Drug Targets 4:257–265
6. Hannemann F, Bichet A, Ewen KM, Bernhardt R (2007) Cytochrome P450 systems–biological variations of electron transport chains. Biochim Biophys Acta 1770:330–344
7. Guengerich FP (1989) Characterization of human microsomal cytochrome P-450 enzymes. Annu Rev Pharmacol Toxicol 29:241–264
8. West SB, Lu AYH (1972) Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds and endogenous substrates. V. Competition between cytochromes P-450 and P-448 for reductase in 3, 4-benzpyrene hydroxylation. Arch Biochem Biophys 153:298–303
9. Saine SE, Strobel HW (1976) Drug metabolism in liver tumors. Resolution of components and reconstitution of activity. Mol Pharmacol 12:649–657
10. Strobel HW, Lu AYH, Heidema J, Coon MJ (1970) Phosphatidylcholine requirement in the enzymatic reduction of hemoprotein P-450 and in fatty acid, hydrocarbon, and drug hydroxylation. J Biol Chem 245:4851–4854
11. Ingelman-Sundberg M (1977) Phospholipids and detergents as effectors in the liver microsomal hydroxylase system. Biochim Biophys Acta 488:225–234
12. Taniguchi H, Pyerin W (1988) Phospholipid bilayer membranes play decisive roles in the cytochrome P-450-dependent monooxygenase system. J Cancer Res Clin Oncol 114:335–340
13. Causey KM, Eyer CS, Backes WL (1990) Dual role of phospholipid in the reconstitution of cytochrome P- 450 LM2-dependent activities. Mol Pharmacol 38:134–142
14. Balvers WG, Boersma MG, Veeger C, Rietjens IM (1993) Kinetics of cytochromes P-450 IA1 and IIB1 in reconstituted systems with dilauroyl- and distearoyl-glycerophosphocholine. Eur J Biochem 215:373–381
15. Autor AP, Kaschnitz RM, Heidema JK, Coon MJ (1973) Sedimentation and other properties of the reconstituted liver microsomal mixed-function oxidase system containing cytochrome P-450, reduced triphosphopyridine nucleotide-cytochrome P-450 reductase, and phosphatidylcholine. Mol Pharmacol 9:93–104

16. French JS, Guengerich FP, Coon MJ (1980) Interactions of cytochrome P-450, NADPH-cytochrome P-450 reductase, phospholipid, and substrate in the reconstituted liver microsomal enzyme system. J Biol Chem 255:4112–4119
17. Reed JR, Kelley RW, Backes WL (2006) An evaluation of methods for the reconstitution of cytochromes P450 and NADPH P450 reductase into lipid vesicles. Drug Metab Dispos 34:660–666
18. Taniguchi H, Imai Y, Iyanagi T, Sato R (1979) Interaction between NADPH-cytochrome P-450 reductase and cytochrome P-450 in the membrane of phosphatidylcholine vesicles. Biochim Biophys Acta 550:341–356
19. Ingelman-Sundberg M, Glaumann H (1980) Incorporation of purified components of the rabbit liver microsomal hydroxylase system into phospholipid vesicles. Biochim Biophys Acta 599:417–435
20. Schwarz D, Gast K, Meyer HW, Lachmann U, Coon MJ, Ruckpaul K (1984) Incorporation of the cytochrome P-450 monooxygenase system into large unilamellar liposomes using octylglucoside, especially for measurements of protein diffusion in membranes. Biochem Biophys Res Commun 121:118–125
21. Ingelman-Sundberg M, Blanck J, Smettan G, Ruckpaul K (1983) Reduction of cytochrome P-450 LM2 by NADPH in reconstituted phospholipid vesicles is dependent on membrane charge. Eur J Biochem 134: 157–162
22. Bosterling B, Trudell JR, Galla HJ (1981) Phospholipid interactions with cytochrome P-450 in reconstituted vesicles. Preference for negatively-charged phosphatidic acid. Biochim Biophys Acta 643:547–556
23. Kawato S, Gut J, Cherry RJ, Winterhalter KH, Richter C (1982) Rotation of cytochrome P-450. I. Investigations of protein-protein interactions of cytochrome P-450 in phospholipid vesicles and liver microsomes. J Biol Chem 257:7023–7029
24. Schwarz D, Pirrwitz J, Ruckpaul K (1982) Rotational diffusion of cytochrome P-450 in the microsomal membrane-evidence for a clusterlike organization from saturation transfer electron paramagnetic resonance spectroscopy. Arch Biochem Biophys 216: 322–328
25. Taniguchi H, Imai Y, Sato R (1987) Protein-protein and lipid-protein interactions in a reconstituted cytochrome P-450 dependent microsomal monooxygenase. Biochem 26: 7084–7090
26. Hjelmeland LM (1990) Solubilization of native membrane proteins. Meth Enzymol 182: 253–264
27. Jackson ML, Schmidt CF, Lichtenberg D, Litman BJ, Albert AD (1982) Solubilization of phosphatidylcholine bilayers by octyl glucoside. Biochem 21:4576–4582
28. Bayerl TM, Werner G-D, Sackmann E (1989) Solubilization of DMPC and DPPC vesicles by detergents below their critical midellization concentration: high-sensitivity differential scanning calorimetry, Fourier transform infared spectroscopy and freeze-fracture electron microscopy reveal two interaction sites of detergents in vesicles. Biochim Biophys Acta 984:214–224
29. Reed JR, Brignac-Huber LM, Backes WL (2008) Physical incorporation of NADPH-cytochrome P450 reductase and cytochrome P450 into phospholipid vesicles using glycocholate and Bio-Beads. Drug Metab Dispos 36:582–588
30. Pike LJ (2004) Lipid rafts: heterogeneity on the high seas. Biochem J 378:281–292
31. Browman DT, Resek ME, Zajchowski LD, Robbins SM (2006) Erlin-1 and erlin-2 are novel members of the prohibitin family of proteins that define lipid-raft-like domains of the ER. J Cell Sci 119:3149–3160
32. Holloway PW (1973) A simple procedure for removal of Triton X-100 from protein samples. Anal Biochem 53:304–308
33. Omura T, Sato R (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem 239: 2370–2378
34. Phillips AH, Langdon RG (1962) Hepatic triphosphopyridine nucleotide-cytochrome c reductase: isolation, characterization, and kinetic studies. J Biol Chem 237:2652–2660
35. Stewart JC (1980) Colorimetric determination of phospholipids with ammonium ferrothiocyanate. Anal Biochem 104:10–14
36. Antonian L, Deb S, Spivak W (1990) Critical self-association of bile lipids studied by infrared spectroscopy and viscometry. J Lipid Res 31:947–951
37. Levy D, Bluzat A, Seigneuret M, Rigaud JL (1990) A systematic study of liposome and proteoliposome reconstitution involving Bio-Bead-mediated Triton×-100 removal. Biochim Biophys Acta 1025:179–190

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