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\(^4,\,5\) (Figure 1A). 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](image1.png) ![Figure 1B](image2.png) ![Figure 2](image3.png)

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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Benefits</th>
<th>Example of studies</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stabilization</td>
<td>Reducing inactivation by the detergent and preserving membrane protein native structure.</td>
<td>cytochrome b$_{6}$f complex bacteriorhodopsin Ca$^{2+}$-ATPase GPCRs</td>
<td>(1-3, 6, 10, 12, 14)</td>
</tr>
<tr>
<td>Functional studies</td>
<td>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.</td>
<td>Ca$^{2+}$-ATPase bacteriorhodopsin nicotinic acetylcholine receptor GPCRs</td>
<td>(3, 6, 7, 10, 12, 14, 15)</td>
</tr>
<tr>
<td>Folding/Refolding</td>
<td>Amphipol A8-35 is a mild surfactant which provides a favorable environment for proteins to fold or refold from denatured state.</td>
<td>GPCRs OmpA and FomA bacteriorhodopsin</td>
<td>(12, 16)</td>
</tr>
<tr>
<td>NMR</td>
<td>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.</td>
<td>OmpX transmembrane β-barrel of OmpA</td>
<td>(8, 11, 17)</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td>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.</td>
<td>mitochondrial Complex I bacteriorhodopsin</td>
<td>(9, 10)</td>
</tr>
<tr>
<td>Immobilization of membrane proteins onto solid supports</td>
<td>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.</td>
<td>nicotinic acetylcholine receptor bacteriorhodopsin cytochrome b$<em>{6}$f complex cytochrome bc$</em>{1}$ complex detection of antibodies or toxin binding by SPR or fluorescence measurements</td>
<td>(15)</td>
</tr>
</tbody>
</table>

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.

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


14. Picard, M., Dahmane, T., Garrigos, M., Gauron, C., Giusti, F., le Maire, M.,
    types of amphipols on the Ca\textsuperscript{2+}-ATPase from sarcoplasmic reticulum: a
15. Charvolin, D., Perez, J.-B., Rouvière, F., Giusti, F., Bazzacco, P., Abdine, A.,
    universal molecular adapters to immobilize membrane proteins onto solid
16. Pocanschi, C. L., Dahmane, T., Gohon, Y., Rappaport, F., Apell, H.-J.,
    integral membrane proteins to their active form. \textit{Biochemistry} \textbf{45}, 13954-
    13961.
    J.-L. (2009). Solution NMR mapping of water-accessible residues in the
    transmembrane \(b\)-barrel of OmpX. \textit{Eur. Biophys. J.}, \textit{in the press}, PMID:
    19639312.
    Complexation of integral membrane proteins by phosphorylcholine-based