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Role of membrane mimetics on biophysical EPR studies of membrane proteins

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ABSTRACT

Biological membranes are essential in providing the stability of membrane proteins in a functional state. Functionally stable homogeneous sample is required for biophysical electron paramagnetic resonance (EPR) studies of membrane proteins for obtaining pertinent structural dynamics of the protein. Significant progresses have been made for the optimization of the suitable membrane environments required for biophysical EPR measurements. However, no universal membrane mimetic system is available that can solubilize all membrane proteins suitable for biophysical EPR studies while maintaining the functional integrity. Great efforts are needed to optimize the sample condition to obtain better EPR data quality of membrane proteins that can provide meaningful information on structural dynamics. In this mini-review, we will discuss important aspects of membrane mimetics for biophysical EPR measurements and current progress with some of the recent examples.

1. Introduction

Earlier fluid mosaic model of the plasma membrane suggested a homogeneous division of lipids in the membrane bilayer plane [1,2]. In this model, protein associated with the membrane can be observed as islands floating in the sea of lipids. The protein associated lipid packing and lipid domain formation have further enhanced the attentiveness in studying the lateral arrangement of biological membranes. The protein-lipid interaction plays a crucial role in organization of plasma membrane. Understanding of tightly controlled and dynamic interplay between membrane-interacting proteins and membrane lipids is crucial to delineate higher order cellular functions [2]. The biological membrane composition is very complex and changes depending on types of cell or cell compartments. The changes in lipid compositions lead to the microdomain formation with varying physical properties. This arises from the interplay between the hydrocarbon chains and the specific lipid headgroups. A membrane bilayer is an energetic mimetic system having various dielectric properties ranging from nonpolar at the hydrocarbon to polar at the interface of the headgroup-solution. Additionally, the interfacial headgroup region can have significant negative charge densities that can cause major implications for identification of membrane-interacting proteins. Obtaining suitable membrane bilayer condition is very challenging for membrane protein studies using biophysical methods. EPR spectroscopy is a very powerful and rapidly growing biophysical technique for studying structural dynamics of membrane proteins in a membrane environment [3–5]. However, solubilizing membrane environments can have influence in the quality of EPR measurements that can introduce an inaccuracy in the structural dynamic information of membrane proteins [5–7]. In this mini-review, we briefly discuss available membrane mimetic systems and their importance in obtaining improved EPR data quality. We further discuss recent developments in membrane mimetic systems for EPR measurements with some examples. Authors refer recent excellent reviews for details of membrane mimetic for biophysical EPR studies [4,5,8].

2. Membrane mimetic systems for biophysical studies of membrane proteins

Membrane proteins are very sensitive to their solubilizing environment. During the extraction of membrane proteins from the plasma membrane and their purification, it is very important to choose a suitable lipid system that can properly solubilize the protein and maintain structure and function of the protein. For example: membrane protein structure can be different in a lipid bilayer environment in comparison

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to that in a micelle [9]. The differences may be due to the integration of the protein into the lipid bilayer membrane in opposition to the micelle because of the poor hydrophobic environment, the curved surface, and hydration differences. Significant efforts have been made in progressing the membrane mimetic systems for biophysical studies of membrane proteins including detergent micelles, bicelles, liposomes, lipodiscs, lipodisq nanoparticles or styrene maleic acid lipid particles (SMALPs) [7,10,11]. Here, we briefly discuss most popular membrane mimetic systems for biophysical EPR spectroscopic studies of membrane proteins.

2.1. Detergent micelles

Detergent micelles are generally utilized to solubilize the membrane protein during purification and elution of the protein while maintaining a significant amount of the protein concentrations required for biophysical studies such as NMR spectroscopy. Detergent micelles have been used to study function of membrane proteins [12,13]. Detergent purification has been very helpful for membrane proteins to answer pertinent questions about the structure and function of the target protein [14–16]. Detergent micelles are widely utilized to solubilize membrane proteins during the extraction from the cell membrane for several biophysical studies including X-ray crystallography [14]. During the protein purification process, concentration of the detergent is kept just above the critical micelle concentration (CMC) to obtain the micelles. The excess detergent can inactivate the protein [14]. Detergents used to solubilize membrane proteins can be categorized depending upon their hydrophilic head groups. These are ionic, nonionic and zwitterionic detergents. Ionic detergents are known as harsh detergents containing head groups either positively or negatively charged. These detergents can disorganize the hydrophobic interactions between protein and biological membranes as well as the hydrophobic interactions in the core of the protein causing unfolding and degrading the protein. Examples of ionic detergents are sodium dodecyl sulphate (SDS), n-laurylsarcosine and bile acids. Non-ionic detergent contains hydrophilic head groups without charges and hence known as mild detergents. These detergents are most popular detergents in the membrane protein chemistry. Some examples are n-octyl-β-D-glucoside (OG), n-decyl-β-D-maltoside (DM), n-dodecyl-β-D-maltoside (DDM), polyoxyethylenes and Triton X-100. Zwitterionic detergents contain both positively and negatively charged groups. They are milder than ionic detergents and hence can be also used for solubilization and purification of several membrane proteins. The most commonly used zwitterionic detergents are 3-[(3-cholamidopropyl)dimethy-lammonio]-1-propanesulfonate (CHAPS), 3-[(3-cholamidopropyl)di-methylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO), lauryldimethylamine-N-oxide (LDAO), n-dodecylphosphocholine (DPC) and n-tetradecylphosphocholine. Fig. 1A shows the chemical structure of some illustrative examples of detergents used to solubilize and purify membrane proteins. Although there are various detergents available to solubilize membrane proteins from plasma membrane, it is often challenging to optimize the suitable detergent condition that can provide the native membrane mimetics. The biophysical data obtained in detergent micelles may not reflect the native state of the membrane proteins.

2.2. Bicelles

Bicelles are artificial lipid bilayer system that can be obtained by mixing a long chain lipid typically having 12–18 carbons and a short chain lipid typically having 6–8 carbons. The structure of bicelles varies depending upon the lipid composition, temperature and hydration [17]. One of the most known bicelle structure formation is a nanodisc form where the long chain lipids assemble in the plane of the disc and the short chain lipids distributed mainly in the disc torus [17]. The detergent-like short chain lipids play a key role in stabilizing the boundary of the bicelle nanodisc. Bicelles are smaller in size and hence they can form homogeneous sample condition for studying membrane proteins via biophysical approaches. However, it is very challenging to obtain the specific lipid and detergent mixture to form bicelles for solubilizing some of membrane proteins that can provide the native structural and functional integrity of the protein. Some examples of the commonly used bicelles for studying membrane proteins using biophysical approaches are 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC)/dihexanoylphosphatidylcholine (DHPC), DMPC/DPC, DMPC/1,2-dicaproylphosphatidyl choline (DCPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)/DHPC, DHPC/dimyristoylphosphatidylglycerol (DMPG) [7,17–20].

2.3. Liposomes

The use of liposomes can provide a more natural way of solubilizing membrane proteins. Liposomes are mostly composed of phospholipids such as phosphatidylcholine and cholesterol. They are compatible with lipid bilayer structures [21]. Liposome properties can vary depending upon the composition of the lipid, surface charge, size, and the sample preparation methods [21]. Depending on the size and number of bilayers, liposomes can be classified into multilamellar vesicles (MLV), large unilamellar vesicles (LUV), small unilamellar vesicles (SUV), and the cochleate vesicles. Liposomes provide lipid bilayers and hence suitable for preserving the native environment. However, the liposome samples are heterogeneous in nature that can introduce several challenges to achieve better biophysical data qualities for membrane proteins. Another challenge for liposome samples is that it is difficult to concentrate causing poor signal-to-noise (S/N) in several biophysical measurements such as NMR spectroscopy and EPR spectroscopy [6,7,22,23]. A high protein concentration (μM to mM amount) and long data acquisition times (hours to several days) are needed to obtain reasonable data quality of membrane protein studies using solution and solid-state NMR techniques [22,23]. The schematic representation of the structure of some examples of lipids utilized to study membrane proteins using biophysical approaches are shown in Fig. 1B.

2.4. Nanodiscs and lipodisq nanoparticles/SMALPs

Nanodiscs have been used as membrane mimetic systems for solubilizing membrane proteins to obtain disperse samples for better data quality of biophysical measurements [24–26]. Nanodiscs are formed by dissolving membrane scaffold protein and phospholipids and protein in a solution that contains detergent. The detergent is later removed from the solution by using methods such as dialysis, incubation with adsorptive beads or size exclusion chromatography [24]. Nanodiscs system can provide lipid bilayer environments to the membrane protein without containing any detergent. Since this method uses membrane scaffold protein (MSP) to control the size of the nanodiscs, this can affect the optical properties of target protein [26]. This can introduce challenges for studying several integral membrane proteins.

Lipodisq nanoparticles or styrene maleic acid lipid particles (SMALPs) are recently emerged membrane mimetic systems that have been very popular in solubilizing membrane proteins for biophysical studies [7,27–30]. Lipodisq nanoparticles system can be formed by solubilizing the phospholipids containing membrane protein by styrene maleic acid (SMA) copolymer in the environment free of detergent. In this approach, styrene maleic acid copolymer is wrapped around the phospholipids containing protein to form a small homogeneous sample (~10–20 nm) of lipodisq nanoparticles [7,31–33]. SMA is obtained by the hydrolysis of precursor styrene-maleic anhydride (SMAnh). SMA contains an alternating styrene and maleic acid moieties that form an amphipathic copolymer. The SMA copolymer can have various ratio of styrene:maleic acid based on the polymerization reaction utilized to prepare SMAnh precursor. The SMA copolymer can be utilized to isolate membrane proteins and their local lipids directly from the crude membrane [33,34]. Membrane proteins incorporated into lipodisq

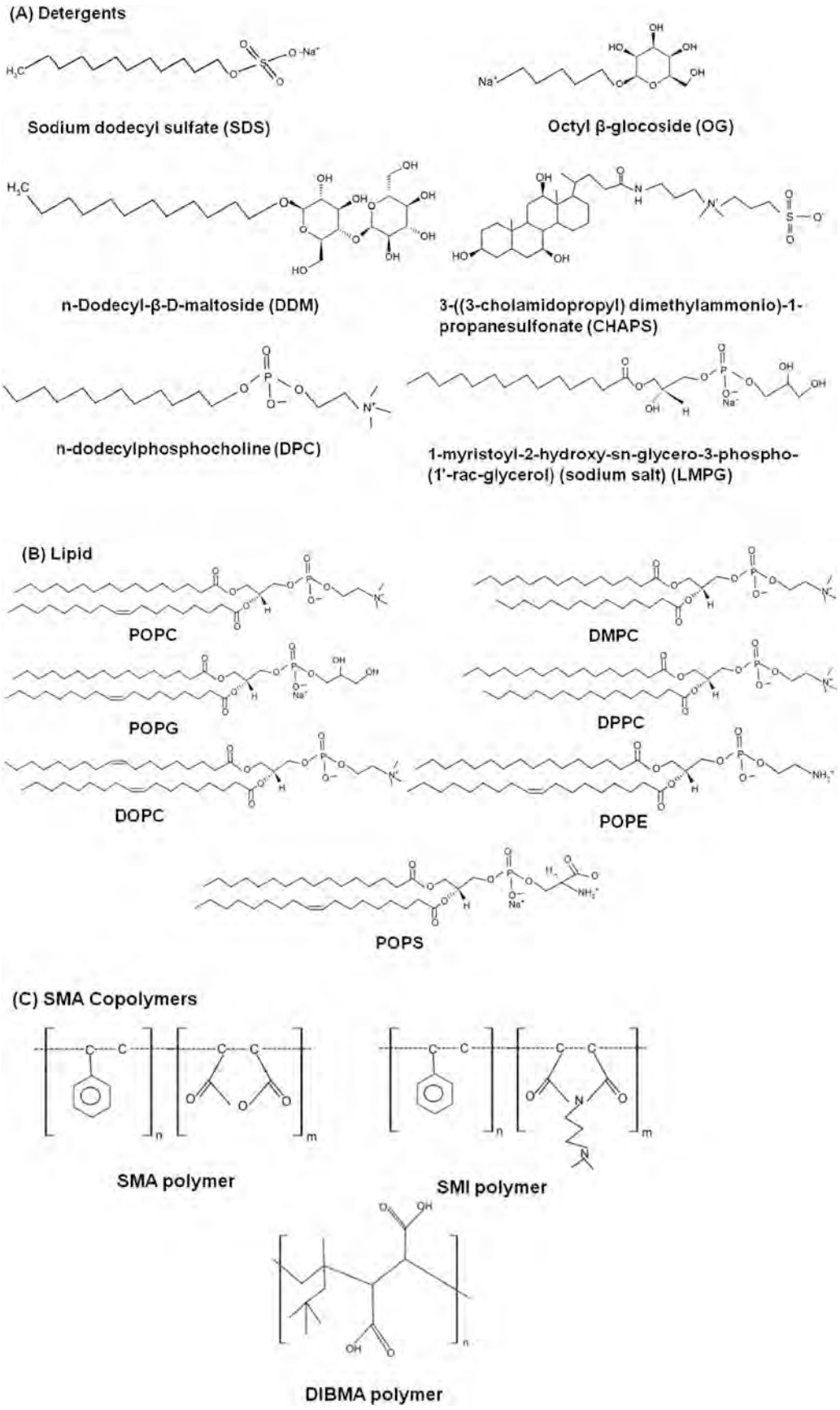

Fig. 1. Chemical structure of examples of detergents (A), lipids (B), and SMA copolymers (C) used for solubilizing membrane proteins for biophysical studies. The chemical structures were prepared using ACD/Sketch (Freeware)-2021.2.1 (www.acdlabs.com).

nanoparticles or SMALPs can preserve their native structure, thermal stability, and functional activities when compared to that with detergent purified proteins [33]. This approach can be applied to various lipids and wide range of membrane proteins for biophysical methods including EPR and NMR spectroscopy. Several forms of SMA copolymers have been developed depending upon their sizes, charges and styrene:maleic acid ratios to solubilize membrane proteins [33–37]. Other alternative SMA copolymers using RAFT synthesis technique and di-isobutylene maleic acid (DIBMA) copolymers have been also introduced for biophysical studies of membrane proteins [38–42]. Recently, a SMALPs has been developed using SMA thin film on a substrate [28]. Fig. 1C shows schematic representation of the structure of some examples of the SMA polymers used for studying membrane proteins using biophysical approaches. Fig. 2 shows schematic representation of different membrane mimetic systems used for studying membrane proteins. The particle sizes (radius) of different model membrane mimetic systems have been recently reported as ~3 Å for micelles, ~9 Å for bicelles, ~250 Å for vesicles and ~12–15 Å for lipodisq nanoparticles [31]. The secondary structural components, interaction, orientation and membrane insertion of proteins/peptides are mostly controlled by physicochemical properties of the constituent lipids of the membrane [43–45]. The anionic bacterial membranes have a high electrostatic affinity for cationic antimicrobial peptides (AMP) while uncharged eukaryotic membranes are more resilient to antimicrobial attack [44]. The lipid bilayer thickness and the intrinsic curvature of the lipid can influence the structure and activity of transmembrane (TM) proteins [46]. The hydrophobic mismatch can destabilize and inactivate some of TM proteins while the thickness of the membrane can adjust with the thickness of the hydrophobic protein surface of other proteins [47–50].

3. EPR spectroscopic measurements of membrane proteins in various membrane-mimetic environments

Different approaches of EPR spectroscopic techniques in connection with site-directed spin labeling (SDSL) can be used to determine structural dynamics of membrane proteins [3–5,51–56]. CW-EPR spectral lineshape analysis can provide spin-label side chain dynamics of membrane proteins incorporated into different membrane environments [3–5,57–59]. EPR power saturation measurements provide membrane protein topology with respect to membrane bilayers by measuring the membrane depth of spin-label side chains from the membrane surface [3–5,60–62]. Continuous wave dipolar broadening EPR approach can be used to determine distances in the range of 8–20 Å which is very useful for obtaining structure and conformational dynamics of membrane proteins in different membrane mimetic systems [63,64]. Pulse EPR approaches can provide very useful information on structure, conformational dynamics and oligomerization states of membrane proteins in different membrane environments [5]. A pulse EPR approach of electron spin echo envelope modulation (ESEEM) can be utilized to measure distances up to ~8 Å between a nitroxide spin label and an individual ^{2}H nucleus providing a great insight on the local secondary structure of membrane proteins in different membrane mimetic systems [19,20,65]. Double electron-electron resonance (DEER) approach of pulse EPR is used to measure distances in the range of 18–60 Å between two spin labels on membrane proteins [66–68]. In site-directed spin labeling EPR approaches, nitroxide based spin label such as methanethiosulfonate spin label (MTSL) is widely used to study structural dynamics of membrane proteins [4,5,69–71]. The motional flexibility of MTSL makes easier to incorporate it at any sites of the protein [71–73]. However, the conformational dynamics of MTSL rotamers may introduce challenges to obtain slow protein dynamics (micro-to-millisecond), inter-spin distance measurements, and orientation measurements for several membrane proteins [74]. A restricted spin label known as bifunctional spin label (BSL) having fewer rotamers compared to the MTSL has been used to study structural dynamics of membrane proteins and peptides [6,58,75–77]. A highly restricted nitroxide spin label known as 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) has been used for obtaining inter-electron distances, orientation and slow backbone motion [78–81]. TOAC is attached at the backbone level of amino acids in protein/peptide sequences [78]. However, there are several challenges while incorporating the TOAC spin label into proteins via molecular biology techniques [64]. Although EPR approaches provide very crucial structural dynamic information on membrane proteins, it is challenging to prepare EPR active membrane protein samples that are properly optimized for homogeneous samples in a near-native membrane environment that can provide high quality of EPR

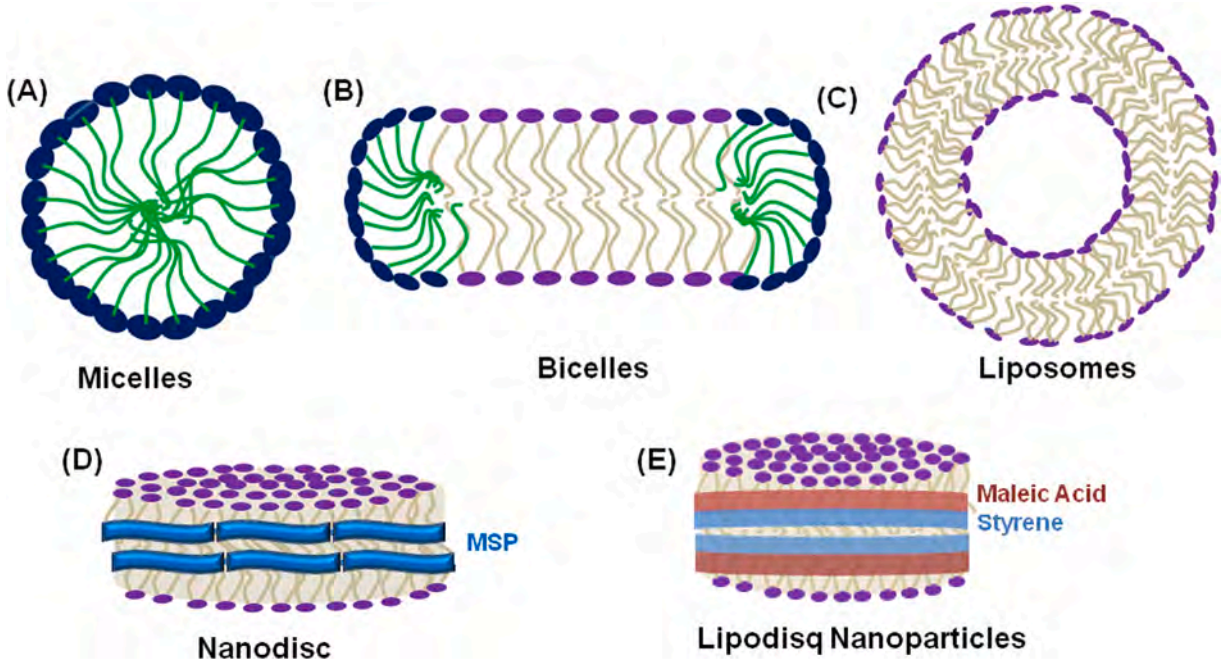

Fig. 2. Schematic representation of different membrane mimetic systems for studying membrane proteins: (A) micelles, (B) bicelles, (C) liposomes, (D) nanodiscs and (E) lipodisq nanoparticles or SMALPs.

measurements [6]. High quality EPR data can provide simplified analysis and interpretation of data to obtain more accurate structural and dynamic information of membrane proteins. Data obtained from different EPR spectroscopic approaches can be analyzed using several available EPR analysis and modeling software tools such as non-linear least squares (NLSL), EasySpin, DEER Analysis, DEFiT, mtsslSuite, and MMM [82–88]. Different lipid membrane environments have been developed to solubilize membrane proteins for EPR spectroscopic studies for reliable information of structural dynamics to understand the functional aspects of the protein in a more native membrane environment [7,10,43,64,75,89,90]. Recent EPR studies have shown that the spin-label side chain motion of several membrane proteins is reduced in the lipid bilayer environment in comparison to that in detergent micelles, and this motion is further reduced in the presence of nanodiscs and lipodisq nanoparticles, some examples of these systems are KCNE1, KCNE3, Human dihydroorotate dehydrogenase (HsDHODH), KCNQ1-VSD, integrin β_{1a}, ABC importer [7,43,59,60,89,91–94].

A recent example of EPR studies of important biological system in different lipid membrane environment is a study of HsDHODH [43,95]. HsDHODH is a flavin-dependent enzyme that is implied in the chemical catalysis of dihydroorotate to orotate. This enzyme is important for the biosynthesis of new pyrimidine molecules and also involved in respiratory complex. Vicente et al. studied association and conformation of the HsDHODH microdomain with different model membranes using CW-EPR spectroscopy [43]. Authors utilized vesicles containing several lipid compositions to examine the association of the HsDHODH microdomain with membrane mimetics to reveal detailed information on the N-t(DH) peptide mechanism of action. Authors analyzed CW-EPR spectral data collected on N-t(DH) peptide derivatives in solution and in the presence of LPC and SDS micelles to obtain rotational dynamics of TOAC spin-label generated at various sites of peptide backbone [43]. These results revealed higher mobility of the TOAC analogues in aqueous solution when compared to that in the other membrane environments suggesting a high degree of motional freedom in aqueous solution. These results further suggested faster rotational motion of peptide derivatives in SDS micelles when compared to that in LPC micelles. EPR experiments were further carried out in different lipid bilayer environments containing individual lipids and mixture of lipids including POPC, POPC:CL, POPC:POPE, and POPC:POPE:CL to understand the mobility of the TOAC-labeled peptides in the lipid bilayer membranes that can provide near-native lipid environment for the HsDHODH microdomain. The analysis of EPR spectra in the liposomes revealed either one or two components based on the different lipid composition of the membrane. These data reveled a more intense immobilized, broad component in EPR spectra in all conditions in the CL-containing membranes irrespective of membrane mimetic [43]. These results suggested that the peptide-membrane interactions were regulated by the lipid composition of the membrane. Additionally, the cardiolipin played a crucial role in the interaction of the peptide with the membrane [43]. In another publication, Vicente et al. reported structural and conformational properties of the interaction of HsDHODH N-terminal microdomain with membranes using four pulse DEER spectroscopic data in DPC micelles and in POPC lipid bilayered vesicles [95]. The analysis of the DEER data of the spin-labeled peptide derivative [Cys^{35}MTSL-$TOAC^{0}$]N-t(DH) showed a notable difference in the spin-spin distances in these two membrane mimetic systems. The average distances obtained across the two spin labels were shorter in micelles (32 ± 4 Å) in comparison to that in liposomes (48 ± 4 Å) [95]. The difference in DEER distances obtained for the peptide analogues suggested that the conformations observed for the HsDHODH N-terminal microdomain are specific to membrane mimetic systems [95].

Another example of using different membrane mimetics for EPR studies of a membrane protein is the structural dynamic studies of a human KCNE1 in different membrane environments [6,64,75,89,93]. KCNE1 is a voltage-gated potassium channel accessory protein having a single pass transmembrane domain (TMD) which regulates the function of several voltage-gated potassium channels (K_v) including KCNQ1. KCNE1 is vital for the repolarization phase for the cardiac action potential. Biophysical EPR studies of KCNE1 in a native membrane state are difficult due to the presence of longer and flanking N- and C-termini of KCNE1. The Lorigan group has extensively studied KCNE1 in different membrane environments to obtain better quality of EPR measurements to get more reliable structural and dynamic properties of the protein in native-like membrane mimetics [6,64,75,89,93]. Coey et al. conducted SDSL CW-EPR measurements on the spin-labeled KCNE1 incorporated into LMPG detergent micelles and POPC/POPG lipid bilayered vesicles to obtain spin-label side chain dynamics of KCNE1 and the topology of the protein with respect to the membrane bilayer [93]. The CW-EPR spectral data suggested that the nitroxide spin-label motion is slower in lipid bilayer membrane when compared to that in detergent micelles and the residue that lies within the membrane is less mobile than those outside [93]. Additionally, the CW-EPR power saturation measurements revealed the spin-label sites in the transmembrane domain are buried into lipid bilayers while the sites outside the transmembrane domain are solvent exposed. Sahu et al. conducted four pulse Q-band DEER measurements on dual spin-labeled KCNE1 in LMPG detergent micelles, POPC/POPG lipid bilayered vesicles and POPC/POPG lipodisq nanoparticles [6]. The analysis of DEER spectroscopic data suggested a significant improvement in the DEER distance measurement quality and experimental throughput with an increase in phase memory time (T_m) by a factor of ~2 and signal-to-noise (S/N) by a factor of ~3 to 4 for POPC/POPG lipodisq nanoparticle samples in comparison to those for proteoliposome samples [6,75]. Sahu et al. conducted CW-EPR titration experiments on the nitroxide spin-labeled KCNE1 reconstituted into POPC/POPG lipodisq nanoparticles for the EPR spectral lineshape analysis to reveal structural and dynamic properties of KCNE3 in an optimized homogeneous sample condition [89]. The CW-EPR titration experimental data showed an increase in the line broadening of EPR spectrum in the presence of the SMA polymer. The increase in the line broadening approaches close to the rigid limit at a lipid to SMA polymer weight ratio of 1:1, leading to a homogeneous solubilization of the protein–lipid complex [89]. The analysis of CW-EPR spectral data for several spin-labeled sites of KCNE1 in POPC/POPG bilayered vesicles and POPC/POPG lipodisq nanoparticles showed a reduced motion of the spin-label side chain of KCNE1 reconstituted into lipodisq nanoparticles in comparison to that in POPC/POPG bilayered vesicles [89]. Fig. 3 shows a cartoon rendering of NMR structure of KCNE1 (PDB ID: 2k21) [96] and the comparison of CW-EPR spectra for several mutants of KCNE1 (inside probes) in POPC/POPG bilayered vesicles and POPC/POPG lipodisq nanoparticles [89].

Another recent example of using different membrane mimetic systems for studying membrane protein is the EPR spectroscopic studies of KCNQ1 voltage sensing domain (Q1-VSD) [7]. KCNQ1 is a voltage-gated potassium channel regulated by the KCNE protein family members. Q1-VSD consists of four transmembrane domains with 149 amino acids that represent the helix one (S1) to helix four (S4) of first four helices of KCNQ1. Q1-VSD is an individual unit of KCNQ1 that can maintain the structural conformation and functional activities in the same way as the full-length of the channel (VSD + pore domain). Sahu et al. performed DEER EPR spectroscopic measurements on a double spin-labeled Q1-VSD in various membrane mimetic systems for distance measurements [7]. Authors analyzed four pulse Q-band DEER data to obtain DEER distance measurements on F123C-S143C mutant of Q1-VSD (inside probe) reconstituted into different membrane mimetic systems including DPC micelles, LMPG micelles, DMPC/DPC bicelles, POPC/POPG lipid bilayers, and POPC/POPG lipodisq nanoparticles [7]. Fig. 4 represents a projected topology of Q1-VSD (100–249) in a lipid bilayer membrane based on previously published solution NMR model and Q-band DEER data of Q1-VSD mutant (Phe123/Ser143) containing two MTSL spin-labels in different membrane environments [7,97]. The major peak DEER distance data showed a close match for each membrane mimetic systems in the range of the uncertainties of the

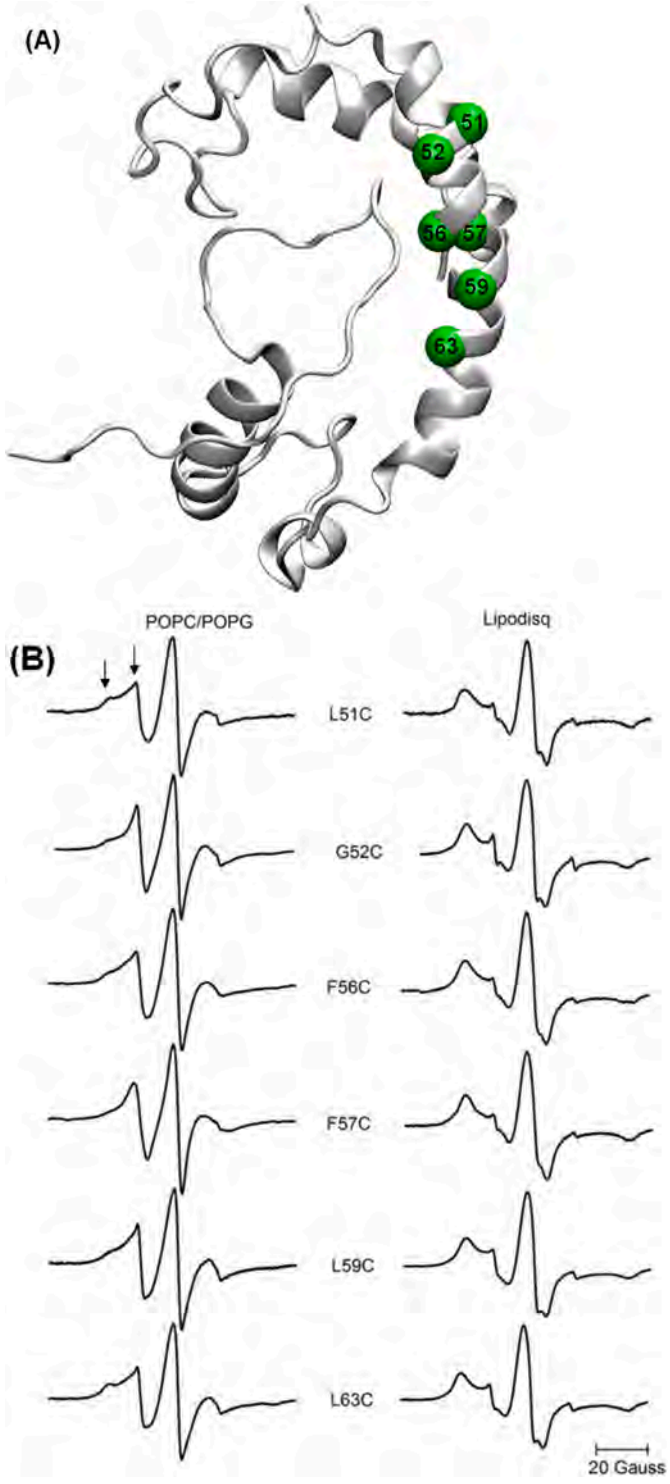

Fig. 3. (A) Cartoon rendering of solution NMR structure of KCNE1 (PDB ID: 2k21) having spin-labeling sites shown by green spheres at the alpha carbons of the protein [96]. (B) CW-EPR spectral data on several mutants of KCNE1 (indie probes) in POPC/POPG bilayered vesicles and POPC/POPG lipodisq nanoparticles (lipid to SMA polymer weight ratio = 1:1) at 297 K. The left arrow shows slower/rigid component and right arrow shows faster motional component. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
(The image was reproduced from ref. [89] with permission.)

experiment. These distance data indicated the close match of the secondary structural conformations of Q1-VSD in all of these membrane mimetic systems [7]. The full-width of the distribution at half maxima (fwhm) for the lipodisq nanoparticle sample (~10 Å) was smaller in comparison to the lipid bilayered vesicle sample (~16 Å) and similar to micelle samples (~9 Å for LMPG and ~11 Å for DPC) and bicelle sample (~12 Å). The DEER data further revealed that the DEER time domain data has an improved signal-to-noise ratio (S/N) for lipodisq nanoparticle sample containing well-defined oscillations with longer data collection time (3 μs) in comparison to the POPC/POPG lipid bilayer sample (2 μs). Additionally, the DEER measurements indicated the phase memory time (T_m) of Q1-VSD can be increased by ~2-fold for lipodisq nanoparticle sample in comparison to the lipid bilayered vesicle sample. This study suggested that the lipodisq nanoparticle is a good membrane mimetic system for studying membrane proteins using EPR spectroscopic approaches. The analysis of CW-EPR spectra obtained for several spin-labeled sites on Q1-VSD showed a slower spin-label side chain motion with a longer rotational correlation times for lipodisq nanoparticle samples in comparison to the liposome samples [7].

One example of studying a membrane protein in different membrane mimetic conditions is an EPR study of C99 domains of amyloid precursor protein (APP) [98]. C99 is a transmembrane C-terminal domain of the amyloid precursor protein obtained by β-secretase cleavage of APP. It contains 99 amino acid residues. Song et al. conducted CW-EPR measurements on spin-labeled C99 incorporated into lipid vesicles with C99-to-lipid mole ratio in POPC:POPG (3:1) lipid vesicles changed from 1:800 to 1:50 to access the dimerization of C99 [98]. The CW-EPR spectra were acquired for C99 having spin-labeled at either on the N-terminal solvent exposed loop (S697C) or on the transmembrane domain (L705C) as a function of the C99-to-lipid mole ratio. The results showed a single narrow component in the lineshape at the lowest concentration of C99 (1:800 C99:lipid) with intermediate motion while the lineshapes become more complicated due to the presence of the second broader components grow with increasing protein:lipid ratio. These data suggested that the position of the monomer-dimer equilibrium is concentration dependent.

4. Future perspectives

Several methodological improvements have been made in solubilizing conditions of the membrane proteins that can provide better quality of EPR data to answer pertinent questions related to the structure and dynamic properties of membrane proteins. The structural dynamic properties of membrane proteins depend on the behavior of the membrane environments while it also complicates the EPR spectroscopic measurements. Different membrane mimetic systems have their own advantages and limitations [7,10,21,28,30,99–102]. Micelles are smaller in size and hence they can provide better quality of EPR data. However, due to the lack of the lipid bilayer, the data obtained from the miceller complex may not reflect the native state of several membrane proteins. Bicelles can provide homogeneous lipid bilayer membrane environments for solubilizing membrane proteins but the requirement of the specific combination of lipid and detergent for the bicelle formation that can maintain the functional integrity of the protein may introduce challenges for some membrane proteins. Liposomes provide lipid bilayers and hence it is good membrane mimetics but it is very heterogeneous in nature that introduces several challenges in obtaining better quality of EPR data. Nanodiscs provide very well dispersed samples that can yield better EPR data quality but the utilization of MSP in the formation of nanodiscs may interfere with the optical properties of the target protein. Lipodisq nanoparticles or SMALPs can provide homogeneous samples to obtain better EPR data quality in near-native environment but a poor tolerance to the pH condition and the poor control to the size of their complex may limit their applications for several membrane proteins [22,102].

The major challenges in structure biology field is to prepare

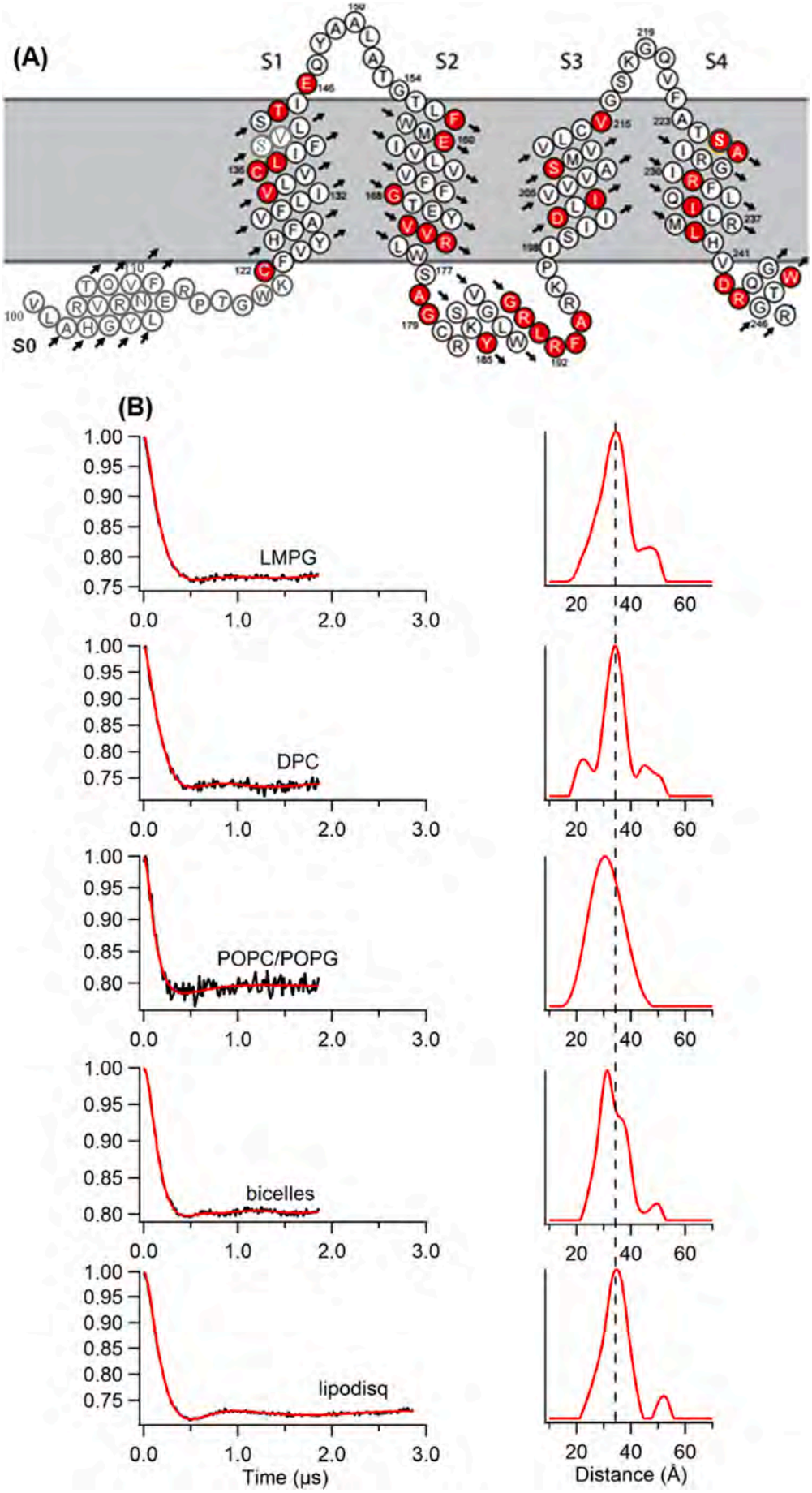

Fig. 4. (A) A projected topology of Q1-VSD (100–249) in a lipid bilayer constructed on previous solution NMR studies [97]. The mutants linked to long QT syndrome are represented by red circles. (B) Four pulse Q-band DEER data of Q1-VSD mutants (Phe123/Ser143) containing two MTSL spin-labels. Background-subtracted time domain data of the indicated mutants (left) and their corresponding distance probability distributions from Tikhonov regularization (right) for LMPG micelles, DPC micelles, proteoliposomes (POPC/POPG = 3:1), bicelles (DMPC/DPC = 3.2:1), and lipodisq nanoparticles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
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homogeneous lipid bilayer samples that can provide minimum spin clustering effect that can improve EPR measurements while maintaining physiological condition of the protein in a native membrane environment. Recent progress in developing several SMA derivatives and their usefulness for EPR measurements has opened an optimistic path for the researchers studying membrane proteins. However, new SMA copolymer derivatives have not been fully characterized for the EPR studies. The challenges are still there to obtain a common membrane mimetic system that can be used to solubilize membrane proteins for preparing EPR active samples that can provide better data quality. Recent improvements in In-cell EPR approaches have also opened a path for researches to obtain reliable structural and dynamic information of membrane proteins in native membrane environments [103].

5. Conclusion

EPR spectroscopic studies are very useful for obtaining structural dynamics of membrane proteins for understanding their function. The EPR structural and dynamic data are dependent on the nature and properties of lipid membrane solubilizing the protein. In this short review, we briefly discussed several important aspects of currently available membrane mimetic systems with special focus on recent progresses in the membrane mimetic systems to improve the quality of the EPR spectral measurements for understanding structural dynamics of membrane proteins.

Abbreviations

CMC	critical micelle concentration
CW	continuous wave
EPR	electron paramagnetic resonance
DEER	double electron-electron resonance
NMR	nuclear magnetic resonance
DPC	dodecyl phosphatidylcholine
SDSL	site-directed spin labeling
SMALPs	styrene maleic acid lipid particles
SMA	styrene maleic acid
SMI	styrene-*co*-maleimide
DIMBA	diisobutylene-maleic acid
MSP	membrane scaffold protein
RAFT	reversible addition–fragmentation chain-transfer
SDS	sodium dodecyl sulfate
OG	n-octyl-β-D-glucoside
DM	n-decyl-β-D-maltoside
DDM	n-dodecyl-beta-maltoside
LMPG	1-myristoyl-2-hydroxy-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (sodium salt)
LMPC	lyso-myristoylphosphatidyl choline
CHAPS	3-[(3-cholamidopropyl)dimethy-lammonio]-1-propanesulfonate
CHAPSO	3-[(3-cholamidopropyl) di-methylammonio]-2-hydroxy-1-propanesulfonate
LDAO	lauryldimethylamine-N-oxide
LPC	lysophosphatidylcholine
MTSL	(*S*-(2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl) methyl methanesulfonothioate)
TOAC	2,2,6,6-tetramethyl-N-oxyl-4-amino-4-carboxylic acid
DHPC	dihexanoylphosphatidylcholine
DMPG	dimyristoylphosphatidylglycerol
POPC	1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine
POPG	1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (sodium salt)
DMPC	1,2-dimyristoyl-*sn*-glycero-3-phosphocholine
DOPC	1,2-dioleoyl-*sn*-glycero-3-phosphocholine
POPS	1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (sodium salt)
DPPC	1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine
POPE	1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine
DCPC	1,2-dicaproylphosphatidyl choline
TMD	transmembrane domain
PDB	protein data bank
ESEEM	electron spin echo envelope modulation

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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