Phase separation in the isolation and purification of membrane proteins

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Phase separation is a simple, efficient, and cheap method to purify and concentrate detergent-solubilized membrane proteins. In spite of this, phase separation is not widely used or even known among membrane protein scientists, and ready-to-use protocols are available for only relatively few detergent/membrane protein combinations. Here, we summarize the physical and chemical parameters that influence the phase separation behavior of detergents commonly used for membrane protein studies. Examples for the successful purification of membrane proteins using this method with different classes of detergents are provided. As the choice of the detergent is critical in many downstream applications (e.g., membrane protein crystallization or functional assays), we discuss how new phase separation protocols can be developed for a given detergent buffer system.

Introduction

Detergents are used to extract membrane proteins from biological membranes and to mediate their solubility in aqueous solutions, which is a prerequisite for further protein purification (1). Purification of membrane proteins is generally tedious (2) because they are removed from their native membrane environment into a detergent buffer that can only partially mimick the physical and chemical properties of a lipid membrane. Thus, many membrane proteins do not retain their biological activity after extraction, or do so only partially or only under very special buffer conditions.

After extraction, purification of membrane proteins is usually accomplished by the same chromatography methods used for soluble proteins, the difference being that detergents must be present in the buffers at all times. Detergents do not interfere with ion exchange or metal chelate chromatography, and only sometimes with other affinity chromatography methods. In size exclusion chromatography, the apparent molecular weight of membrane proteins is increased by the bound detergent.

Phase separation is a powerful alternative or addition to chromatography-based purification protocols. It can be used directly on solubilized membranes, separating membrane proteins from soluble proteins and other hydrophilic impurities. Such crude purification protocols are used in membrane proteomics or as a first purification step followed by chromatography (3,4). Alternatively, phase separation can be exploited as a simultaneous concentration and polishing step at a later stage of purification (5).

Using phase separation steps in the purification of membrane proteins has a number of benefits. The protocols are simple to use, do not require complex laboratory equipment, are easily scaled up to large volumes, and are compatible with most chromatographic methods. Especially, the removal of hydrophilic compounds is very efficient. Moreover, membrane proteins can be simultaneously purified and concentrated, comparable in efficiency with precipitation protocols for soluble proteins that employ $(NH_4)_2SO_4$ or other salts. A possible disadvantage of the method are the high detergent concentrations involved, which can be unfavorable for protein stability and can interfere with biochemical assays and binding processes. Dialysis or other methods like detergent absorption or gel filtration might be necessary to remove excess detergent from the solution.

Here, we describe the physical and chemical parameters that influence phase separation of the different classes of detergents commonly used in membrane protein purification. We discuss successful phase separation protocols and give guidelines as to how such protocols can be adapted to new detergent buffer systems.

General Properties of Detergents

Detergents are amphipatic molecules usually consisting of a polar or charged headgroup and an extended hydrophobic hydrocarbon chain. At very low concentrations, these molecules are soluble in water as monomers. When increasing the detergent concentration above the so-called critical micelle concentration (CMC), the detergent molecules form aggregates with a very narrow size distribution, called micelles. The size of the micelles is dependent on the type of detergent; for detergents typically used in membrane biochemistry, the aggregation number (i.e., the number of detergent molecules per micelle) can range from 2 to 3

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for sodium cholate to approximately 140 for Triton® X-100 (6). Moreover, micelle size and CMC depend on the ionic strength and the temperature of the detergent solution. While the CMC of ionic detergents decreases with increasing salt concentrations but is hardly affected by temperature, the CMC of nonionic detergents is only little affected by the presence of salts but increases with increasing temperature (7). Other factors that influence the size of the micelle and the CMC are pressure, pH, and the presence of impurities (6).

Cloud Point and Phase Separation

The so-called cloud point can be reached by increasing the detergent concentration or by changing the temperature or the salt concentration of an aqueous micellar detergent solution. The micellar solution then becomes turbid; the micelles become immiscible with water and form large aggregates that will separate from the water phase. Most but not all of the detergent partitions into the detergent-rich phase, which in turn still contains a substantial amount of water. Depending on the detergent and the buffer conditions, the detergent-rich phase can be completely clear or turbid and can be found on top or below the detergent-poor phase. This process is called phase separation or, sometimes, cloud point extraction. Phase separation occurs due to a temperature-dependent difference in entropy between the one-phase and two-phase system. The effect is similar to protein precipitation using polyethelene glycol (PEG) or $(NH_4)_2SO_4$ where not enough free water is available to keep the protein fully hydrated and thus, soluble. Likewise, the detergent micelles aggregate and form a separate phase in which less water covers their surface, and this aggregation behavior is influenced by temperature, salts, and polymers.

Phase Diagrams

Figure 1 shows two simplified examples of detergent phase diagrams in aqueous solution, displaying the phase behavior of two different detergents upon increase in temperature and/or detergent concentration. All

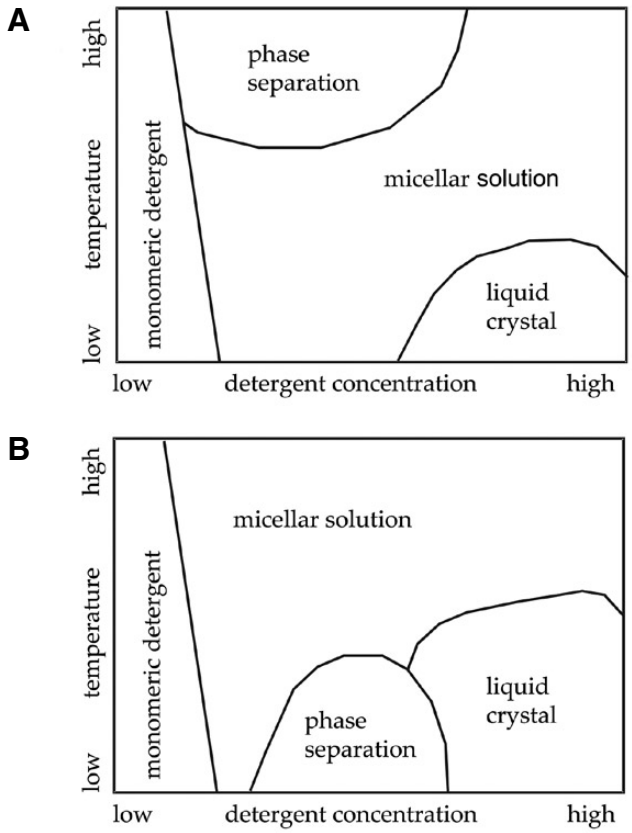

Figure 1. (A) A simplified phase diagram of a detergent with a lower consolute boundary. Detergents of this type are usually nonionic. All poly ethylene glycol (PEG)-based detergents fall into this category. (B) A simplified phase diagram of a detergent with an upper consolute boundary, which is frequently observed for zwitterionic detergents and for glycosidic detergents.

detergents are monomeric in solution at low concentrations. The line separating the monomeric detergent solution from the micelle solution represents the CMC that by itself is dependent on temperature. Above this concentration, the detergent forms micelles of a defined size. The borderline between micellar solution and phase separation in the phase diagram is called either upper or lower consolute boundary. In Figure 1A, the detergent's cloud point is reached by increasing the temperature of a micelle solution of intermediate concentration to cross the lower consolute boundary, while the cloud point of the detergent in Figure 1B is reached by decreasing the temperature of a micelle solution of intermediate detergent concentration to cross the upper consolute boundary. Phase behavior as in Figure 1A is typical for PEG-based detergents, while an upper consolute boundary as in Figure 1B is observed for many zwitterionic and glycosidic detergents. When the cloud point is reached, the detergent will form a separate phase, and the water phase is depleted of detergent. At extremely high detergent concentrations and low temperature (usually at concentrations not relevant for biochemical purposes), a liquid crystalline phase will form which contains well-ordered detergent molecules. Liquid crystalline phases can be classified into cubic, hexagonal, or lamellar phases (8) depending on many factors not to be discussed here. It is important to note that temperature versus concentration phase diagrams can change dramatically when the ionic strength of the solution is varied. Thus, the cloud point of a micelle solution can be reached by adding salt instead of changing the temperature. This has been extensively studied for detergents of the Triton family (9). Other additives like glycerol or urea strongly affect the phase behavior of a detergent, as does the presence of lipids in mixed

Table 1. Detergents Used for Phase Separation in the Purification of Membrane Proteins

Detergent	CMC (mM)	Cloud point at Low Ionic Strength	Conditions and Additives to Induce Phase Separation	Other
Triton X-100	0.17–0.3 (30)	64°–65°C (23,24,30)	Temperature increase (23). Addition of 9-23% $(NH_4)_2SO_4$ or 16%–25% NaCl decreases cloud point to room temperature (9). Addition of dextran and PEG 40000 decreases cloud point (17).	Solid (liquid crystal?) phase above 20% $(NH_4)_2SO_4$ (9).
Nonidet P-40	0,3 (6)	63°–67°C	Temperature increase (23). Addition of 6-16% $(NH_4)_2SO_4$ or 10-25% NaCl decreases cloud point to room temperature (9).	Solid (liquid crystal?) phase above 18% $(NH_4)_2SO_4$ (9).
Triton X-114	0.2–0.35 (30)	20°–25°C (23,30)	Temperature increase (23). Addition of 20% Glycerol reduces cloud point to 4°C (25).	
Tween-20	0.04–0.06 (30)	76°C (29)	Addition of dextran and PEG 40000 decreases cloud point (17).	
Tween-80	0.01–0.02 (30)	93°C (30)	Addition of dextran and PEG 40000 decreases cloud point (17).	
C8POE	6.6 (73)	58°C (73)	Temperature increase. Addition of 20% $(NH_4)_2SO_4$ decreases cloud point to room temperature (5).	Can be removed easily by dialysis (5).
C8E4	6.5–8.5 (30,73)	35°–40°C (22,30)	Temperature increase.	Can be removed easily by dialysis.
C10E4	0.64–0.81 (30)	19.5°–21°C (22,30,31)	Temperature increase.	
C12E5	0.45–0.65 (30)	26°–32° C (22,30,31)	Addition of dextran and PEG 40000 decreases cloud point (17).	
C12E8	0.07–0.11 (30)	74°–79°C (22,30,31)	Addition of glycerol or dextran and PEG 40000 decreases cloud point (to 43°C at 60% gylcerol) increases cloud point (17).	
Brij35	0.06–0.1 (30)	>100°C (30)	Dextran and PEG 40000 decrease cloud point (17).	
β-OG	25 (49)	<0°C	Temperature decrease and addition of PEG (35). Addition of dextran or PEG 40000 (17), lipids (10), PEG increases (35,38), glycerol reduces cloud point (38).	Can be removed easily by dialysis.
β-DM	0.15 (6)	<0°C	Temperature decrease and addition of PEG (35). Addition of dextran or PEG 40000 increases cloud point (17).	
β-OTG	9 (38)	7°C (38)	Temperature decrease and addition of PEG (38). Addition of PEG 6000 increases, glycerol reduces cloud point (38).	
LDAO (DDAO)	1.7–2.2 (30,40)		pH shift at low ionic strength; phase separation occurs at a pH range from pH 6.0–7.5 (39). Addition of small amounts of cationic detergents influences cloud point (39).	Phase separation occurs in small droplets (40).
Digitonin			Temperature decrease and addition of PEG 6000 (42).	No CMC available from literature; properties may vary between different charges.

All concentrations given as percentages are w/v unless otherwise stated. CMC, critical micelle concentration.

micelles (10) and the addition of polymers (see next section).

Using Phase Separation to Purify Membrane Proteins

Detergent-based phase separation was developed from polymer-based phase separation. When two sufficiently different water-soluble polymers (e.g., dextran and PEG) are mixed in solution at high concentrations, two immiscible aqueous phases form. Different proteins will partition into the two phases depending on their physical properties and the properties of the polymers (11). When using polymers of different charges (e.g., cationic or anionic PEG derivatives), a protein can be shifted from one phase to the other simply by changing the pH from above to below its isoelectric point at low ionic strength of the buffer system (12). Variations of this method are two-phase systems comprising PEG and salt (13). Aqueous two-phase systems have been adapted for industrial-scale processes (14), but are only infrequently used in laboratory applications.

Polymer-based aqueous two-phase systems can be used for detergent-solubilized membrane proteins where the properties of the bound detergent will influence the partitioning of the membrane protein in question. As this involves two polymers in addition to the protein and the detergent, such systems are difficult to handle, and the removal of polymers for downstream applications is an additional challenge. But the PEG-dextran system in combination with the detergent Triton X-100 has been successfully used to purify *Escherichia coli* outer membrane phospholipase (15) and *Micrococcus lysodeikticus* cytochrome b556 (16).

Detergents can phase-separate in the presence of only one polymer, reducing the complexity of the system. In this case, the detergent micelles themselves take the role of the second polymer. This works for many nonionic detergents in combination with either PEG or dextran and has been used in membrane protein purification (17). When a hydrophobic tag is added to otherwise soluble proteins, they can partition into the detergent-rich phase in such systems (18). Phase separation can also be accomplished in systems without other polymers, provided that the cloud point of the detergent in question is at a temperature not harmful to protein structure and function. If this is not the case, the cloud point can be modified by the addition of salts or a change of pH, as discussed in the following sections for the different classes of detergents and shown in Figure 2. Phase separation of detergents is easily scaled up and applied to industrial processes (19–21). Table 1 summarizes the phase separation properties of detergents that have been successfully used for membrane protein purification.

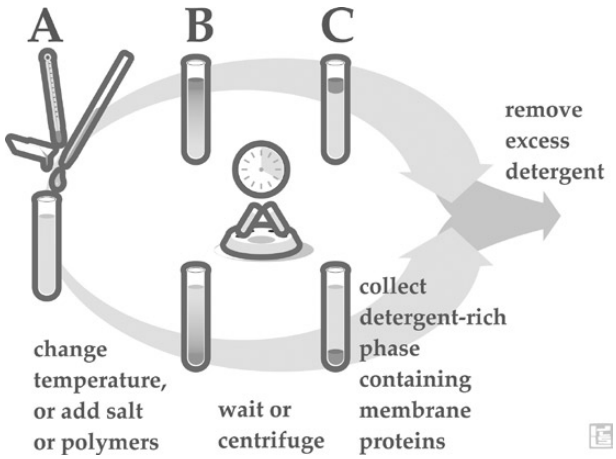

Figure 2. Detergent-based phase separation can be used to purify or to concentrate membrane proteins. When the consolute boundary of a detergent solution is crossed due to a change in temperature or ionic strength of the buffer or due to the addition of polymers (A), a separate detegent-rich phase is formed (B). Time (usually several hours) or centrifugation is necessary to fully separate the two phases. Note that the detergent-rich phase containing the membrane proteins can be found either above or below the detergent-depleted phase, depending on the density of the buffer system (C). As the detergent-rich phase is typically 1%–10% of the total volume, significant concentration factors can be reached.

The Triton Family

Most published protocols for phase separation rely on detergents of the Triton family. *tert*-octylphenol poly (ethyleneglycolether)$_n$ is commercially available under different trademarks. The slight differences between the products are in the average size *n* and the size distribution of the PEG-based headgroup. In Triton X-100, Nonidet® P-40 (NP-40), and Igepal® CA-630, *n* is 9.6, 9.0, and 9.5, respectively (22).

The classic phase separation used for membrane proteins relies on the detergent Triton X-114 (n = 7–8) and was developed by Bordier in 1981 (23). This detergent has a cloud point at about 22°C at concentrations relevant for membrane protein work, allowing for easy separation at room temperature while only one phase is present when samples are kept on ice. Many different membrane proteins have been extracted and purified using the Triton X-114 system from animal and plant tissues, as well as from microorganisms (22). For better separation of the two phases, a 6% sucrose cushion can be used; after centrifugation at room temperature, the detergent-rich phase can be collected from below the sucrose cushion (24). In cases where room temperature is not favorable because of limited protein stability, the cloud point of Triton X-114 can be reduced to 4°C by adding 15%–20% glycerol (25).

Triton X-100 has a cloud point of 64°C (23) and thus needs additives that lower the working temperature for phase separation with membrane proteins. Adding >9% of $(NH_4)_2SO_4$ or >16% NaCl reduces the cloud point of 2% Triton X-100 to room temperature (9), which can be exploited for the fractionation of membrane proteins (26). The detergent-rich phase becomes solid at $(NH_4)_2SO_4$ concentrations >20%, probably forming a liquid crystalline phase as discussed previously. NP-40 behaves similarly but needs slightly lower salt concentrations for phase separation at room temperature (9).

All *tert*-octylphenol-based detergents strongly absorb UV light, which can interfere with optical assays. Moreover, they have rather low CMCs and cannot be removed from solution by dialysis. Thus, they are not an ideal choice for many applications, albeit they are mild detergents that rarely denature membrane proteins.

The Tween Family

Tween® detergents are polyoxyethylene sorbitan esters of fatty acids that are used for membrane protein solubilization because they are relatively mild detergents that rarely interfere with enzymatic assays (6). As for Triton and other nonionic detergents, the salt effects on the CMC and aggregation number of Tween detergents are low (27). Phase separation of a 2% solution of Tween 20 or Tween 80 can be initiated by addition of 16% or 12 % $(NH_4)_2SO_4$, respectively (9), or by the addition of PEG or dextran (17). Tween 80 in combination with two complex polymers has been used to purify human and recombinant apolipoprotein A1 (28). Frequently, Tween detergents are only used to solubilize proteins that are then phase-separated using the well described Triton X-114 method because of their very similar properties. Tween 20 and Tween 80 can replace Triton X-100 in phase separation protocols using polymers (29).

Polyoxyethylene Glycol Monoether Detergents

Polyoxyethylene glycol monoethers are commercially available in many different chain lengths, either as pure substances or mixtures of a certain size distribution. A list of available PEG-monoether detergents and their cloud points, CMCs, and aggregation numbers can be found in various physical chemistry reviews (30,31). They are named C_xE_y according to their alkyl chain length (x) and the number of polyoxyethylene glycol units in the headgroup (y). Frequently, these detergents are better known by their trade names (e.g., Brij® 35 for $C_{12}E_{23}$ or Emulgen 147 for $C_{12}E_{25}$). The cloud point of these detergents depends on the length of the alkyl chain as well as of the head group; at constant alkyl chain length, the cloud point increases with head group size (e.g., from 5°–8°C for C_8E_3 to 96°C for C_8E_8). At the same time, the cloud point decreases with increasing alkyl chain size (e.g., from 43°C for C_8E_4 to 4°C for $C_{12}E_4$) (31). In spite of this wide variability of cloud points that should allow for phase separation under almost any buffer condition at ambient temperature, few protocols exist for the purification of membrane proteins using PEG monoether phase separation. Recently, C_8POE, a C_8E_x mixture with x = 2–9, has been used in the purification of a bacterial outer membrane protein. The major component of C_8POE is C_8E_4, whose cloud point is 43°C, but the cloud point was modified to room temperature with the help of 20% $(NH_4)_2SO_4$ (5). A major advantage of C_8POE over Triton X-114 is its high CMC, which allows for easy removal of excess detergent by dialysis. $C_{10}E_4$ displays the same, favorable properties with the added advantage that phase separation occurs at room temperature already in low-salt buffers (32).

As with detergents of the Triton and Tween series, polyoxyethylene glycol monoethers can be used in polymer-based phase separation with dextran or PEG. A detailed study with the membrane proteins bacteriorhodopsin and cholesterol oxidase was done for the detergents $C_{12}E_5$, $C_{12}E_8$, and $C_{12}E_{23}$ (i.e., Brij 35) in combination with dextran T500 or PEG 40000. Both polymers shifted the cloud points of the detergents such that phase separation occurred at room temperature while only one phase existed at 4°C (17). Similarly, polyvinylpyrrolidone can be used to induce phase separation of PEG-based detergents (33).

Glycosidic Detergents

Detergents with glycosidic headgroups are frequently used in membrane protein crystallography. The most common detergents of this class are alkyl β-glucosides and alkyl β-maltosides, with alkyl chain lengths typically ranging from 8 to 14. It seems that glycosidic detergents have unique properties compared with detergents having other headgroups, making them especially suitable for the solubilization and stabilization of membrane proteins; the interface region of β-dodecyl-maltoside (β-DM) micelles provides an aqueous-like microenvironment, which is not the case for other (non-glycosidic) detergents (34). In buffer systems containing PEG, phase diagrams of glycosidic detergents display an upper consolute boundary and thus undergo phase separation at low temperature (35) (Figure 1B). This can be exploited for the purification of membrane proteins, using PEG (or dextran) with β-octylglucoside (β-OG), β-dodecyl-maltoside (17,36), or β-octylthioglucoside (β-OTG) (37,38). Depending on the ratio of detergent and lipid, phase separation of β-OG can occur during membrane solubilization without the addition of polymers and has been used to purify nicotinic acetylcholine receptor (10).

Other Nonionic Detergents

N,N-dimethyldodecylamine-N-oxide (DDAO; also called N,N-lauryldimethylamine-N-oxide or LDAO) has been successfully used to purify bacterial reaction centers via phase separation. In this special application, a shift from pH 8.0 to a pH <7.0 was used to initiate phase separation. The detergent-rich phase has a density very similar to the water phase in this system, resulting in an emulsion that does not separate readily upon centrifugation (39). The phase separation is promoted by an increase of temperature, but is inhibited by addition of salt (40).

Digitonin, a mild detergent used mainly for the selective solubilization of eukaryotic plasma membrane proteins (41), can be used in phase separation procedures. The phase separation takes place after addition of 13% PEG 6000 at 0°C to digitonin-solubilized membranes (e.g., of staphylococci) (42).

Many other classes of nonionic detergents are used in membrane protein biochemistry and crystallography for which no detailed information exists on phase diagrams and cloud points. Examples of such detergents are alkyl-N-methylglucamides (MEGA-8 to MEGA-10) (43) and 6-O-(N-heptylcarbamoyl)-methylglucosid (HECAMEG), all of which are easily dialyzable and do not denature

membrane proteins even at high concentrations.

Zwitterionic Detergents

Many phase diagrams of zwitterionic detergents display an upper consolute boundary (44), similar to that of glycosidic detergents (Figure 1B). Their cloud point increases with increasing alkyl chain size. Unfortunately, detailed physicochemical studies exist only on alkyl dimethylammonioethylsulfates and alkyl dimethylammoniopropylsulfates that are used for extraction of small hydrophobic organic compounds and not for membrane protein biochemistry (44,45). Alkyl dimethylammoniopropylsulfonates (alkyl chain lengths between 10 and 16, better known as sulfobetaine detergents or Zwittergents®) are mild detergents used for the solubilization of membrane proteins (46). Their cloud points lie below 0°C; thus, their upper consolute boundary cannot be crossed to induce phase separation by temperature decrease unless buffer additives bring the cloud point to room temperature (44). The cholate-based zwitterionic detergent 3-[(3-cholamidopropyl)-dimethylammnio]l-propane sulfonate (CHAPS) (47) is frequently used to solubilize membrane proteins; however, no information on phase diagrams or cloud points exists, while C_8-lecithin is well studied for its phase separation behavior, but has not been used in the extraction of membrane proteins (48).

Anionic Detergents

Sodium dodecyl sulfate (SDS) is frequently used in protein applications, but usually denatures proteins. As for other anionic detergents, the solution behavior of SDS is strongly dependent on the ionic strength of the buffer system. The aggregation number of SDS more than doubles from 62 to 132, while its CMC is reduced 16-fold from 8.1 to 0.5 mM when the NaCl concentration is increased from 0–500 mM at 25°C (49). To induce SDS phase separation, 0.4–0.5 M NaCl is added to the SDS protein mixture (50,51). Obviously, most proteins will denature when exposed to high SDS concentrations, but it is conceivable that extremely stable membrane proteins (e.g., some bacterial outer membrane proteins) can be purified using SDS. The anionic detergents cholate and desoxycholate seem to stabilize membrane proteins very well, but they precipitate upon addition of salts and thus cannot be used for phase separation (9).

Cationic Detergents

Cationic detergents denature most proteins, just like SDS does (52). However, dodecyltrimethylammonium bromide (DTAB or C_{12}TAB) has been successfully used to isolate intact rhodopsin from bovine retina (53). Thus, also cationic detergents could be used in phase separation procedures for the isolation of membrane proteins. Tricaprylylmethylammonium chloride (Aliquat®-336) in combination with Na_2SO_4 allows to concentrate peptide toxins from water samples through phase separation (54). Several phase separation protocols use mixtures of cationic with nonionic detergents. Addition of small amounts of DTAB induces the phase separation of LDAO in the purification of bacterial reaction centers (39). Mixtures of $C_{10}E_4$ with C_{10}TAB improve the phase separation-based purification of glucose-6-phosphate dehydrogenase (55).

Mixtures of Detergents

In some cases, it can be favorable to mix different detergents to modify the phase separation properties of a detergent buffer. Mixtures of nonionic with cationic detergents have been used sucessfully to purify membrane proteins (see section entitled Cationic Detergents), and mixtures of $C_{10}E_4$ and OTG lead to improved phase separation properties in the purification of bacterial reaction centers compared with the single detergents (56). A mixture of Triton X-114 and the zwitterionic detergent SB-10 ensures complete solubilization and enrichment of membrane proteins via phase separation for proteomics studies, while each single detergent does not (3). Many detergents are commercially available as mixtures of different alkyl or headgroup chain lengths, which makes them cheap and thus applicable to large-scale processes [e.g., Triton (57), Angrimul® (58), or Lorol® (21)]. Whether using a mixture of detergents is acceptable for the purification of a membrane protein will mainly depend on the downstream applications; in membrane protein crystallography where the purity of the detergent influences crystal quality, this will certainly be an issue.

Detergent Removal

Membrane proteins partition into the detergent-rich phase during phase separation. The high concentrations of detergent in this phase might be harmful to the protein, and the viscosity of the phase poses technical problems for liquid handling (e.g., for pipeting of precise volumes). Collecting the detergent-rich phase and diluting it is the most simple way to re-establish a one-phase micellar solution. Dialysis against a buffer with a defined detergent concentration will work only for detergents with a high CMC, but it has the advantage of retaining the high protein concentration of the detergent-rich phase. Gel filtration can be used for buffer exchange (59), as can other chromatography methods like ion exchange or affinity chromatography. Alternatively, detergents can be bound and removed specifically, either using hydrophobic polystyrene resins (Bio-Beads®, Calbiosorb™, or Amberlite® XAD2, among others) (60) or cyclodextrins. Cyclodextrins bind to detergent monomers. They can be added to the solution and removed by dialysis together with bound detergent because they are smaller than the micelles of non-dialyzable detergents (61). Cyclodextrins can also be coupled to chromatography resins for detergent removal (62). Care has to be taken because the membrane proteins will precipitate if the detergent concentration is reduced to below the CMC.

How to Adapt the Protocol to Your Needs

The starting point for the development of a phase separation procedure is a detergent buffer whose components are determined by the membrane protein to be purified, as most solubilized membrane proteins are stable only in certain detergents (63,64).

This buffer is then tested for its phase separation properties. In the most simple cases, phase separation occurs upon an increase or decrease in temperature (see Table 1 and Figure 1). Frequently, phase separation can be initiated by adding high concentrations of $(NH_4)_2SO_4$ or other salts. Similarly, most detergents will undergo phase separation upon addition of PEG, dextrans, or other high-molecular weight polymers. The stability of the membrane protein in the presence of high salt or polymer concentrations needs to be tested. Moreover, care has to be taken that the protein will not denature when exposed to the high detergent concentrations that occur during phase separation. But if these conditions are met, phase separation conditions can be found for most cases, even though phase diagrams are not available for many specialty detergents. Information on phase separation conditions can also be gained from membrane protein crystallization trials, as many membrane proteins crystallize from detergent solutions under conditions close to the cloud point of the detergent (65–67).

A simple titration of the detergent buffer of choice with salts or polymers is usually enough to establish a useful protocol. Phase separation can be observed by eye, by turbidity measurements in a spectrophotometer, or by static light scattering (68). A more detailed analysis of phase separation properties and kinetics can be obtained using combined transmission and static light scattering measurements (69). Adding a hydrophobic dye to the solution can improve this cloud point assay in combination with ultraviolet-visible spectroscopy (UV-VIS) or fluorescence detection (70). Sometimes, phase separation occurs only above or below a certain temperature even after addition of salts or polymers; we recommend to do the titrations at room temperature and at 4°C. Centrifugation can speed up the separation of the phases.

After collecting the detergent-rich phase, excess detergent and salt or polymer has to be removed as discussed previously. Alternatively, phase separation can be done using the established protocols reviewed here, followed by detergent exchange using dialysis or chromatographic methods. A promising new phase separation method is the use of polymers containing metal-chelating groups able to bind polyhistidine tags. His-tagged membrane proteins can then partition into the polymer phase (instead of the detergent-rich phase), allowing for a separation of tagged from untagged membrane proteins and the protection of the tagged protein from contact with extreme detergent concentrations (71). This method can also be used to purify membrane vesicles containing His-tagged protein (72).

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

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