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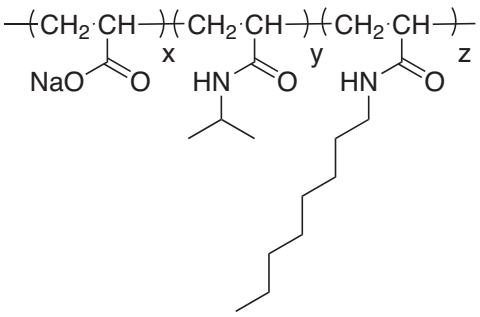
Technical Bulletin 140

Amphipol A8-35: Amphipathic Polymer for Membrane Protein Studies

Amphipols are a new class of polymers that serve as stabilizers of membrane proteins in aqueous solutions. In general, amphipols are used to replace detergents after the solubilization step. Membrane proteins trapped in amphipols are soluble in detergent-free, aqueous solutions and biochemically stabilized[1-4].

Amphipol A8-35 from Anatrace® 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, 5, 6] (Figure 1A). Amphipol A8-35 is highly water-soluble (> 200 gm/lt depending on pH, ionic strength of the solutions, and the concentration of divalent cations)[5-7]. The high solubility is due to the anionic charges (~12 per molecule) carried by the carboxylate groups. The average molecular mass of individual A8-35 molecules is ~4.3 kDa[8]. In aqueous solutions (pH > 7.0), Amphipol A8-35 self-assembles into globular particles, each comprising ~9 average A8-35 molecules, with an average mass of ~ 40 kDa and a Stokes radius of ~ 3.15 nm[6] (Figure 1B). The critical aggregation concentration is so low (~ 0.002 g/L)[9] as to be negligible under most circumstances[3, 4].

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[2-4].



X = 0.35, Y = 0.25, Z = 0.40

Figure 1A

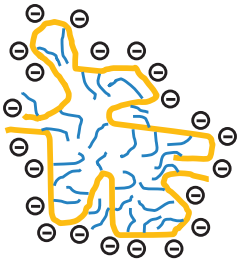


Figure 1B

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 a detailed protocol of a trapping procedure, see ref. 10 (Figure 2) shown on page 2. To date, amphipols have been used to trap more than three dozen different types of membrane proteins reviewed in ref. 11, ranging in molecular weight from 5 kDa to > 1MDa. Small proteins, like bacteriorhodopsin, may bind ~ 50 kDa of amphipols[12], the mass of amphipol bound increasing slowly with the size of the transmembrane region[11]. The protein/amphipol complexes thus formed are slightly larger than those formed with classical detergents[12, 13]. Although there can be exceptions[7, 14] in most cases, trapping by Amphipol A8-35 affects neither the binding of ligands or substrates nor the functionality of membrane proteins[12, 15-17]. A list of applications is given in Table 1 (shown on page 3).

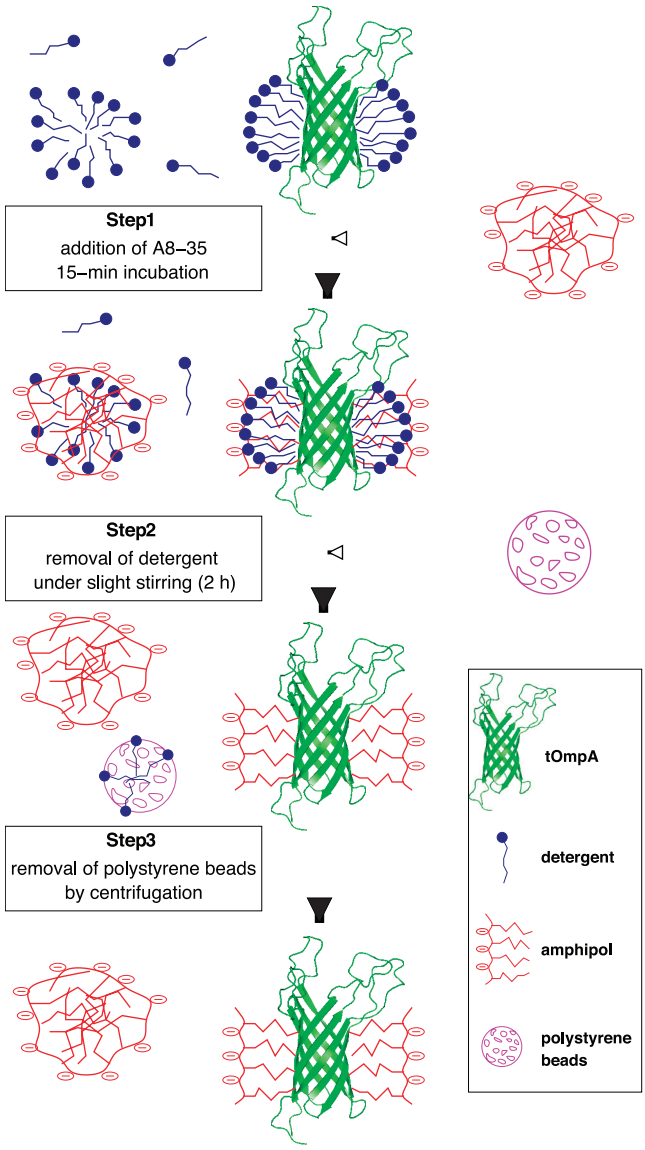


Figure 2. An example of trapping procedure. Figure reproduced from "NMR study of a membrane protein in detergent-free aqueous solution." (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 8893-8898, Zoonens, M., Catoire, L. J., Giusti, F. and Popot, J.-L. Copyright (2005) National Academy of Sciences, U.S.A.

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Table 1. Applications of Amphipol A8-35 to membrane protein studies.
For a more complete bibliography, see ref. 11 and http://tinyurl.com/amphipolbibliography.

Application	Benefits	Example of studies
Stabilization	Reducing inactivation by the detergent and preserving membrane protein native structure, most likely due to limitation of hydrophobic sink, preservation of MP/lipid interactions, and damping of transmembrane domain conformational excursions	Bacteriorhodopsin[12] Ca^{2+}-ATPase[7, 14] GPCRs[15] cytochrome **bc₁** complex[18] MOMP[19]
Functional studies and ligand binding	Avoiding functional perturbations by detergents such as most MPs are functional in APols, but the enzymatic cycle of the calcium ATPase is slowed down, possibly due to damping of large-scale transmembrane conformational changes. Ligand binding is generally unperturbed.	Bacteriorhodopsin[12] nicotinic acetylcholine receptor[16] GPCRs[15] Ca^{2+}-ATPase[7, 14]
Folding/Refolding	Amphipol A8-35 is a mild surfactant which provides a favorable environment for proteins to fold or refold from denatured state.	OmpA[20, 21], FomA[20], OmpT[22] and PagP[22] Bacteriorhodopsin[20, 23] GPCRs[12]
NMR	Stabilizing the native structure of membrane proteins by amphipols facilitates working for extended periods at relatively high temperature. Note, however, that membrane protein/Amphipol A8-35 complexes cannot be handled at acidic pH. Addition of EDTA improves the spectra.	OmpX[24, 25] transmembrane β-barrel of OmpA[26] BR[27, 28] GPCRs[29] MOMP[30]
Electron microscopy (Cryo-EM)	Stabilizing native structure and/or conformational changes of membrane proteins and supercomplexes that are easily disrupted by detergent. Mitochondrial Complex I/Amphipol A8-35 particles were observed to spread better than Complex I/detergent ones in cryo-EM single-particle experiments.	mitochondrial respirasome[31] TRPA1[32], TRPV1[33, 34] mitochondrial Complex I[35] Bacteriorhodopsin[12] OmpF[36]
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 **b₆f** complex, cytochrome **bc₁** complex, detection of antibodies or toxin binding by SPR or fluorescence measurements[17, 37, 38]
Light spectroscopy	UV and visible absorption, fluorescence, CD and SR-CD spectroscopies can all be used. All current APols interfere with IR absorption spectroscopy in the amide band region, but resonance Raman spectroscopy is accessible.	Bacteriorhodopsin[4, 12]
Mass spectrometry	MALDI-TOF, ESI-MS and ESI-IMS-MS have all been validated. Subunits and lipids can be detected, and the folded and unfolded states of the proteins distinguished. A8-35 facilitates whole-proteome trypsinolysis and identification of tryptic peptides.	OmpT and PagP[22] Bacteriorhodopsin, cytochrome **b₆f** complex, cytochrome **bc₁** complex, tOmpA[39]
Delivery of membrane proteins to pre-existing membranes	Amphipol A8-35 can be used to transfer membrane proteins to lipidic mesophases where they crystallize. Also, amphipols do no lyse target membranes (lipid vesicles or black films, cell plasma membrane) and can, therefore, be used to deliver to them hydrophobic cargoes. Note that amphipols will remain associated to the target membrane.	Bacteriorhodopsin[40] OmpA[20] DAGK[41]
Vaccination	Stabilizing membrane proteins used as immunogenes. Co-delivering them along with amphipol-bound or co-trapped adjuvants. The A8-35-trapped major outer membrane protein (MOMP) from *Chlamydia trachomatis* offers a much better protection to vaccinated mice than its detergent-solubilized counterpart.	MOMP[19, 42]

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