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Hydrophobic variants of ganglio-tripod amphiphiles for membrane protein manipulation

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ABSTRACT

Membrane proteins operate in unique cellular environments. Once removed from their native context for the purification that is required for most types of structural or functional analyses, they are prone to denature if not properly stabilized by membrane mimetics. Detergent micelles have prominently been used to stabilize membrane proteins in aqueous environments as their amphipathic nature allows for shielding of the hydrophobic surfaces of these bio-macromolecules while supporting solubility and monodispersity in water. This study expands the utility of branched diglucoside-bearing tripod agents, designated ganglio-tripod amphiphiles, with introduction of key variations in their hydrophobic sections and shows how these latter elements can be fine-tuned to maximize membrane protein solubilization while preserving characteristics of these molecules that afford stabilization of rather fragile assemblies. Their efficacy rivals benchmark detergents heavily used today, such as *n*-dodecyl-β-D-maltoside.

1. Introduction

Integral membrane proteins play central roles in many cellular processes including ion exchange, signal transduction, and material transfer between cells and their environments. It is estimated that roughly one third of human genes encode this protein class [1,2] and at least 50% of all commercially available pharmaceuticals target membrane proteins [3]. Despite such prevalence and functional importance, only a few hundred membrane protein structures are available – which is less than 1% of the soluble protein counterpart – indicating notorious difficulties in preparation and manipulation [4]. The discrepancy between biodiversity and sparse structural information is partly due to their low natural abundance, necessitating the development of expression systems tailored specifically for efficient membrane protein production [e.g., 5–7]. In addition, these bio-macromolecules are unstable in non-native aqueous environments due to their amphipathic characteristics [8].

In most experiments, detergents are used as complementary amphipathic agents to facilitate the solubilization, stabilization and structural and functional characterization of membrane proteins. Detergents readily self-assemble into micelles in aqueous media with a hydrophilic exterior and a hydrophobic interior — driven by the hydrophobic effect [9]. These assemblies have the ability to associate with entities bearing complementary surfaces. In particular, detergent micelles can dismantle cellular membranes and readily bind to (or shield) the hydrophobic portions of membrane proteins [10,11]. Once solubilized, membrane proteins suspended by detergent molecules are routinely referred to as protein-detergent complexes (PDCs). As it stands currently, detergent sets may be thought to be enough for membrane protein science because more than 100 detergents are commercially available. However, only a small number of detergents are generally useful, and the scope of their present application is embarrassingly narrow. Consequently, PDCs are often not stabilizing enough to prevent membrane protein denaturation and aggregation — the two main routes by which membrane protein structural and functional integrity is lost [12–14]. It is presumed that application coverage by conventional detergents is mainly limited because of their lack of structural variation [15,16]. Typical, conventional agents are commonly built biologically or synthetically from a flexible alkyl chain and a simple, single hydrophilic group (e.g. glucose, maltose or *N*-oxide). Finding a compatible detergent that stabilizes the membrane protein during the multi-step purification, functional characterization, and/or crystallization process(es) can prove to be a potentially painstaking trial-and-error adventure — but one that is exceedingly critical to the success of such experiments.

Alternative mimetic systems have been developed and pursued for membrane protein manipulation over the past several decades. Amphiphilic polymers (amphipols) [17,18], nanodiscs (NDs) [18–21], and lipodisqs [22] are innovative approaches to overcome the limitation of current tools for membrane protein stability. Tandem facial amphiphiles (TFAs) [23] and hemifluorinated surfactants (HFSs) [24–26] are other

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examples of recent inventions that have shown to be excellent in retaining the native structures of delicate membrane proteins. Amphipathic peptides such as lipopeptide detergents (LPDs) [27] and short designer peptides [28] proved to be effective for several classes of membrane protein systems as well. Also, amphiphiles bearing rigid hydrophobic groups have shown promising behaviors in membrane protein manipulation [29–32]. However, most of these agents were not designed for extracting/solubilizing membrane proteins from native membranes and, more importantly, have been unsuccessful in facilitating membrane protein structure determination via techniques, like X-ray crystallography, that require growth of high-quality, three-dimensional crystals. These types of membrane mimetics have been generally found more useful in structural characterization by solution-based approaches, such as NMR, EPR, or complementary optical spectroscopies.

The development of tripod amphiphiles (TPAs) as membrane mimetics has worked to coordinate the chemical and physical properties of these molecules and their associated micelles to ensure utility in membrane protein research [33–37]. Since one cannot *a priori* predict these properties, they need to be tested empirically. Improvements are made in an iterative process, balancing experimental efficacy with efficiency in synthesis. The TPA architecture is unique in terms of the presence of a quaternary carbon with three hydrophobic appendages [33]. This carbon in the lipophilic region limits the conformational freedom of this class of molecules, thereby making them rigid relative to conventional detergents. This rigidification likely enabled us to solve the crystal structures of several *N*-oxide TPAs themselves [37] and has the potential to facilitate the crystallization of a wide array of membrane proteins; for example, TPA-solubilized bR and potassium channel from *Streptomyces lividans* have been crystallized, although their structures have not yet been solved [34,36]. Recent TPA advances had led to a series of molecules with bifurcated glucose headgroups with favorable solubilization and stabilization efficacy [35]. With a successful lead molecule identified and a large number of alternative, aromatic-group-containing, Grignard reagents available, the current variations on the central theme of a ganglio-TPA template were envisioned. Herein, we show that some of these new analogs also display favorable (even superior) properties in terms of membrane protein solubilization as compared to the previously described TPAs and conventional agents, while preserving their ability to stabilize membrane proteins and membrane protein complexes. The systematic variation of the hydrophobic groups in combination with an efficient, inexpensive, information-rich solubility/stability screen enables us to contemplate detergent structure–property relationships that have been, to date, experimentally inaccessible.

2. Materials and methods

2.1. Detergent design and synthesis

The structures of the hydrophobic group variations of the ganglio-TPA template (TPA-2) are illustrated in the six examples, TPA-6, TPA-7, TPA-8, TPA-9, TPA-10 and TPA-11 (Fig. 1). These amphiphiles share a quaternary carbon projecting three hydrophobic entities and a branched diglucoside headgroup. These agents vary in either aromatic hydrocarbons: TPA-6 (methylphenyl), TPA-7 (isopropylphenyl), TPA-8 (*t*-butylphenyl) and TPA-9 (biphenyl), or in the chain length of the two alkyl groups: TPA-10 (pentyl) and TPA-11 (hexyl). These hydrophobic groups were introduced into the lipophilic region via straightforward chemical reactions according to Scheme 1. Briefly, an alkylidene (A) with an alkyl group (R = butyl, pentyl or hexyl) was reacted with a Grignard reagent (R_1MgBr) in the presence of Cu(I)CN, producing dinitrile-functionalized tripodal derivative (B). These dinitrile products were then subjected to hydrolysis in strong basic conditions at high temperature (200 °C). The resulting carboxylic acids (C) were activated using 1-hydroxybenzotriazole (HOBt) and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (EDCI) and were coupled with serinol to give amide coupling products (D). The two hydroxyl groups of these products were utilized for glycosylation with perbenzoyl-protected glucosyl bromide, followed by the removal of the protecting groups under Zemplén's conditions [38], providing the final diglucoside-containing tripod amphiphiles

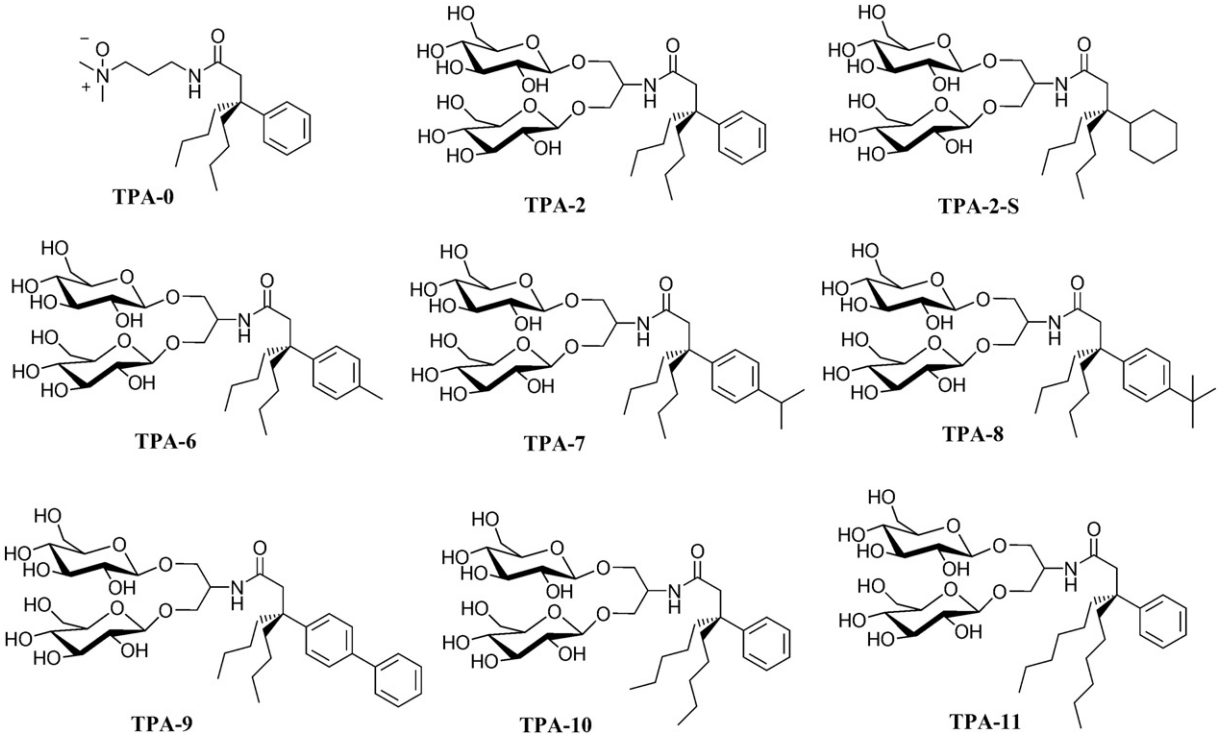

Fig. 1. Chemical structures of TPAs used as lead compounds (TPA-0, TPA-2 and TPA-2-S) and ganglio-tripod amphiphiles with the hydrophobic variations (TPA-6, TPA-7, TPA-8, TPA-9, TPA-10, and TPA-11).