REVIEW

Aqueous polymer two-phase systems: Effective tools for plasma membrane proteomics

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Plasma membranes (PMs) are of particular importance for all living cells. They form a selectively permeable barrier to the environment. Many essential tasks of PMs are carried out by their proteinaceous components, including molecular transport, cell-cell interactions, and signal transduction. Due to the key role of these proteins for cellular function, they take center-stage in basic and applied research. A major problem towards in-depth identification and characterization of PM proteins by modern proteomic approaches is their low abundance and immense heterogeneity in different cells. Highly selective and efficient purification protocols are hence essential to any PM proteome analysis. An effective tool for preparative isolation of PMs is partitioning in aqueous polymer two-phase systems. In two-phase systems, membranes are separated according to differences in surface properties rather than size and density. Despite their rare application to the fractionation of animal tissues and cells, they represent an attractive alternative to conventional fractionation protocols. Here, we review the principles of partitioning using aqueous polymer two-phase systems and compare aqueous polymer two-phase systems with other methods currently used for the isolation of PMs.

Keywords:
Affinity partitioning / Countercurrent distribution / Plasma membrane / Subcellular fractionation / Two-phase system

1 Introduction

Plasma membranes (PMs) represent the interface between biological cells and their external milieu. Thus, all communication of a cell with its environment has to occur via PMs. Each PM has therefore a bewildering variety of proteins which facilitate the passage of larger molecules, like amino acids and sugars, as well as flow of information. The protein repertoire includes receptors for hormones [1, 2], growth factors [2], and neurotransmitters [3], transporters [4, 5], channels [6, 7], cell adhesion molecules [8], and molecules that regulate vesicular transport mechanisms such as endocytosis, exocytosis, or transcytosis [4]. Many of these proteins represent important targets for pharmacological action. Currently, PM proteins account for ~70% of all known drug targets [9]. Hence, their in-depth identification and functional characterization has become a central theme in biology, biochemistry, biomedicine, and related disciplines [10–16].

The advent of proteomic techniques, such as highly sensitive MS [17] or multidimensional LC [18] for gel-free separation of hydrophobic proteins, allow efficient global profiling of proteins. Yet, the analysis of PM proteomes represents a true challenge. PM proteins are often less abundant, which renders their identification difficult in the bulk of other, more abundant proteins of the cytoskeleton, the energy metabolism, the endoplasmic reticulum and alike [11, 14, 19]. Furthermore, each PM is unique, with cell-type specific functions and consequently a specific set of proteins. An extreme example is the olfactory epithelium of rodents,

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Abbreviations: **CD**, countercurrent distribution; **PM**, plasma membrane; **PEG**, poly(ethylene glycol)

in which ~1000 different olfactory receptor proteins are all expressed each in a different cell. Hence, at least 1000 different sensory cells can be expected in a tissue of the surface of few cm^2 [20–22]. This cell-type specific composition of the PM requires high anatomical resolution in any proteomic approach aiming at identifying important PM proteins for a given function or condition. Additionally, in biomedical research, only small amounts of biopsy material are available for diagnostic purposes or target screens. Consequently, highly selective protocols are needed to reduce the contamination of low-abundance PM proteins by other proteins, and which efficiently reduce the loss of PM proteins during fractionation [12, 14, 15].

A highly selective and efficient method to isolate PMs is partitioning of membranes in aqueous polymer two-phase systems, which separate membranes due to their different affinity for two immiscible aqueous polymer phases. In an early report, a 30–40-fold enrichment of liver PMs was obtained, as determined by enzymatic marker activities [23]. A more recent proteome analysis of liver PM, purified by two-phase partition, identified 428 proteins, of which 67% were PM proteins according to gene ontology [16]. Adaptation of the method to neural PMs resulted in a 12-fold enrichment of a PM marker enzyme. Characterization of 506 proteins by MS and stringent database search revealed that ~ 40% were true PM proteins [15]. In addition to the high selectivity of aqueous polymer two-phase systems, only small amounts of tissue are required. Whereas purification of PM from fat cells by centrifugation required 16–24 rats, only 1–2 animals were used when applying two-phase partitioning [24]. The efficacy of aqueous polymer two-phase systems was also underlined by a recent study of the PM proteome of the cerebellum that used material from a single rat [15]. Finally, the low interfacial tension, the non-denaturing conditions, and the high content of water (>80%) of aqueous polymer two-phase systems provide a mild environment that preserves protein structure and biological activity [25].

In aqueous polymer two-phase systems, the concentration and composition of the phase-forming polymers, salts, temperature, and biospecific ligands affect partitioning of PMs. First, we describe basic principles essential to the effective purification of PMs. In the second part, we present alternative methods for PM isolation and briefly discuss their strengths and drawbacks.

2 History of aqueous polymer two-phase systems

In 1896, Beijerinck [26] observed that aqueous solutions of gelatine and agar, or gelatine and soluble starch, when mixed, resulted in the separation into two liquid layers. The bottom layer contained most of the agar or starch, the top layer most of the gelatine. These findings were confirmed by studying a large number of different polymer pairs, soluble either in organic solvents or water [27, 28]. In most cases, demixing and phase separation occurred. In the mid-1950s, Albertsson made use of aqueous polymer two-phase systems to separate biological material [29, 30]. However, the selectivity obtained was often insufficient to separate different subcellular compartments. To overcome this limitation, biospecific affinity ligands, specifically recognizing a certain protein or a certain type of membrane, were introduced by conjugating them to one of the phase polymers [31, 32]. The resulting polymer-ligand adduct will partition in the corresponding polymer phase, selectively pulling the target molecules into this phase. This strategy was first employed to purify membranes rich in cholinergic receptors from the electric organ of *Torpedo californica* using bis-α-ω-trimethylamino poly(ethylene oxide) or bis-*p*-trimethylammonium phenylamino poly(ethylene oxide) as ligand-polymers [31]. The approach was taken a step forward using wheat germ agglutinin as an affinity ligand to purify the entire PM from rat liver [23]. More recently, an antigen-antibody interaction was exploited to purify caveolae from liver and lung PMs by using anti-caveolin-1, a biotinylated secondary antibody, and NeutrAvidin coupled to dextran [33].

3 Aqueous polymer two-phase systems

Above a certain concentration, most aqueous mixtures of two structural distinct water-soluble polymers give rise to a two-phase system with each phase enriched in one of the two polymers. This concentration is called the critical concentration (Fig. 1). A similar effect is observed when mixing a polymer solution with salt above a critical concentration. In this review, we focus on polymer/polymer two-phase systems, because the typically required 10–15% salt content in polymer/salt two-phase systems is detrimental to the isolation of native membrane structures. Phase-forming polymers can be chosen according to their structure, molecular weight, and ionic group. The most widely used aqueous polymer two-phase systems consist of poly(ethylene glycol) (PEG) and dextran, with the top phase enriched in PEG and the bottom phase enriched in dextran. Both polymers are cheap, require only moderate concentrations, separate rapidly, have moderately low viscosities, and can be easily buffered and rendered isotonic [25, 34].

Phase formation in two-phase systems can be attributed to the high molecular weight of the polymers and interactions between molecules of the same polymer [34, 35]. Depending on the difference in the density of the two phases, phase separation requires a few minutes to a few hours and can be accelerated by low-speed centrifugation. Partition of biomaterial in two-phase systems is based on different affinities of the components for either of the two phases. After adding biomaterial to the system and mixing, phases settle and thereby separate the different components of the biomaterial on the basis of their affinity for either of the two phases.

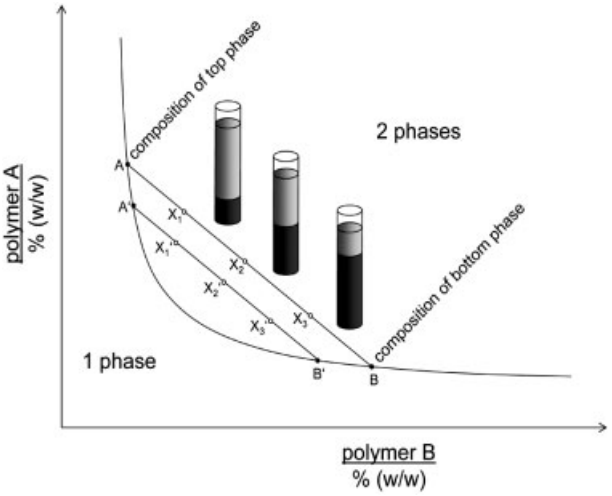

Figure 1. Schematic phase diagram. Two-phase systems are characterized by phase diagrams. The curved line (binodal curve) separates two regions of possible composition of the phase system: polymer compositions above the binodal curve give two phases, all compositions represented by points at or below the binodal curve result in one phase. All points on the binodal curve represent polymer compositions of either top or bottom phase. Each point on the lines connecting the composition of a top phase and a bottom phase in equilibrium (tie lines) represents two-phase systems with the same polymer composition in the top phase and in the bottom phase, respectively. They only differ in the volumes of the two phases. For a detailed explanation of phase diagrams, see text.

3.1 Phase diagram

The main properties of a pair of polymers are usually summarized in a phase diagram (Fig. 1), in which the concentrations of both polymers are plotted against each other. In total, three different values have to be considered: the concentrations of the two polymers in the entire system, and their respective concentrations in the top phase and bottom phase. A curved line (binodal curve) separates the diagram into two regions. Pairs of polymer concentrations above the binodal curve (open circles in Fig. 1) give rise to two phases, whereas all compositions represented by points at or below the binodal curve result in a one-phase system. In general, the concentration of polymers required for phase separation increases with decreasing molecular weight of the polymers. Straight lines (tie lines) represent polymer concentrations of the entire system. Their intersection with the binodal curve marks at one end the concentration of each of the two polymers in the top phase (intersection A) and in the bottom phase (intersection B). Tie lines become shorter with reduced polymer concentrations. At the critical point, the polymer concentrations in the top and the bottom phase equal each other, resulting in a single phase. Close to the critical point, two-phase systems are very sensitive to alterations.

All points on the same tie line result in two-phase systems with identical polymer concentrations in the top and bottom phase, respectively. They only differ in the volume of each phase [36]. The volume ratio of the phases is described by equation (1):

$$\frac{V_{top}}{V_{bottom}} = \frac{d_{bottom}}{d_{top}} \times \frac{BX}{AX} \quad (1)$$

where V_{top} and V_{bottom} are the volumes of the phases, d_{top} and d_{bottom} are the densities of the phases, and BX and AX are the distances between A and B (composition of the top phase and the bottom phase on the binodal curve, respectively) to point X on the tie line, which indicates the concentrations of the two polymers in the entire system (*i.e.*, X_1, X_2, or X_3 in Fig. 1) [34]. As the phase densities are usually close to 1, the volume ratio is mainly governed by BX:AX. Increase of BX results in an increase of the volume of the top phase, whereas an increase of AX results in an increase of the volume of the bottom phase. This easy manipulation of volumes can be exploited for particle concentration in one of the two phases. Biomolecules that preferentially partition into the top phase can be several hundredfold concentrated by a concomitant decrease in the concentration of polymer A and increase in polymer B in the entire system [25].

Phase diagrams depend not only on the polymer composition and concentration, but also on temperature, which therefore should be kept constant (Table 1). For example, in the most widely used PEG/dextran system, reduced temperature requires lower polymer concentrations for phase separation.

Table 1. Influence of different parameters on membrane partitioning[a)]

Increase in	Partition coefficient
Polymer concentration	−
Molecular weight of prevailing polymer in top phase	−
Molecular weight of prevailing polymer in bottom phase	+
Temperature	−
Salt	+/−[b)]
Affinity ligand coupled to prevailing polymer in top phase	+
Affinity ligand coupled to prevailing polymer in bottom phase	−

a) Increasing the parameters mentioned result in an altered partitioning behavior of membranes of different subcellular origin. An increase in the partitioning coefficient is equivalent with an increased partitioning of the membrane to the top phase. −, decrease in the partition coefficient; +, increase in the partition coefficient.

b) Depending on partition coefficient of the ions and charge of the biomolecule.

The partition behavior of particles in a two-phase system is usually expressed as the partitioning coefficient K, defined as the ratio of the concentrations partitioned into the top phase (c_{top}) and partitioned into the bottom phase (c_{bottom}):

$$K = \frac{c_{top}}{c_{bottom}} \quad (2)$$

Sometimes the distribution is expressed as the percentage of material found in one of the two phases.

3.2 Partition behavior of membranes in aqueous polymer two-phase systems

Close to the critical point of the commonly used PEG/dextran two-phase system, membranes, regardless of subcellular origin, tend to partition into the top phase. As outlined above, increase in polymer concentrations results in larger differences in the composition of the two phases (Fig. 1). As a result, membranes tend to partition to the interface or the bottom phase (Table 1), which is caused by an increase in surface tension at the boundary of the two phases. This dependency of the partitioning behavior on the polymer concentrations can be exploited to selectively enrich PMs in the top phase. In plants and in animals, PMs show the highest affinity for the more hydrophobic top phase, followed by Golgi vesicles, lysosomes, the endoplasmic reticulum and mitochondria [37, 38]. These different affinities are attributed to differences in hydrophilic and hydrophobic surface properties of the various membranes, which might arise, among others, from differences in their phospholipid composition [39]. An important step in the successful enrichment of PMs is therefore the determination of the polymer concentrations where all membranes except for the PM partition already by and large to the interface.

In addition, partition of membranes is influenced by the molecular weight of the polymers (Table 1). In the PEG/dextran system, increase of the molecular weight of PEG results in decreased partition of the membranes into the top phase, whereas increase of the molecular weight of dextran give rise to increased partition of the PM to the top phase [34].

3.3 Influence of salt

Both the composition and concentration of salts strongly affect the partition of charged membranes in aqueous polymer two-phase systems. Ions usually have different affinities for either of the two phases [40, 41]. However, the requirement of electroneutrality in each phase forces one ion to co-partition together with a counter ion. Therefore, the differently charged ions of the salt can not partition independently between the two phases in the absence of additional charged material [25, 42]. When introducing additional charged molecules like biological membranes in the two-phase system, the differences in the affinities of the ions for one phase result in an interfacial potential ΔU:

$$\Delta U = \frac{RT}{(z^+ + z^-)F} \ln \frac{K_-}{K_+} \quad (3)$$

where R is the gas constant, F is the Faraday constant, T the absolute temperature, z^+ and z^- the charges of the cations and anions, respectively, and K_+ and K_- the hypothetical partitioning coefficients of cations and anions in the absence of the potential. The larger the difference of K_- and K_+, the larger will be the interfacial electrostatic potential generated by the salt. This potential results in a driving force that affects the partition of membranes, which have a net negative charge at neutral or alkaline pH. This maintains electroneutrality of both phases in equilibrium.

The preference of cations for the top phase is in the following order: $Li^+ > NH_4^+ > Na^+ > Cs^+ \cong K^+$. Anions preferentially partition to the bottom phase as follows: borate > citrate ≅ sulfate ≅ hydrogen phosphate > dihydrogen phosphate > halides [34]. Consequently, the negatively charged membranes can be pushed to the top phase by adding Li_2SO_4, as Li^+ preferentially partitions into the top phase and SO_4^{2-} into the bottom phase. The opposite effect can be obtained by the addition of KCl, as K^+ preferentially partitions to the bottom phase and Cl^- to the top phase [15, 38]. Since the net charge of membranes is pH dependent, pH adjustment can be used to further promote favorable partitioning behavior (Table 1). However, most protocols use neutral or only slightly alkaline conditions in order to preserve the native structure of the biomaterial.

3.4 Countercurrent distribution

The isolation of PMs by aqueous polymer two-phase systems can not be achieved through a single-step procedure. Under optimized polymer and salt concentrations, at best ~70% of PMs and at least ~30% of intracellular membranes partition to the top phase [15]. This results in considerable loss of PMs and considerable contaminations (Fig. 2A, 1st row, bold numbers represent partition of PMs, italics represent partition of intracellular membranes). Therefore, multiple extraction procedures such as countercurrent distribution (CD) experiments are often applied [43]. The principle of CD, which is illustrated in Fig. 2A, makes use of the fact that the partitioning coefficient K of particles or molecules remains constant during multiple extractions under identical conditions (equation 2). In CD experiments, the top phase of two-phase system A is therefore transferred sequentially to a fresh bottom phase with the same polymer composition as the initial system A. After phase separation, again 70% of the remaining PM will partition into the top phase and 30% into the bottom phase. Thus, 49% (0.7×0.7) of the initial amount of PMs are recovered in the top phase and 21% (0.7×0.3) are collected in the bottom phase of two-phase system B (Fig. 2A, 2nd row, bold numbers). In addition, re-extraction of the bottom phase of two-phase system A with a fresh top phase of the same composition as before is performed. This results in 21% (0.3×0.7) of the initial PMs in the new top

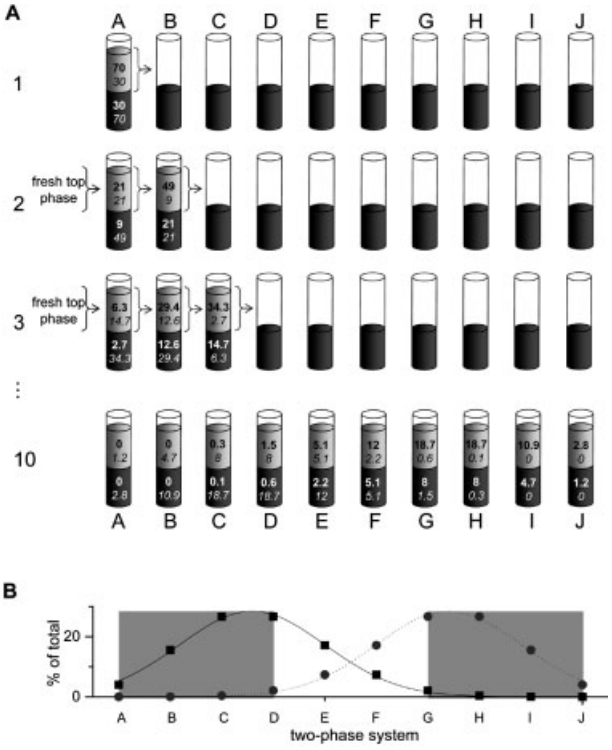

Figure 2. Scheme for CD. (A) Of a given structure (*e.g.,* PMs), 70% might partition to the top phase and 30% to the bottom phase (bold numbers) in a given two-phase system. Of the other structures (*e.g.,* intracellular membranes), 30% might partition to the top phase and 70% to the bottom phase (italic numbers). In CD experiments, the top phase of the initial two-phase system A is transferred to a fresh bottom phase B and the bottom phase of two-phase system A is re-extracted with a fresh top phase resulting in the distribution indicated. After nine iterations this results in an efficient separation of the biomaterial. (B) The outcome of the CD procedure is illustrated for each of the resulting two-phase systems. Intracellular membranes (squares) and PM (circles) are well separated in the gray-shaded two-phase systems.

phase and 9% (0.3×0.3) in the bottom phase. In further rounds, all top phases are transferred one step further onto the next bottom phase, always re-extracting the bottom phase of two-phase system A with fresh top phase, and the top phase of the latest two-phase system with fresh bottom phase. After nine transfers, PMs are distributed as indicated in Fig. 2A (last row, bold numbers). Figure 2B summarizes the distribution of PMs (circles) and intracellular membranes (squares) for a CD experiment in each of the resulting two-phase systems. In the gray-shaded areas, intracellular membranes and PMs are well separated and of high purity. Based on the requirements on yield and purity of PMs, entire two-phase systems and top phases can be pooled. Using CD for the separation of subcellular membranes, the sample might be highly diluted in the end. However, this problem can easily be overcome by pelleting the sample using high-speed ultracentrifugation.

CD experiments have already been applied to the fractionation of rat liver organelles [44], vesicles from the endoplasmic reticulum from rat pituitary homogenates [44], synaptosomal membranes [45] or region-specific PMs of rat liver [38]. Aside of membrane vesicles, this technique has also been applied to the separation of whole cells [46–48].

3.5 Affinity two-phase partition

In addition to polymers and salts, partitioning of membranes in aqueous polymer two-phase systems can be affected by affinity ligands, such as lectins, antibodies or receptor agonists and antagonists. Lectins are proteins, which specifically recognize carbohydrate residues and thus bind glycoproteins and glycolipids of membranes. The same holds true for antibodies, agonists and antagonists, which bind to specific proteins of the desired membranes. Affinity ligands are usually covalently linked to one of the two polymers. This results in co-partitioning of the targeted membrane together with the ligand-polymer conjugate, as under the usually applied non-denaturing conditions, not only the targeted molecules, but also the associated membrane patches are pulled into the phase with the ligand. In the PEG/dextran two-phase system, both polymers can be used for conjugation. As the dextran concentration in the PEG-enriched top phase is lower than the PEG-concentration in the dextran-enriched bottom phase, dextran is better suited as carrier polymer for affinity ligands [34]. Furthermore, the higher molecular weight of dextran is less influenced by the ligand [49]. This will prevent major alterations in polymer distribution after conjugation. Finally, dextran has several potential ligand coupling sites, whereas PEG has only two sites per molecule. The coupling of ligands to dextran can thus be varied in a wider range compared to PEG.

Affinity partitioning aims at the enrichment of a certain type of membrane by selectively pulling it to one phase. For that purpose, usually two-phase systems are chosen in which the bulk of membranes partitions to one phase, whereas the membrane of interest is pulled into the other phase via an affinity ligand coupled to the prevailing polymer in this phase [50] (Fig. 3). To favor partition of the ligand-polymer conjugate to one phase, a two-phase system far from the critical point, i.e. with higher polymer concentrations, is desirable (Fig. 1). A drawback of such a system is the partitioning of membranes to the interface (see Section 3.2). To overcome this problem, first a conventional two-phase system is used with polymer concentrations best suited to enrich the desired membrane, *e.g.,* PMs, in the PEG-enriched phase, and the intracellular membranes in the dextran-enriched phase. In a second step, the so-called affinity partitioning, the PEG-enriched phase is mixed with an affinity ligand conjugated dextran-enriched phase (*e.g.,* wheat germ agglutinin-conjugated dextran in the case of PMs). The selected membrane will therefore be pulled to the dextran-enriched phase. To counteract partitioning of the residual intracellular membranes from the PEG-enriched top phase

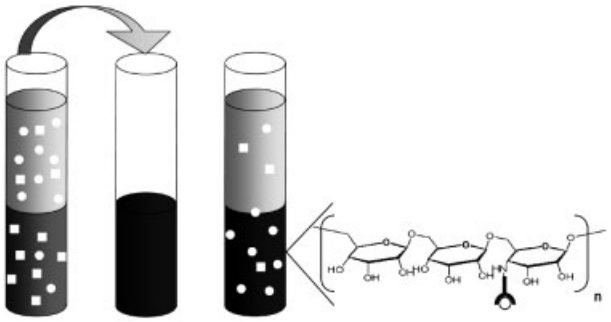

Figure 3. Principle of affinity partitioning for PM enrichment. PMs (circles) are enriched in the top phase of a PEG/dextran two-phase system and separated from intracellular membranes (squares), which mainly partition to the bottom phase. In a second step, PMs are further purified by pulling them to the bottom phase by an affinity ligand coupled to dextran. Partition of intracellular membranes into the bottom phase is counteracted by increasing the molecular weight of dextran or adding suitable salts.

to the bottom phase, dextran of higher molecular weight or addition of salts such as Li_2SO_4 is recommended. This will increase the partitioning coefficients of the undesired membranes (Table 1).

After the initial isolation of membranes enriched in cholinergic receptors from *Torpedo california* [31, 51], affinity partitioning was applied to isolate synaptic membranes enriched in opiate-binding receptors using naloxene [52] or to purify PMs from rat liver [23], lung [53], or brain [15] using wheat-germ agglutinin. Recently, the isolation of caveole using anti-caveolin-1, a biotinylated secondary antibody, and NeutrAvidin coupled to dextran was reported [33].

3.6 Defined conditions are required for membrane partitioning

As stated above, aqueous polymer two-phase systems are sensitive to salt, temperature and several other factors and their use hence requires proper and careful handling at defined conditions. Contaminations of buffers or stock solutions by salt might already change the partitioning of membranes considerably. Gierow *et al.* [38] recovered 75% of PM marker activity in the top phase in a two-phase system with 6% PEG and dextran buffered with 15 mM Tris-H_2SO_4, pH 7.8. The addition of 5 mM KCl reduced the recovery to only 35%. One has therefore to keep in mind that already the homogenization of tissues directly in the two-phase system introduces different amounts of various ions that can influence membrane partitioning. Regarding only the single ion K^+, homogenizing directly tissue, amounting to 10% of the entire system, in the two-phase system, results in a final concentration of 9 mM K^+ in the two-phase system (assuming an intracellular concentration of 100 mM and an extracellular concentration of 5 mM in the ratio 9:1 in the tissue). Aqueous polymer two-phase systems have therefore to be adapted to each tissue and to the amount of tissue applied. Another problem might arise from the fact that no kit is available and affinity ligand-coupled polymers have to be synthesized in the lab. Each novel batch has hence to be checked for the coupling degree and for its functionality. For the synthesis, chemical knowledge is of advantage.

4 Alternative techniques for PM preparation

As for most subcellular compartments, a variety of alternative techniques are available to enrich PMs. Due to the many different protocols, we focus on the principles of the most commonly used techniques and some emerging approaches and discuss their strengths and shortcomings. For a more detailed coverage of the various techniques and their underlying principles, the reader is referred to several excellent reviews [54–60].

4.1 Centrifugation

The most often used technique for membrane purification is centrifugation, which separates subcellular compartments according to differences in size, shape or density [61]. A commonly used method to start with is differential centrifugation, which exploits these differences to separate a cell homogenate into different subcellular fractions. Usually, cell lysates are first centrifuged at a speed that sediments only cell components larger and denser than the desired organelle. Thereafter, another centrifugation step at a higher speed is performed to pellet mainly the compartment of interest. This technique is rapid and simple, and the subcellular organelles are not damaged by exposure to hypertonic gradient media as can occur in density gradient centrifugation. The major problem encountered in this type of centrifugation is the heterogeneity of the isolated material, since light particles close to the bottom of the centrifuge tube will sediment along with heavier particles from the upper part of the tube during centrifugation.

To overcome this limitation, differential centrifugation is often combined with isopycnic or density gradient centrifugation to yield highly enriched subcellular fractions. Isopycnic centrifugation separates particles mainly due to differences in their buoyant density. Particles move in a density gradient under the force of the centrifugal field until their density equals the density of the medium. At the point of isodensity, further centrifugation will not cause any further sedimentation of the particles. This should result in homogenous membrane preparations. In practice, however, the similar density and the heterogeneity within the different cellular membranous compartments results in an overlap of their densities [61]. Thus, different membranes are not readily separated and several time- and material-consuming combinations of differential and isopycnic centrifugations are required, which ultimately results in low yields. Further-

more, some media used for density gradients, such as sucrose, interfere with the fractionated material by introducing osmotic changes during centrifugation, which can alter the density of the membrane vesicles. Other media are rather expensive such as Nycodenz. Despite these limitations and drawbacks, combinations of different centrifugation techniques have been proven to be extremely valuable and have become the workhorse for subcellular fractionation experiments in cell biology. Centrifugation techniques were successfully applied to enrich PMs from tissue such as liver [62, 63], smooth muscle, skeletal muscle, and heart muscle [64], brain [65], the postsynaptic density of neurons [66], or from cell lines such as lymphoblasts [67, 68], monocytes [69], or cultured retinal pigment epithelium [70]. Recently, a combination of various protein extraction procedures with density centrifugation demonstrated the potential of this classical technique. PM proteins from as little tissue as the hippocampus from a single mouse (10–20 mg tissue) were successfully isolated, albeit the purity was rather low [14].

4.2 Immunoprecipitation

Another approach to isolate PMs is immunoprecipitation, also called affinity adsorption. This technique is based on the specific interaction of PMs with a solid phase that contains an antibody highly selective for a PM protein. The solid phase can consist of various materials such as Sepharose, polyacrylamide, or magnetic beads. This technique has been applied to the PM from mature and immature dendritic cells [19], PM substructures such as cholinergic terminals of the mammalian brain [71], or caveolae [71, 72]. Drawbacks of this approach are the high costs for antibodies and the nonspecific adsorption of contaminating membranes to the supporting matrix, leading to a mixture of membranes on the bead surface. Thus, this technique is often used in combination with other purification methods. Furthermore, elution of the PM from the solid support requires harsh conditions, which render most proteins functionally inactive.

4.3 Surface labeling

Another strategy makes use of PM-specific tagging prior to isolation. In one approach, the cell surface is exposed to polycationic colloidal silica polymers that interact with the anionic cell surface [73]. After cross-linking, the increased density of the PM is exploited for purification by density gradients. First applied to the cell surface from *Dictyostelium discoideum*, it was more recently used for the purification of PMs from endothelial cells of various normal and neoplastic tissues [74–76], or to profile fibroblasts and mammary carcinoma cells [77].

A variant of this approach consists in global biotinylation of cell surface proteins in intact cells. After homogenization, biotinylated PMs are isolated by exploiting the high affinity and specificity of avidin-biotin interactions. Using this technology, various human cancer cell lines were profiled [11–13]. While surface labeling holds great potential as it resorts to well-established methods and principles, a serious limitation is the requirement of free access to the cell surface. This is easily accomplished when using cell cultures or epithelial cells, but precludes its application to bulky tissues or organs.

4.4 Liquid-phase electrophoresis

Recently, a number of gel-free, electromigration-based instruments became available for fractionation of biological samples according to their isoelectric characteristics. These systems include the free-flow electrophoresis system, initially developed by Hannig and coworkers [78, 79]. In free-flow electrophoresis, continuously injected proteins or organelles are separated in an electric field perpendicular to the direction of flow [80]. Other, related liquid-phase devices include the rotofor, where proteins are separated into distinct chambers [81], the gradiflow, which consists of several molecular weight cutoff membranes in a cartridge formation positioned between electrodes [82], or a multi-compartment electrolyzer operating with isoelectric membranes [83]. So far, only free-flow electrophoresis has been applied to the purification of entire PMs, as it requires no artificial membranes which impair the flow of cellular compartments. This device was part of the purification of detergent-resistant membranes [84], and was used to separate the basolateral membrane from the brush border membrane of renal cells [85]. The advantage of liquid-phase instruments is the large loading capacity, but robust protocols are not yet available. Furthermore, systems such as the free-flow electrophoresis instrument necessitate high initial investments and long hands-on experience to run it appropriately.

5 Conclusion

In summary, aqueous polymer two-phase partitioning represents an attractive alternative to currently used methods for the isolation of PMs from eukaryotic cells. Its main advantages are the high yield and purity, together with rapid processing. Different factors such as molecular weight and concentrations of the polymers and salts can be explored to optimize the partition behavior. After establishment of the appropriate conditions, the entire partitioning protocol can be easily performed within a few hours. Furthermore, no expensive equipment is required.

After completion of this review, two novel articles appeared on the use of two-phase systems. One reported the isolation of rat liver PMs by an aqueous two-phase system [86] and the other article reported on the development of a novel two-phase system, composed of *n*-butanol, $(NH_4)_2SO_4$, and water [87].

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