CHAPTER

11

Synthetic mimics of membrane-active proteins and peptides

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11.1 Introduction

Biomembranes are functional interfaces that not only compartmentalize the cytoplasmic components from the extracellular environment but also play various essential roles in biological functions such as intracellular communication, material transport, and energy production (Gennis, 1989). These advanced and complex functions of cell membranes are established by the well-organized supramolecular network of lipid bilayers and proteins. Phospholipids, which assemble into a bilayer structure in most cases, sometimes exhibit a variety of structural polymorphisms depending on the lipid composition or in response to the condition of the surrounding environment such as temperature, pressure, and pH (Cullis & Dekruijff, 1979; Tate et al., 1991). In addition, many biologically active proteins and peptides have exhibited their function through the rearrangement of lipid assemblies in the cell membrane (Farsad & De Camilli, 2003; Last et al., 2013; McMahon & Gallop, 2005). Such a rearrangement of the lipid assembly induced by the proteins or peptides significantly alters the morphology and the physicochemical properties of the membrane, resulting in membrane deformation, fusion, fission, and permeabilization.

A common structural feature of membrane-binding domains in proteins and membrane-active peptides is their amphiphilic structure, which is originated in the localization of hydrophobic and hydrophilic amino acid residues on the same molecular backbone. The spatial arrangement of the hydrophobic and hydrophilic residues on the protein is defined by forming the higher-order structure. For example, in the case of transmembrane proteins, the domains in contact with the hydrophobic core in a lipid membrane are enriched in hydrophobic residues, while the regions exposed on the surface of the membrane are mainly composed of hydrophilic amino acids (White & Wimley, 1999). Also,

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TABLE 11.1 Comparison between natural proteins and synthetic polymers.

	Natural proteins	Synthetic polymers
Molecular species	Single	Mixture
Monomer sequence	Specific	Random
Higher-order structure	α-helix, β-sheet	Random coil
Production cost	High	Low
Large scale synthesis	Difficult	Easy
Chemical modification	Difficult	Easy

some membrane-active peptides form higher-order structures upon interaction with membranes, resulting in the localization of hydrophilic and hydrophobic residues in the same molecule (Last et al., 2013).

Previously, several synthetic polymers have been designed to exhibit biological functions through the interaction with membranes by mimicking the amphiphilic structures of membrane-binding domains of proteins and membrane-active peptides. To design the protein or peptide-mimetic synthetic polymers, it is necessary to understand the differences in the characteristics between natural proteins and synthetic polymers (Table 11.1). Compared to proteins, synthetic polymers can be easily synthesized on a large scale at a low cost. Also, their molecular structures are freely modified by the introduction of functional groups by established synthetic routes. However, there are significant differences between proteins/peptides and synthetic polymers in their molecular structures and compositions. Proteins and peptides are also macromolecules like synthetic polymers, but they have a specific amino acid (monomer) sequence and are composed of a single molecular species. In contrast, synthetic polymers are generally obtained as a mixture of multiple molecular species with different molecular weights and monomer sequences although great efforts have been made to establish living polymerization techniques that can reduce the distribution in their molecular weights. Proteins and peptides fold into higher-order structures such as α-helix and β-sheet depending on their amino acid sequences whereas synthetic polymers generally do not form such higher-order structures. According to these characteristics, it seems to be challenging to reproduce the structure of proteins or peptides with synthetic polymers. However, recently, it has been demonstrated that even synthetic polymers can display functions similar to membrane-active proteins or peptides by mimicking their physicochemical characteristics. In this chapter, we introduce an attempt to design the membrane-active synthetic polymers that mimic membrane-active antimicrobial peptides as well as a lipid nanodisc-forming apolipoprotein.

11.2 Synthetic mimics of membrane-active antimicrobial peptides

In nature, most multicellular organisms including mammals have antimicrobial peptides called host defense peptides (HDPs) that have been used as a primitive immune system (Zasloff, 2002). HDPs are effective against a wide range of pathogens, including Gram-positive bacteria, Gram-negative bacteria, Mycobacterium, and fungi, without developing resistance. Furthermore, this class of peptides does not show significant toxicity to host cells. Thus, HDPs have been attracting attention in recent years as an alternative to conventional antibiotics, which have been associated with the problem of drug-resistant bacteria (Aslam et al., 2018). Most HDPs exert antimicrobial activity by selectively disrupting bacterial cell membranes. The action mechanism of HDPs is completely different from that of conventional antibiotics, which target specific proteins in a lock-and-key manner. In this chapter, we will introduce the design principles and features of synthetic membrane-targeted antimicrobial polymers that have been designed by mimicking HDPs.

11.2.1 Characteristics of membrane-active host defense peptides

HDPs are relatively short cationic peptides consisting of 20−50 amino acid residues that selectively attack bacterial membranes over the membranes of the host cell. One of the most studied examples of HDPs is Magainin 2 isolated from the skin of the African clawed frog *Xenopus laevis* (Fig. 11.1) (Zasloff, 1987). Magainin 2 contains five cationic amino acid residues, such as lysine (K) and histidine (H), that can selectively recognize the bacterial membrane through electrostatic interaction. Arginine (R) is also frequently found as a cationic residue in other HDPs. Bacterial membranes are essentially negatively charged because a significant amount of acidic lipids such as phosphatidylglycerols and cardiolipins are exposed to the outer surface of the membrane. On the other hand, in the plasma membrane of multicellular organisms, negatively charged phosphatidylserines are localized in the cytoplasmic side, and the outer leaflet of the membrane is not charged. Magainin 2 does not form specific high-order structures in water, but the peptide is folded into an α-helix upon binding to the bacterial membrane. In the folded state, Magainin 2 forms a facially amphiphilic structure where the hydrophobic residues are segregated on one side of the helix, and the hydrophilic residues are localized on the other side. After the binding to the surface of the bacterial membrane, multiple numbers of peptides will assemble to form transmembrane pores (toroidal or barrel-stave model) or will partially solubilize the membrane (carpet model) (Shai, 2002; Shai & Oren, 2001), thereby increasing

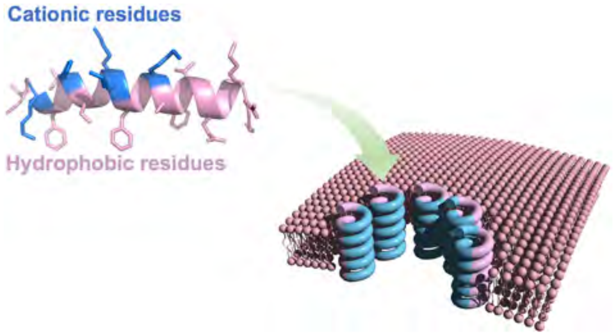

FIGURE 11.1 Facially amphiphilic structure of Magainin 2 and the pore formation in a membrane (PDB ID: 2MAG).

membrane permeability. Membrane disruption by HDPs induces the leakage of cytoplasmic components and loss of membrane potential, resulting in the death of the bacteria. In the previous study of Magainin 2 variants, their antimicrobial activity was found to depend on the propensity for secondary structure formation and the balance of hydrophobic and cationic residues (Maloy & Kari, 1995). Thus, the amphiphilicity of the entire molecule is recognized as a key determinant in the membrane disruption and associated antimicrobial activity.

11.2.2 Designing the synthetic mimics of antimicrobial peptides

Many HDP-mimetic polymers have been developed to date by employing various types of different synthetic molecular frameworks since the 1980s (Fig. 11.2). The common guideline for the design of HDP-mimetic polymer is the introduction of both hydrophobic and cationic groups on the same polymer backbone, which provides global amphiphilicity of the polymer. Mimicking HDPs with synthetic molecular frameworks offers several advantages in practical uses such as improved pharmacokinetics due to the resistance to protease degradation in vitro and large-scale production at low cost. In the earliest stage, Ikeda et al. developed an antimicrobial polystyrene derivative (1) with long alkyl chains and quaternary ammonium groups in the side chain, which is a similar structure to benzalkonium chloride, a commonly-used antibacterial surfactant (Ikeda et al., 1984). This polymer displayed antimicrobial activity against Gram-positive and Gram-negative bacteria, but its toxicity was not assessed. From the approach of peptidomimetics, HDP-mimetic synthetic agents were synthesized using non-natural analogs of amino acids. For instance, peptides of D-amino acids (Bessalle et al., 1990; Won et al., 2011), peptoids (N-substituted glycine oligomers) (Patch & Barron, 2003), β-peptides (Porter et al., 2000), and α/β-peptides (Schmitt et al., 2004) have been employed to mimic HDPs. These molecules were designed to mimic the folded helical structure of HDPs by employing a simplified sequence consisting of a few different types of monomer units. Although these molecules do not have a complex sequence like natural HDPs, they displayed excellent antimicrobial activity comparable to or higher than natural HDPs with low hemolytic toxicity against mammalian erythrocytes. The approaches to designing antimicrobial agents have also been made by employing the molecular framework of synthetic polymers that do not have specific secondary structures. DeGrado et al. designed antimicrobial cationic arylamide derivatives (2) (Choi et al., 2009). They introduced intramolecular hydrogen bonding to limit the conformational freedom of the backbone that fixes the orientation of the cationic side chains. This molecule successfully displayed high antimicrobial activity against Gram-negative *E. coli* and Gram-positive *S. aureus* with minimized hemolytic toxicity. Tew et al. employed rigid polymer backbones such as poly(oxanorbornene) (3) (Lienkamp & Tew, 2009) and poly(phenylene-ethynylene) (4) (Yang et al., 2007) to design HDP-mimetic polymers with a fixed special arrangement of cationic and hydrophobic groups.

In contrast, there are several examples of highly-active and non-toxic HDP-mimetic polymers with a flexible molecular backbone. Gellman et al. designed antimicrobial nylon-3 derivatives (5), which are polyamides with cationic and hydrophobic groups (Mowery et al., 2009). This compound displayed excellent antimicrobial activity with low hemolytic toxicity

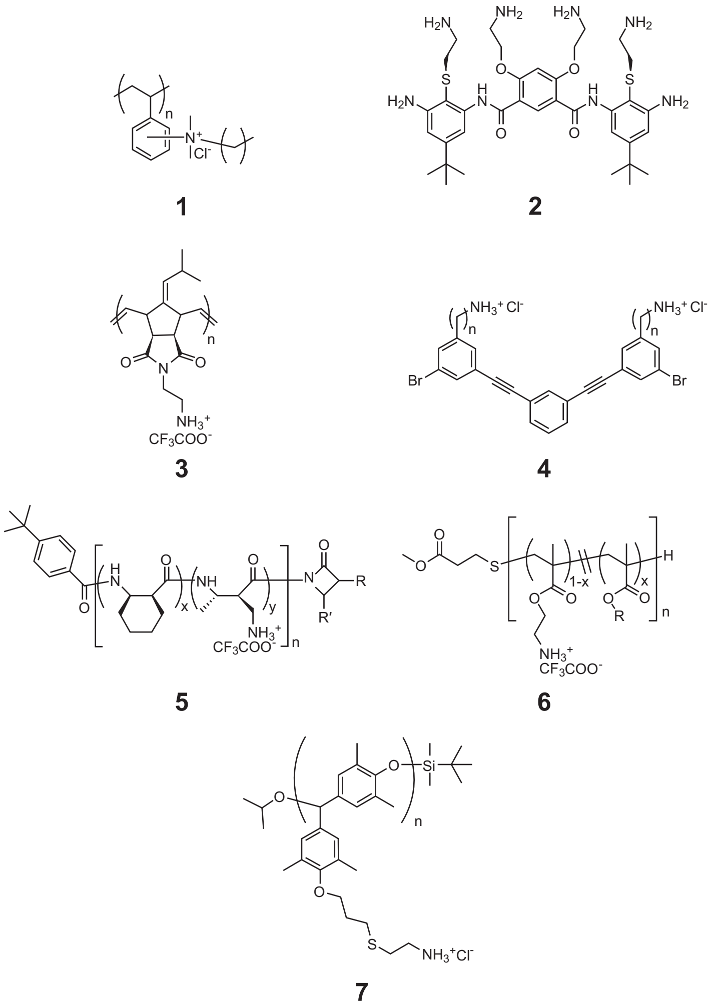

FIGURE 11.2 Chemical structures of HDP-mimetic synthetic polymers. HDPs, *host defense peptides*.

although the cationic or hydrophobic groups are not fixed due to the flexibility in the backbone. They proposed that the presence of hydrophilic/hydrophobic moieties in the flexible backbone provides an induced amphiphilic structure upon binding to the membrane where the hydrophobic groups are faced to the hydrophobic core of the membrane whereas cationic groups are oriented to the aqueous phase. Kuroda et al. developed HDPs-mimetic polymers based on amphiphilic polymethacrylate random copolymers (6). Extensive evaluation of the correlation between structures of the copolymers and their biological activity revealed that the biological activities of polymethacrylate copolymers depend on (1) the fraction and structure of hydrophobic monomers (Kuroda et al., 2009), (2) the molecular weight of the polymer (Kuroda & DeGrado, 2005), and (3) the molecular structure of the cationic groups (Palermo & Kuroda, 2009). In a recent study, it was also demonstrated that HDP-mimetic polymethacrylates are effective not only against bacteria but also cancer cells (Takahashi et al., 2019). Since negatively charged phosphatidylserine is known to be exposed on the surface of cancer cells, the cationic polymethacrylate derivatives are expected to recognize it in the same way as bacterial cell membranes. A polymethacrylate copolymer was able to kill prostate cancer cells even in a dormant state by the disruption of the cell membrane.

Various functions can be added to HDP-mimetic polymer by chemical modification. Most of the molecular frameworks used in the HDP-mimetic antimicrobial polymers introduced in this section are not biodegradable, which may lead to unexpected toxicity in vivo in long-term usage. Several biodegradable HDP-mimetic polymers have been developed by employing polyesters (Mizutani et al., 2012), polycarbonates (Ng et al., 2014), and polyurethanes (Mankoci et al., 2017). These molecular backbones are randomly cleaved by hydrolysis or enzymatic reactions into less-toxic small fragments. In recent years, self-immolative antimicrobial polymers were developed in which spontaneous depolymerization is induced by external triggers. Palermo et al. have developed an HDP-mimetic polymer based on a poly(benzyl ether) with cationic primary ammonium side chains and a silyl ether group at the polymer end (7). The addition of fluorine ion initiates the sequential decomposition of the polymer into monomers from the terminal, resulting in a significant reduction in hemolytic toxicity (Ergene & Palermo, 2017). Self-adhesive HDPs-mimetic polymers were also developed by the modification of a catechol group, which mimics the 3,4-dihydroxyphenylalanine (DOPA) found in the glue protein of mussels (Han et al., 2011). This polymer can prepare an antimicrobial surface effective to *E. coli*, *S. aureus*, and *A. baumannii* by a simple dip-coating technique.

11.2.3 Revealing the action mechanism of synthetic antimicrobials using model membranes

Biophysical studies using model membranes are a powerful approach to analyze the action mechanisms of HDP-mimetic antimicrobial molecules. Model membrane system consisting of only lipids allows the evaluation of the mechanism at the molecular level easier compared to complex intact cells containing a variety of molecular species. Liposomes, which are spontaneously formed hollow capsules by various lipid compositions in water, have been used as common model membranes. A binary lipid system containing a negatively-charged lipid such as phosphatidylethanolamine-phosphatidylglycerol mixture is used to mimic the cell membranes of Gram-negative bacteria including *E. coli* whereas phosphatidylethanolamine-cardiolipin mixture mimics Gram-positive bacteria such as *S. aureus*. To prepare a model mammalian membrane, zwitterionic phosphatidylcholine is usually used. The basic assay for the membrane disruption induced by HDP-mimetic polymer is the dye-leakage experiment using fluorescent dye entrapping liposomes. For this assay, water-soluble fluorescent dye such as calcein is entrapped in a liposome at a high enough concentration to cause self-quenching (Allen & Cleland, 1980). Alternatively, a pair of fluorophore and quencher such as 8-aminonaphthalene-1,3,6-trisulfonic acid disodium salt (ANTS)/p-xylene-bis-pyridinium bromide (DPX) can be used for this assay

(Ellens et al., 1984). The leakage of the entrapped fluorescent dye induced by the membrane disruption is detected as a recovery of the fluorescence due to the dilution of the dye in the outer aqueous phase. Epand et al. reported that HDP-mimetic nylon-3 polymer effectively disrupts *E. coli* membrane-mimicking liposome formed by a 8:2 mixture of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and 1,2-dioleoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (DOPG) whereas mammalian model 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) liposomes remained intact (Epand, Mowery, et al., 2008). Similar lipid composition-dependent membrane disruption induced by HDP-mimetic polymer has also been reported for polymethacrylate (Palermo et al., 2009) and polynorbornene derivatives (Eren et al., 2008).

Thermal analysis techniques provide an evaluation of the interaction between antimicrobial polymers and lipid bilayers without using any additives such as fluorescent probes. Isothermal titration calorimetry (ITC) can analyze the interaction between HDP-mimetic polymers and lipid bilayers by detecting the heat of adsorption generated by binding. Epand et al. reported that HDP-mimetic nylon-3 polymer strongly binds to DOPE/DOPG (8:2) membrane whereas it does not bind to DOPC membrane, which is consistent with the selectivity of their bioactivities (Epand, Mowery, et al., 2008). Differential scanning calorimetry (DSC) can evaluate the effect of HDP-mimetic polymers on the phase transition of lipid bilayers. DSC measurements of bacterial model membranes consisting of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and tetraoleoylcardiolipin (TOCL) revealed that oligo-acyl-lysine (Epand, Rotem, et al., 2008) and nylon-3 polymers (Epand, Mowery, et al., 2008), which have multiple cationic groups, induce clustering of anionic lipids (TOCL) to form phase-separated domains. The boundary of the domain structure at which lipid molecules are loosely packed behaves as structural defects in the membrane and consequently increase the membrane permeability.

The topology of the supramolecular assembly formed by lipids and HDP-mimetic polymers can be characterized by small-angle X-ray scattering (SAXS). Wong et al. reported that HDP-mimetic phenylene ethynylene derivatives induced the structural transition in the membranes formed with DOPC/DOPG (8:2) as well as *E. coli* extract lipid from lamellar phase to inverted hexagonal phase with water channels with a diameter of 3.4 nm (Yang et al., 2007). In a similar experiment, cationic polymethacrylate derivatives induced a transition from a lamellar phase to a bicontinuous cubic phase, which also has a water channel (Hu et al., 2013).

Cell-sized giant vesicles allow the in situ microscopic observation of the spatial and time-dependent response of the membrane due to the interaction with HDP-mimetic polymers. HDP-mimetic phenylene ethynylene derivatives induced the leakage of a low molecular weight (1 kDa) fluorescent marker but not a high molecular weight (10 kDa) marker entrapped in a giant vesicle formed by *E. coli* extracted lipids, suggesting the formation of a leakage pore with a defined size (Yang et al., 2007). Kuroda and Yasuhara et al. reported that HDP-mimetic polymethacrylate derivatives disrupt membranes by different mechanisms depending on the chemical structure of the hydrophobic side chains. Specifically, polymers with butyl groups as hydrophobic chains form pores in the membrane whereas polymers with methyl groups induced the rupture of vesicles (Tsukamoto et al., 2021).

Molecular dynamics (MD) simulations can provide atomic-scale information on the interaction between HDP-mimetic polymer and lipids. The MD simulation of the interaction between HDP-mimetic polymethacrylate and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC)/POPG (7:3) membrane suggested that the hydrophobic side chains are oriented toward the hydrophobic core of the membrane, while the cationic side chains are faced to the water-membrane interface when the polymer is bound to the membrane (Palermo et al., 2012). In addition, the polymer with longer alkyl spacer in the cationic side chain was inserted in the membrane deeper than the short spacer one, suggesting that the spacer length determines how deeply polymers are inserted in the membrane. Similar polymethacrylate derivatives induced the clustering of the negatively charged POPG in membranes, inducing the demixing of lipids in the membrane (Baul et al., 2014).

As described here, a variety of synthetic antimicrobial polymers have been developed by mimicking the HDPs in nature. Although this class of polymers does not have specific monomer sequences like peptides, they display biological activities as well as the mode of actions that are similar to natural HDPs.

11.3 Apolipoprotein-mimetic polymers for lipid nanodisc formation

Apolipoprotein A-I (Apo A-I) is a protein responsible for cholesterol transport in vivo by forming high-density lipoproteins (HDLs). Primitive HDLs form nanometer-scale disk-like molecular assemblies (Forte et al., 1971) in which a lipid bilayer is surrounded by two belt-like Apo A-I proteins (Fig. 11.3) (Borhani et al., 1997). Apo A-I consists of 243 amino acid residues that form ten alpha-helix domains. Each helix has an amphiphilic structure in which hydrophilic amino acid residues are segregated on one side, and hydrophobic residues are localized on the other. The discoidal assembly is stabilized by two amphipathic α-helices that cover the hydrophobic edge of a lipid bilayer. In this section, we introduce the molecular design and characteristics of lipid nanodisc-forming polymers that have been developed to mimic the structure of Apo A-I.

11.3.1 Membrane scaffold proteins and nanodisc-forming peptides

Sligar et al. developed a membrane scaffold protein (MSP), which is optimized for the formation of lipid nanodiscs by modifying the structure of Apo A-I to provide a molecular

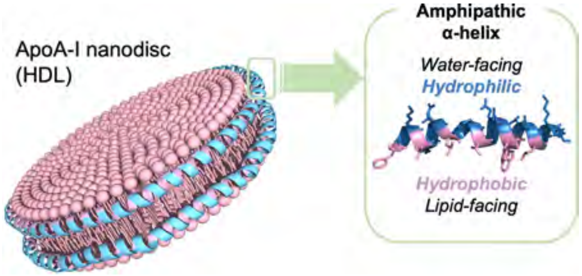

FIGURE 11.3 Schematic illustration of a lipid nanodisc formed with Apo A-I.

platform for the analysis of membrane proteins. The first MSP was designed by eliminating the globular N-terminal domain from Apo A-I, which is not involved in the interaction with lipid bilayers (Bayburt et al., 2002). To form lipid nanodiscs, the MSP is mixed with phospholipids in the presence of a surfactant (sodium cholate), which is then removed by dialysis. Each nanodisc has a diameter of 10 nm and contains 160 and 125 lipid molecules for saturated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and unsaturated POPC, respectively. It has also been reported that nanodiscs with different diameters ranging from 9.8 to 12.9 nm can be prepared by the MSP mutants with different lengths of amphipathic α-helices (Denisov et al., 2004). MSP nanodiscs that can provide a more native-like environment compared to conventional surfactants have been applied to the structural and functional analysis of a wide variety of membrane proteins in combination with NMR and biochemical assays (Denisov & Sligar, 2017).

Many attempts have been made to form HDL-like lipid particles using short peptides that form amphipathic α-helices instead of a full-length MSP. A family of amphiphilic peptides—the so-called class A peptide—has been designed to mimic the amphipathic α-helices of apolipoproteins, which have hydrophobic and hydrophilic faces. In a hydrophilic face of the class A peptide, anionic amino acid residues are located in the center, whereas cationic residues are placed at the boundary near the hydrophobic face (Segrest et al., 1990). 18 A is the first example of the class A peptide that has 18 amino acid residues with the sequence of DWLKAFYDKVAEKLKEAF (Anantharamaiah et al., 1985). Although 18 A peptide has no sequence homology with apolipoproteins, it has formed discoidal molecular assemblies with phospholipids in water. Ac-18A-NH_2, which increased the helicity of the peptide due to acetylation and amidation of N- and C-terminuses, respectively, enhanced the affinity for lipid molecules. Additionally, a 4 F peptide has been designed that has increased hydrophobicity by the substitution of two leucine residues with phenylalanine in Ac-18A-NH_2 (Mishra et al., 2008). These peptides not only form HDL-like lipid aggregates but also exhibit pharmacological activities such as anti-inflammatory and anti-atherosclerotic effects. Fragment peptides of Apo A-I and their oligomers have been known to form nanodiscs with phospholipids (Zhao et al., 2013). A 23-residue peptide (CGVLESFKASFLSALEEWTKKLQ-NH_2) and a truncated 16-residue peptide (CGSFLSALEEWTKKLQ-NH_2) were designed by the slight modification of helix 10 in Apo A-I, which is known to be essential in lipid binding and cholesterol transport. The oligomers of up to tetramers of these peptides were also synthesized by a native chemical ligation technique. All these peptides and their oligomers successfully formed nanodiscs with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC). This nanodisc displayed the activity of cholesterol efflux confirmed by in vitro study using macrophage J774 cells. Furthermore, oligomerization improved the stability of nanodiscs against enzymatic degradation, resulting in improved pharmacokinetics. These peptide-based studies indicated that the amphiphilic α-helix is an essential structure rather than the amino acid sequence itself for the design of molecules that form HDL-like nanodiscs through the complexation of phospholipids.

11.3.2 Synthetic polymer-based agents for lipid nanodisc formation

Several synthetic polymers have been designed so far to spontaneously form nanodiscs by the complexation with lipid molecules (Fig. 11.4). Synthetic polymer-based approaches

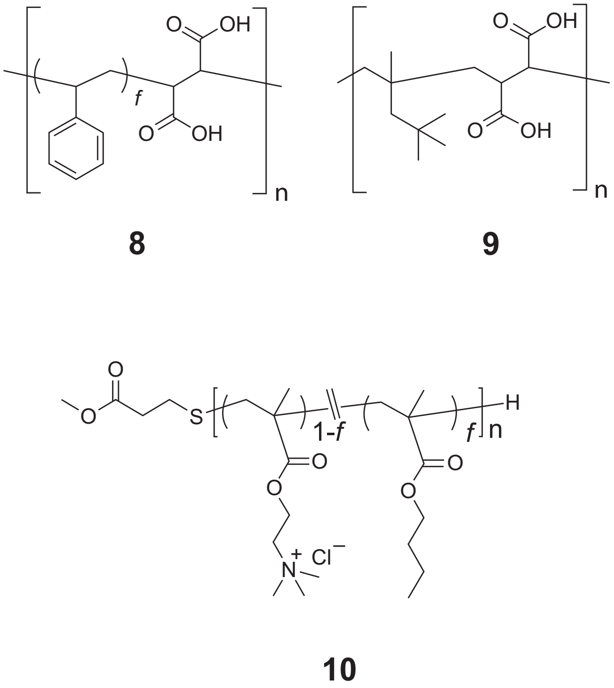

FIGURE 11.4 Chemical structures of nanodisc-forming synthetic polymers.

for the formation of lipid nanodisc have several advantages: (1) easy synthesis enables large-scale production at low cost, (2) functional groups such as fluorophores can be modified, (3) nanodiscs can be prepared spontaneously without the aid of surfactants, and (4) the overlap of spectroscopic signals, and unexpected interaction due to the presence of nanodisc-forming proteins/peptides can be avoided. Watts et al. reported the spontaneous formation of nanodiscs using amphiphilic styrene-maleic acid copolymer (SMA, **8**) (Orwick et al., 2012). A similar nanodisc-forming amphiphilic polymer (DIBMA, **9**) was designed by introducing diisobutylene as hydrophobic groups to diminish the absorption in the UV region derived from the styrene group in SMA (Oluwole et al., 2017). Ramamoorthy et al. have designed derivatives of styrene-maleic acid copolymers with various hydrophilic groups for the formation of nanodiscs with different surface charges that can minimize the unexpected interaction with incorporated membrane proteins (Ravula et al., 2018). SMA can produce nanodiscs not only from phospholipid vesicles but also from intact cell membranes. Dorr et al. demonstrated that tetrameric potassium channel KcsA was directly isolated from *E. coli* membrane using SMA (Dorr et al., 2014). Taking advantage of these features, SMA-based nanodiscs have been applied to the analysis of a variety of membrane proteins to date (Dorr et al., 2016).

Yasuhara et al. designed a lipid nanodisc-forming polymer by employing a polymethacrylate molecular framework (PMA, **10**) (Yasuhara et al., 2017). This nanodisc-forming polymer is a random copolymer with hydrophilic choline groups and hydrophobic butyl groups in the side chains. This polymer can be synthesized in one pot by free radical polymerization which is a great advantage to developing the molecular library with a variety of structural variations. Systematic screening of the molecular library using DMPC liposome revealed that the polymers containing about 40%−60% hydrophobic monomers and with average molecular weights of 3000−9000 g/mol spontaneously induced fragmentation of the lipid bilayer to form lipid nanodiscs. These characteristics of the polymer structure (molecular weight and hydrophobicity) are somewhat similar to those of the amphiphilic class A peptides described above. Since the expected length of the polymer from the molecular weight is significantly shorter than the periphery of the nanodisc, the edge part of the nanodisc is assumed to be covered with several polymer chains. PMA produced various sizes of nanodiscs in a range of 8−20 nm by changing the polymer/lipid ratio as confirmed by dynamic light scattering (DLS) measurement and transmission electron microscope (TEM) observation. Such a tunability of the nanodisc size is another advantage of a polymer-based system compared to MSP. In addition, the gel to the liquid crystalline phase transition of the lipid bilayer was observed in the differential scanning calorimetry (DSC) analysis, confirming that the native-like membrane property was maintained in the nanodiscs. The lipid nanodiscs formed by PMA do not show significant absorption in the UV and visible regions, which is a great benefit for the spectroscopic study. Furthermore, amyloid fiber formation of human islet amyloid polypeptide (hIAPP), which has been linked to type 2 diabetes, was evaluated using the nanodiscs formed with PMA. Fluorescence measurement using thioflavin T revealed that the presence of the lipid nanodiscs markedly inhibited the fiber formation of hIAPP. Circular dichroism (CD) measurements found that hIAPP forms a β-sheet structure as amyloid fibers elongate in the absence of nanodiscs whereas the α-helix structure of hIAPP is maintained in the presence of nanodiscs. The observed inhibition of the amyloid formation may reflect that hIAPP is trapped in an oligomeric state due to the small surface area of the nanodiscs, which limits the number of hIAPP that can bind to the membrane. Similarly, PMA-based nanodiscs can inhibit the fiber formation of an amyloid-β protein associated with Alzheimer's disease (Sahoo et al., 2018).

These synthetic polymer-based nanodiscs have so far been used as a molecular platform for the analysis of membrane proteins as well as membrane-binding peptides. We expect that the biological activity of the polymer-based nanodiscs themselves will be explored in future studies.

11.4 Conclusion

In this chapter, we have introduced the approach to design biologically active synthetic polymers inspired by proteins and peptides that induce the rearrangement of lipid assemblies. HDP-mimetic and apolipoprotein-mimetic polymers have been successfully developed by mimicking the amphiphilic structures found in peptides and proteins. Interestingly, all synthetic polymers featured in this chapter successfully simulate biological activities as well as action mechanisms of parent proteins or peptides although they have very simplified chemical structures. These studies suggest that it is more important to mimic the physicochemical characteristics of the entire molecule rather than strictly reproducing the molecular structure or amino acid sequence of proteins and peptides. This approach to molecular design using artificial molecules is expected to provide us with not only the principle to developing new biologically active molecules but also new insights into how to reconstruct biological systems with minimal elements.

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