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Review

Membrane biology visualized in nanometer-sized discs formed by styrene maleic acid polymers

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ABSTRACT

Discovering how membrane proteins recognize signals and passage molecules remains challenging. Life depends on compartmentalizing these processes into dynamic lipid bilayers that are technically difficult to work with. Several polymers have proven adept at separating the responsible machines intact for detailed analysis of their structures and interactions. Styrene maleic acid (SMA) co-polymers efficiently solubilize membranes into native nanodiscs and, unlike amphipols and membrane scaffold proteins, require no potentially destabilizing detergents. Here we review progress with the SMA lipid particle (SMALP) system and its impacts including three dimensional structures and biochemical functions of peripheral and transmembrane proteins. Polymers systems are emerging to tackle the remaining challenges for wider use and future applications including in membrane proteomics, structural biology of transient or unstable states, and discovery of ligand and drug-like molecules specific for native lipid-bound states.

1. Introduction to polymer-based membrane solubilization

1.1. Polymers used for direct extraction of membrane proteins

Detailed analysis of molecular assemblies in their membrane environment requires isolation of the desired states. While detergents have proven adept at removing lipids, they can compromise protein stability and are inadequate at mimicking the true chemical and physical properties of biological bilayers. Just as soluble proteins are necessarily surrounded by water to retain stability and functionality, membrane proteins are coated by lipids that help preserve their biological activity. Hence there has been a search for molecular “scissors” that can excise a working section of membrane while leaving protein:lipid complexes intact. Since their discovery as efficient extractors of functionally intact proteins including the PagP palmitoyl transferase and bacteriorhodopsin [1] from membranes, SMA has appeared in over 80 publications (Fig. 1), and a grassroots SMALP network has formed to push the potential of this new field of native nanodisc technology. This community effort is harnessing the unique abilities of SMA polymers and overcoming technical barriers, and builds on decades of work on amphipols [2], bicelles [3] and membrane scaffold protein (MSP)-encircled nanodiscs [4] (Fig. 2). These systems each offer a growing array of new possibilities for membrane protein preparation and analysis, thus driving their rise in popularity and the emergence of practical solutions.

Several formulations of SMA have been shown to be effective at liberating active membrane protein assemblies. All contain styrene and maleic acid subunits arranged in a semi-randomly alternating pattern in a linear chain (Fig. 2). They offer differing ratios of hydrophobic groups, which insert into lipid bilayers. The polar moieties maintain pH-dependent solubility of the polymer. At a critical concentration, these polymers fragment membranes, yielding nanometer-sized discs.

Various free radical syntheses are used to generate these polymers, resulting in a variable number of styrene groups separated by single maleic anhydride groups. Acid hydrolysis is used to form maleic acid from the maleic anhydride group, yielding an active amphipathic polymer that is highly soluble in aqueous solution. They spontaneously form styrene maleic acid lipid particles (SMALPs) when the polymer solution and a membrane fraction or whole cells are mixed, yielding a clear emulsion (Fig. 3). These particles are stable in aqueous solutions, and can be freeze-dried after incorporation and then readily reconstituted. Initial studies reported the use of hydrolyzed versions of SMA2000 and SMA3000 from Total Cray Valley, which contain styrene to maleic acid ratios of 2:1 and 3:1, respectively, as do the related Lipodisq™ products. Polyscope offers XIRAN 30010 and 25,010 reagents, which have comparable activities and styrene to maleic acid subunit ratios of 2.3:1 and 3:1, respectively, as well as lacking cumene end-groups and offering distinct molecular size distributions. Based on comparative data from many studies, the commercially available XIRAN reagents and the various SMA2000 versions are the most

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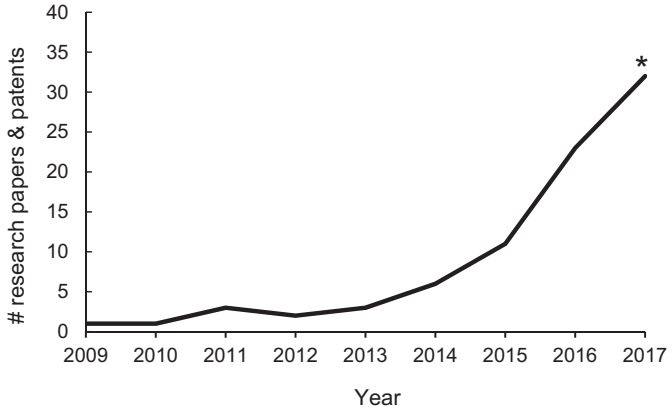

Fig. 1. Growth in the SMALP literature. The number of publications and patents describing SMA since the first paper describing their discovery appeared in 2009. The asterisk for 2017 indicates a partial year count.

effective and widely used SMA polymers for solubilizing a range of membrane proteins from single-transmembrane α-helices [5] to oligomeric complexes containing 36 transmembrane helices [6] and even larger assemblies.

For comparison, a range of SMA types have been tested for solubilization of three different membrane proteins expressed in *Escherichia* (*E.*) *coli* [7]. These included the homodimeric BmrA multidrug efflux pump which contains six transmembrane helices and a nucleotide binding domain, the LeuT symporter with 12 transmembrane helices, and ZipA, which spans the membrane once and has a large extramembrane domain. Yields of ~55% from total protein were purified, with SMA(2:1) producing 8–9 nm diameter nanodiscs with the highest protein yields, purities, and ligand binding activities. In contrast, smaller nanodiscs with diameters of about 5 nm could be produced by various SMA(3:1) polymers. The SMA(2:1) nanodiscs tolerated up to 4 mM magnesium chloride, while the varieties with 3:1 ratios of styrene to maleic acid show precipitation at cation concentrations over 1 mM due to cation chelation by the maleic acid groups. Altogether, the SMA(2:1) polymers with an average molecular mass of 7.5–10 kDa appear optimal for isolating these *E. coli*-expressed assemblies.

1.2. Replacement of detergents with SMA to solubilize and purify membrane assemblies

Conventional detergents work by sequestering lipid molecules within micelles. They dramatically disrupt bilayers, thus perturbing the stability and functionality of proteins. Hence only stable systems and those with reliable assays have been tractable. In contrast, SMA is much less disruptive and acts as a mild solubilizing agent by inserting into bilayers and, at a critical polymer concentration, spontaneously forming nanodiscs [8,9]. The optimal pH depends on the SMA polymer, but is in the range of 7.0 to 9.0, with lower pH values causing polymer aggregation, while low salt conditions generally help keep the SMA soluble [10]. The lipid bilayer structure appears to be less affected by polymers containing a lower amount of styrene to maleic acid, with a 2:1 ratio being less disruptive than a 3:1 ratio [11]. The exchange of lipids between particles is relatively rapid compared to other nanodisc systems, and is mediated by diffusion of monomeric lipid molecules out of discs and, particularly at high concentrations, by collisions between discs [12]. The dynamic nature of the SMA presumably allows lipids to be introduced or removed from a SMALP relatively easily, and also allows proteins to be transferred back into a liposome or other bilayer system [13].

The specificity of SMA for lipids has been investigated by multiple studies. They all show promiscuous interactions with various phospholipid bilayers and native biological membranes. Nonetheless, there is strong selectivity for fluid phase bilayers, while densely packed or ordered bilayers resist insertion of SMA [14]. There are differences in the rates of SMA-mediated solubilization, with easier insertion and disruption of bilayers formed of lipids having acyl chains that are shorter or unsaturated or having cylindrical shapes [15].

Eight SMA types have been surveyed for their abilities to solubilize monomeric, dimeric, trimeric and tetrameric forms of the *Rhodobacter sphaeroides* reaction center [16]. The most effective are those with lower molecular weights in the 10 kDa range and having 2:1 and 3:1 ratios of styrene to maleic acid. Increasing oligomer size or tight protein packing results in lower solubilization efficiencies. Fusing cellular membranes with synthetic or biological source lipid can boost protein yields, and large oligomers can be better solubilized in longer chain SMA forms in the 80–120 kDa range. In addition to small nanodiscs, larger membrane fragments with diameters of 50–100 nm can also be discerned by transmission electron microscopy (EM).

SMA perforates and solubilizes plasma and intracellular membranes at different rates, as seen by live cell fluorescence microscopy [17]. Addition of XIRAN 30010 to HeLa cell cultures causes the plasma membrane to perforate first. The polymer can then perforate intracellular membranes next, releasing fluorescent test proteins contained in their organelles shortly after those in the cytosol. There is no apparent preference of SMA for particular organelles in the cell,

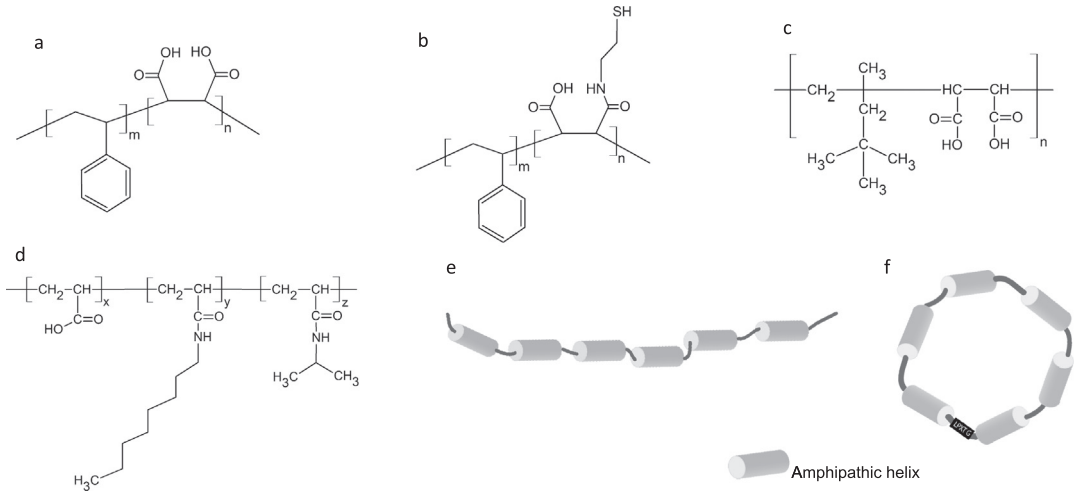

Fig. 2. Chemical structures of polymer-based solubilizing systems. Different synthetic polymers and schematic diagram of protein-based polymers that have been used for the preparation of membrane protein nanodiscs. a) SMA, b) SMA-SH, c) DIBMA, d) amphipols (A8-35), e) linear MSP and f) circularized MSP.