New Glycotripod Amphiphiles for Membrane Protein Solubilization and Stabilization : Importance of Branching in the Hydrophilic Portion

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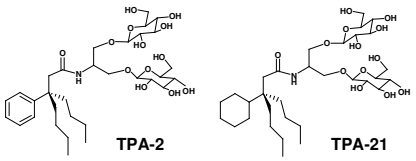
Abstract

Membrane proteins play central roles in biology. Elucidating the structures of membrane proteins is crucial for elucidating their functions. Isolation and physical characterization of membrane proteins remains a central challenge in biomolecular science. Three-dimensional structure determination for membrane proteins has been successful only within the past two decades, and the set of known membrane protein structures is far smaller than the set of known soluble protein structures. It is therefore important to explore new molecular tools that facilitate the stabilization of membrane proteins after removal from their native lipid bilayers, and ultimately their crystallization. Synthetic amphiphiles, typically detergents, are crucial tools in this field: they are used to extract embedded proteins from the membranes in which they naturally occur and maintain native protein conformation in the solubilized state. Physical characterization is often carried out with protein-amphiphile complexes, and such complexes are usually the basis for crystallization efforts; growth of high-quality crystals is a rate-limiting step in structure determination. In light of the central role played by synthetic amphiphiles in membrane protein science, surprisingly little effort has been devoted to exploration of non-traditional architectures for these small molecules. We describe new synthetic amphiphiles that display favorable solubilization and stabilization properties and that therefore represent attractive candidates for future functional and structural studies. Two of these new "glycotripod amphiphiles" are illustrated below. We show that these molecules are superior to conventional detergents for solubilization and stabilization of the LHI-RC photosynthetic superassembly from *Rhodobacter capsulatus*.



Introduction

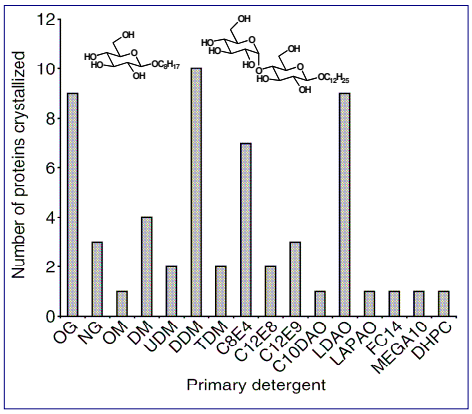


Figure 1. Number of proteins crystallized by primary detergents.

Our hypothesis

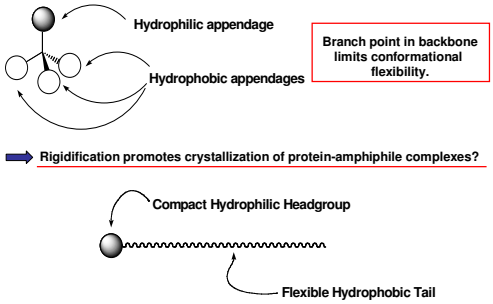
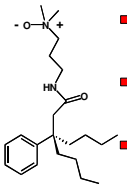


Figure 2. Schematic representation of the architecture of tripod amphiphiles and that of conventional detergents called as MPAs.

Previous studies



solubilization of bacteriorhodopsin from purple membrane and bovine rhodopsin from ROS

Potassium Channel from *S. lividans* (R. MacKinnon, personal communication, late 1990s)

Bacteriorhodopsin (Chiu et al. *Biochim. Biophys. Acta* 1751:213 (2005))

Figure 3. Chemical structure of lead tripod amphiphile (TPA 0) from initial studies and crystallization efforts by using TPA 0.

(TPA 0 is now manufactured and sold by Anatrace, Inc.)

Synthetic scheme of tripod amphiphiles

Molecular structures of TPAs and MPAs

Detergent screening protocol

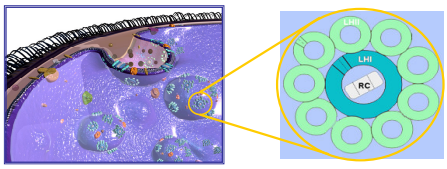


Figure 4. *Rhodobacter capsulatus* membranes without LHII was used.

Detergent screening protocol : solubilization and purification

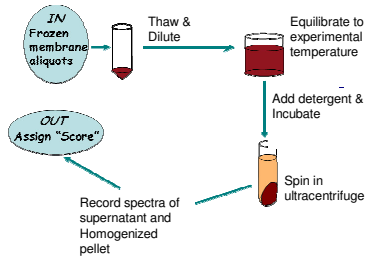


Figure 5. Schematic representation of solubilization protocol.

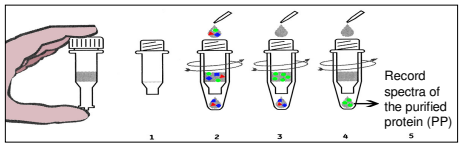


Figure 6. Schematic representation of purification protocol.

Superior properties of TPA 21

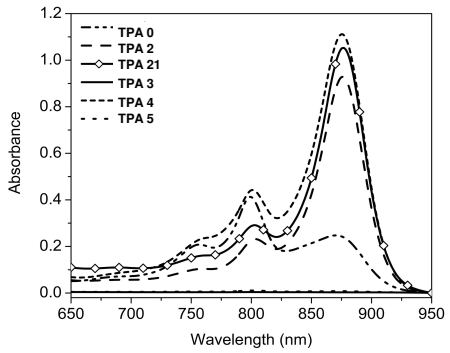


Figure 7. Spectroscopic comparison of solubilized protein complexes extracted from intracytoplasmic membrane of *R. capsulatus* by tripod amphphiles.

Stabilization effect of TPA 2 and TPA 21 : importance of branching in the hydrophilic portion of tripod amphiphiles

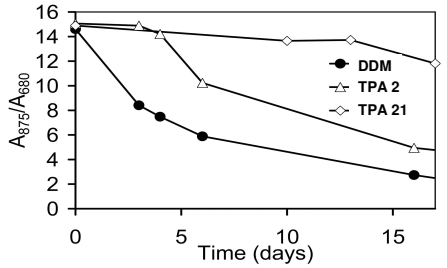


Figure 8. Stabilization effect of the protein purified by DDM, TPA 2 and TPA 21.

Conclusion

- Branching in hydrophilic as well as lipophilic portions of TPAs generates optimal behavior in stabilization toward delicate protein superassembly.
- The best amphiphile – TPA 21 is clear superior to conventional biochemical detergents with regard to long-term stability of solubilized LHI-RC superassembly.
- Overall, the TPA *vs.* MPA or DDM comparisons indicate that the new amphiphile design strategies can produce useful alternatives to conventional detergents for membrane protein manipulation.

References

1) Walian, P.;Cross, T.A.; Jap, B.K., *Genome Biology* **2004**, *5*, 215.
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3) Yu, S. M.; McQuade, D. T.; Quinn, M. A.; Hackenberger, C.P.R.; Krebs, M.P.; Polans, A. S.; Gellman. S. H. *Protein Sci.* **2000**, 9, 2518-2527.