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Technical Note 114

Amphipol A8-35: A Polymeric Surfactant for Membrane Protein Studies

Amphipols are a new class of surfactants that serve as stabilizers of membrane proteins in aqueous solutions. Amphipols can substitute traditional detergents used to extract membrane proteins, keeping them soluble in detergent-free aqueous solutions while stabilizing them biochemically[1-3]. Amphipol A8-35 is the most thoroughly characterized amphipol and is becoming widely used for membrane protein research. It consists of a strong hydrophilic polyacrylate chain onto which octylamine and isopropylamine have been randomly grafted[1, 4, 5] (Figure 1 A). Amphipol A8-35 is highly water soluble (> 200 g/L depending on pH and ionic strength of the solutions)[4, 5]. The high solubility is due to the anionic charges (~25 per molecule) carried by the carboxylate groups[4]. The average molecular mass of individual A8-35 molecules is 9-10 kDa[1, 4, 5]. In aqueous solutions (pH > 7.0), Amphipol A8-35 self-assembles into globular particles, each comprising ~4 molecules, with an average mass of ~40 kDa and a Stokes radius of ~3.15 nm (Figure 1B)[5]. The critical aggregation concentration is so low as to be negligible under most circumstances[3].

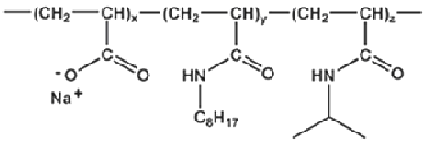

Figure 1A

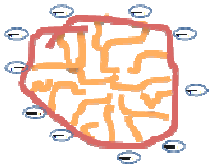

Figure 1B

Figure 2

Due to its amphipathic character, Amphipol A8-35 is able to "trap" solubilized membrane proteins by adsorbing onto their hydrophobic transmembrane surface, stabilizing their native structure and preserving their functionality[2, 3] (Figure 2).

Applications

Although its detergency is too weak to effectively extract and solubilize most membrane proteins [for some exceptions, see ref. 2], Amphipol A8-35 has been very successfully used to replace the detergent after the solubilization step and handle the extracted proteins in their native state in detergent-free solutions [for an example of trapping procedure, see ref. (8) (Figure 3)]. To date, amphipols have been used to trap ~30 different types of membrane

Table 1. Applications of Amphipol A8-35 to membrane protein studies.

Application	Benefits	Example of studies	References
Stabilization	Reducing inactivation by the detergent and preserving membrane protein native structure.	cytochrome b_6f complex bacteriorhodopsin Ca^{2+}-ATPase GPCRs	(1-3, 6, 10, 12, 14)
Functional studies	Reducing inactivation by the detergent and preserving membrane protein native structure and function. Avoiding perturbations of the latter by detergents. In the vast majority of cases, trapping by Amphipol A8-35 has no effect on ligand/substrate binding.	Ca^{2+}-ATPase bacteriorhodopsin nicotinic acetylcholine receptor GPCRs	(3, 6, 7, 10, 12, 14, 15)
Folding/Refolding	Amphipol A8-35 is a mild surfactant which provides a favorable environment for proteins to fold or refold from denatured state.	GPCRs OmpA and FomA bacteriorhodopsin	(12, 16)
NMR	Maintaining the solubilized membrane protein soluble without detergent, thus stabilizing the native structure. Note, however, that membrane protein/Amphipol A8-35 complexes cannot be handled at acidic pH. Addition of EDTA improves the spectra.	OmpX transmembrane β-barrel of OmpA	(8, 11, 17)
Electron microscopy	Stabilizing native structure. Mitochondrial Complex I/Amphipol A8-35 particles were observed to spread better than Complex I/detergent ones in cryo-EM single-particle experiments.	mitochondrial Complex I bacteriorhodopsin	(9, 10)
Immobilization of membrane proteins onto solid supports	Appropriate functionalization of Amphipol A8-35 turns it into a sort of double-faced tape that can be used to anchor amphipol-trapped membrane proteins onto solid surfaces such as chips or beads for ligand binding studies.	nicotinic acetylcholine receptor bacteriorhodopsin cytochrome b_6f complex cytochrome bc_1 complex detection of antibodies or toxin binding by SPR or fluorescence measurements	(15)

proteins, ranging in molecular weight from 5 kDa to > 1MDa[2, 3]. Small proteins may bind ~50 kDa of Amphipols[10], the mass of Amphipol bound increasing slowly with the size of the transmembrane region[2]. The protein/Amphipol complexes thus formed are slightly larger than those formed with classical detergents [6, 8, 10, 13]. Although there can be exceptions[3, 6, 14]) in most cases, trapping by Amphipol A8-35 affects neither the binding of ligands or substrates nor the functionality of membrane proteins[3, 7, 10, 12, 15]. A list of applications is given in Table 1.

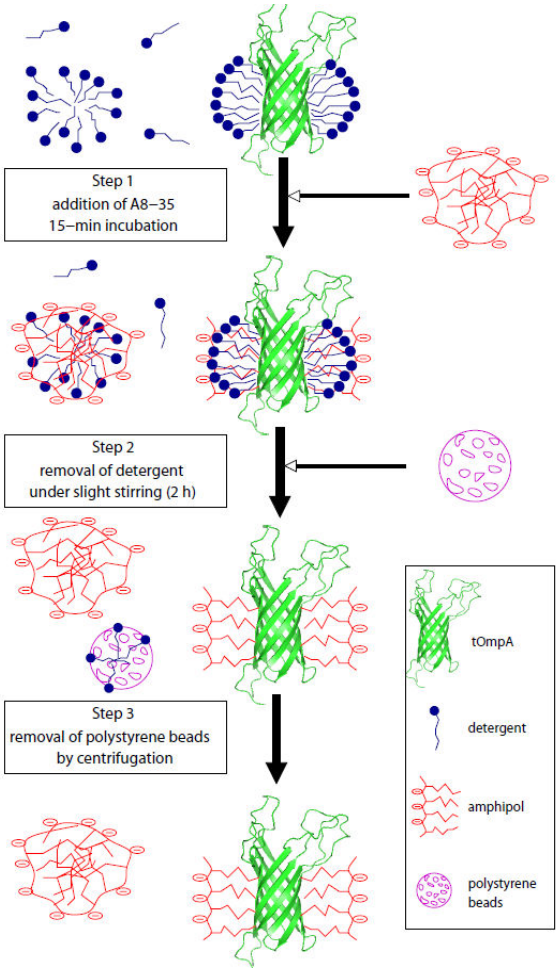

Figure 3. An example of trapping procedure. Figure reproduced from "NMR study of a membrane protein in detergent-free aqueous solution." *Proc. Natl. Acad. Sci. USA*. 2005, 102, 8893-8898, Zoonens, M., Catoire, L. J., Giusti, F. & Popot, J.-L. Copyright (2005) National Academy of Sciences, U.S.A.

Anatrace Offering

Anatrace is proud to be the exclusive manufacturer and supplier of Amphipol A8-35. Anatrace offers Amphipol A8-35 in three packaging sizes: 50 mg, 100 mg and 500 mg.

AP835	Amphipol A8-35	ANAGRADE

<MW> = 9-10 kDa (Amphipols are intrinsically polydisperse)
Purity: Conforms to HPLC standard
Appearance: white powder
Solubility: >200g per liter in water

Storage and Handling

Amphipol A8-35 can be stored at room temperature in tightly sealed containers
Stability: 1 year at room temperature

References

1. Tribet, C., Audebert, R. & Popot, J.-L. (1996). Amphipols: polymers that keep membrane proteins soluble in aqueous solutions. *Proc. Natl. Acad. Sci. USA* **93**, 15047-15050.
2. Popot, J.-L., Berry, E. A., Charvolin, D., Creuzenet, C., Ebel, C., Engelman, D. M., Flötenmeyer, M., Giusti, F., Gohon, Y., Hervé, P., Hong, Q., Lakey, J. H., Leonard, K., Shuman, H. A., Timmins, P., Warschawski, D. E., Zito, F., Zoonens, M., Pucci, B. & Tribet, C. (2003). Amphipols: polymeric surfactants for membrane biology research. *Cell. Mol. Life Sci.* **60**, 1559-1574.
3. Popot, J.-L. (2010). Amphipols, nanodiscs, and fluorinated surfactants: three non-conventional approaches to studying membrane proteins in aqueous solutions. *Annu. Rev. Biochem., in the press*.
4. Gohon, Y., Pavlov, G., Timmins, P., Tribet, C., Popot, J.-L. & Ebel, C. (2004). Partial specific volume and solvent interactions of amphipol A8-35. *Anal. Biochem.* **334**, 318-334.
5. Gohon, Y., Giusti, F., Prata, C., Charvolin, D., Timmins, P., Ebel, C., Tribet, C. & Popot, J.-L. (2006). Well-defined nanoparticles formed by hydrophobic assembly of a short and polydisperse random terpolymer, amphipol A8-35. *Langmuir* 22, 1281-1290.
6. Champeil, P., Menguy, T., Tribet, C., Popot, J.-L. & le Maire, M. (2000). Interaction of amphipols with the sarcoplasmic reticulum Ca^{2+}-ATPase. *J. Biol. Chem.* **275**, 18623-18637.
7. Martinez, K. L., Gohon, Y., Corringer, P.-J., Tribet, C., Mérola, F., Changeux, J.-P. & Popot, J.-L. (2002). Allosteric transitions of *Torpedo* acetylcholine receptor in lipids, detergent and amphipols: molecular interactions *vs.* physical constraints. *FEBS Lett.* **528**, 251-256.
8. Zoonens, M., Catoire, L. J., Giusti, F. & Popot, J.-L. (2005). NMR study of a membrane protein in detergent-free aqueous solution. *Proc. Natl. Acad. Sci. USA* **102**, 8893-8898.
9. Flötenmeyer, M., Weiss, H., Tribet, C., Popot, J.-L. & Leonard, K. (2007). The use of amphipathic polymers for cryo-electron microscopy of NADH:ubiquinone oxidoreductase (Complex I). *J. Microsc.* **227**, 229-235.
10. Gohon, Y., Dahmane, T., Ruigrok, R., Schuck, P., Charvolin, D., Rappaport, F., Timmins, P., Engelman, D. M., Tribet, C., Popot, J.-L. & Ebel, C. (2008). Bacteriorhodopsin/amphipol complexes: structural and functional properties. *Biophys. J.* **94**, 3523-3537.
11. Catoire, L. J., Zoonens, M., van Heijenoort, C., Giusti, F., Popot, J.-L. & Guittet, E. (2009). Inter- and intramolecular contacts in a membrane protein/surfactant complex observed by heteronuclear dipole-to-dipole cross-relaxation. *J. Magn. Res.* **197**, 91-95.
12. Dahmane, T., Damian, M., Mary, S., Popot, J.-L. & Banères, J.-L. (2009). Amphipol-assisted *in vitro* folding of G protein-coupled receptors. *Biochemistry* **48**, 6516-6521.
13. Zoonens, M., Giusti, F., Zito, F. & Popot, J.-L. (2007). Dynamics of membrane protein/amphipol association studied by Förster resonance energy transfer. Implications for *in vitro* studies of amphipol-stabilized membrane proteins. *Biochemistry* **46**, 10392-10404.

14. Picard, M., Dahmane, T., Garrigos, M., Gauron, C., Giusti, F., le Maire, M., Popot, J.-L. & Champeil, P. (2006). Protective and inhibitory effects of various types of amphipols on the Ca^{2+}-ATPase from sarcoplasmic reticulum: a comparative study. *Biochemistry* **45**, 1861-1869.
15. Charvolin, D., Perez, J.-B., Rouvière, F., Giusti, F., Bazzacco, P., Abdine, A., Rappaport, F., Martinez, K. L. & Popot, J.-L. (2009). The use of amphipols as universal molecular adapters to immobilize membrane proteins onto solid supports. *Proc. Natl. Acad. Sci. USA* **106**, 405-410.
16. Pocanschi, C. L., Dahmane, T., Gohon, Y., Rappaport, F., Apell, H.-J., Kleinschmidt, J. H. & Popot, J.-L. (2006). Amphipathic polymers: tools to fold integral membrane proteins to their active form. *Biochemistry* **45**, 13954-13961.
17. Catoire, L. J., Zoonens, M., van Heijenoort, C., Giusti, F., Guittet, E. & Popot, J.-L. (2009). Solution NMR mapping of water-accessible residues in the transmembrane *b*-barrel of OmpX. *Eur. Biophys. J., in the press*, PMID: 19639312.
18. Diab, C., Tribet, C., Gohon, Y., Popot, J.-L. & Winnik, F. M. (2007). Complexation of integral membrane proteins by phosphorylcholine-based amphipols. *Biochim. Biophys. Acta* **1768**, 2737-2747.

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