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# Systematic analysis of the use of amphipathic polymers for studies of outer membrane proteins using mass spectrometry

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ABSTRACT

Membrane proteins (MPs) are essential for numerous important biological processes. Recently, mass spectrometry (MS), coupled with an array of related techniques, has been used to probe the structural properties of MPs and their complexes. Typically, detergent micelles have been employed for delivering MPs into the gas-phase, but these complexes have intrinsic properties that can limit the utility of structural studies of MPs using MS methods. Amphipols (APols) have advantages over detergent micelles and have been shown to be capable of delivering native MPs into the gas-phase. Comparing six different APols which vary in mass and charge, and the detergent *n*-dodecyl-β-D-maltopyranoside, we aimed to determine which APols are most efficient for delivery of native outer membrane proteins (OMPs) into the gas-phase. We show that maintaining the solution-phase folding and global structures of three different OMPs (PagP, OmpT and tOmpA) are independent of the APol used, but differences in OMP activity can result from the different APol:OMP complexes. ESI-IMS–MS analysis of OMP:APol complexes shows that the A8-35 APol is most proficient at liberating all three OMPs into the gas-phase, without altering their gas-phase conformations.



## 1. Introduction

Membrane proteins (MPs) are vital components of many biological systems and represent a proportion of drug targets greater than their abundance in the genome [1]. Despite this, high resolution structural data from conventional biophysical techniques such as X-ray crystallography and NMR spectroscopy are sparse when compared with those available for soluble proteins. This is a result of the poor aqueous solubility of MPs and the difficulty in expressing and purifying MPs in yields required for structural analysis [2]. Mass spectrometry (MS) has recently been used to probe the topology, stability and stoichiometry of MPs and their complexes, to assess specific binding of lipids and detergents to MPs, and to determine the influence of these solubilising partners on the gas-phase stability of MPs [3–7]. MS has also been used in combination with other techniques, such as ion mobility spectrometry (IMS), photo-oxidative labelling (FPOP) and hydrogen–deuterium exchange (HDX), to provide further structural and dynamic information of MPs in the solution and gas-phase [8–10].

Detergents above their critical micelle concentration (CMC) form water soluble micelles, and these are commonly used to maintain the native structure of MPs [11,12]. The dynamic and curved nature of micelles, however, can perturb MP structure and dynamics, and high concentrations of detergents are required to maintain MP solubility [11–13]; additionally the dissociating character of detergents can bring about deactivation of membrane proteins [14]. Alternative solubilisation techniques have been developed to provide a more stable and native-like environment, including bicelles [12,15,16], nanodiscs [17] and amphipols (APols) [14,18,19], all of which have been used for the analysis of MPs by electrospray ionisation-mass spectrometry (ESI-MS) [20,21]. Bicelles and nanodiscs have been developed to provide an environment more closely resembling that of a native membrane, whilst APols (or amphipathic polymers) are highly non-native yet provide a very stabilising environment for MPs [14,18]. A8-35 (Fig. 1) is the best characterised of previously described APols [14,18,21–23]. It is a polyacrylic acid derivative randomly grafted with octylamine (~25%) and isopropylamine (~40%) groups for

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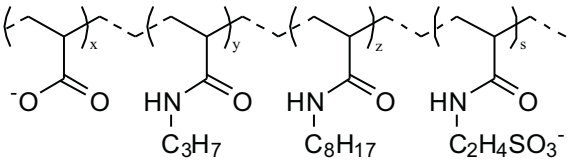
a)




| | A8-35 | A8-75 | SAPol | A34-35 | A34-75 |
|---|---|---|---|---|---|
| X | ~35% | ~75% | ~35% | ~35% | ~75% |
| Y | ~40% | - | - | ~40% | - |
| Z | ~25% | ~25% | ~25% | ~25% | ~25% |
| S | - | - | ~40% | - | - |
| Mw (kDa) | 4.3 | 4.1 | 5.6 | 17 | 16 |

b)

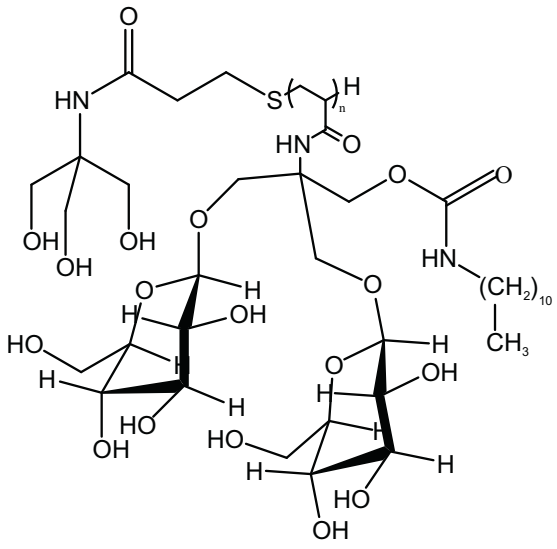



**Fig. 1.** (a) Average compositions of A8-35, A8-75, SAPol, A34-35 and A34-75. The table shows the % of free acid groups that remain ungrafted (x) or grafted with isopropylamine (y), octylamine (z) or taurine (s). Also shown are (approximate) number average masses of the respective APol [19]. (b) Structure of NAPol, a non-ionic amphipol (number average mass 11.3 kDa) [29].

increased hydrophobicity, whilst the remaining free acid groups (~35%) allow the solubility of APols with trapped MPs. However, as A8-35 is dependent on having ionised carboxylic acid groups for solubility, its usage is limited to pH >7 [18,23–25]. This has inspired the development of a variety of APols to expand the range of possible applications. Non-ionic amphipols (NAPols), sulphonated amphipols (SAPols) and phosphocholine based amphipols (PCAPols) use alternative chemical groups in place of, or in addition to, carboxylic acids to maintain solubility, and alleviate the pH restriction [14,18,26–30]. Synthesis of these and other variant forms of A8-35 has expanded the repertoire of physical properties of APols (Fig. 1). For example, A8-75 is a variant of A8-35 that uses the same precursor polyacrylic acid but lacks the isopropylamine grafting, resulting in a higher proportion of free acid groups (~75%); A34-35 and A34-75 are equivalent in their grafting to A8-35 and A8-75, respectively, but use a larger polyacrylic acid precursor [31]. The broad variability and the resulting properties of APols allow them to be applied in conjunction with an array of biophysical techniques for MP structural and function studies, including SEC, SAXS, EM and NMR [18,19,32–39].

Matrix-assisted laser desorption ionisation (MALDI)-MS analysis has been used for mass measurements of bacteriorhodopsin and the cytochrome $b_6f$ MP complex following release from NAPol and A8-35, respectively [35,40]. However, due to the denaturing

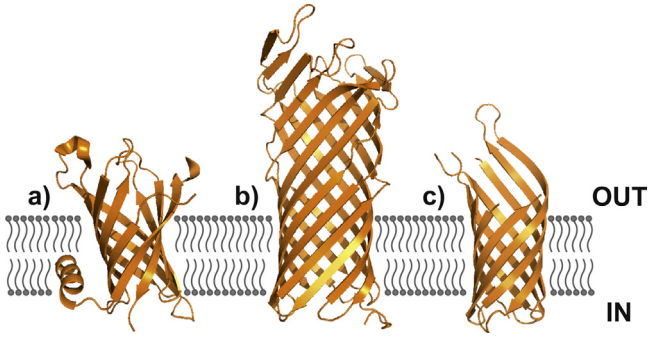



**Fig. 2.** Crystal structures of (a) PagP (PDB file 1THQ) [45], (b) OmpT (PDB file 1I78) [42] and (c) tOmpA (PDB file 1QJP) [44].

nature of MALDI ionisation, few conclusions can be drawn about the gas-phase structures of these MPs. Conversely, ESI-IMS–MS showed OmpT and PagP to populate a native-like conformation in the gas-phase when liberated from A8-35 (as determined by collision cross-section (CCS) values estimated from IMS data [21]). In the following study, we describe how the physical properties of a range of APols (A8-35, A8-75, SAPol, A34-35, A34-75, and NAPol) influence the introduction of native OMPs into the gas-phase, using three OMPs: PagP, OmpT and tOmpA (Fig. 2). OmpT and PagP have been shown previously to be released from the APol A8-35 into the gas-phase in a native-like state [21,23] but analysis of tOmpA (the transmembrane region of OmpA, residues 1–171) from A8-35 using ESI-IMS–MS is previously unreported. PagP (20.2 kDa) and tOmpA (18.9 kDa) are eight-stranded β-barrels (Fig. 2) that function as a palmitoyl transferase enzyme and porin, respectively [41–44]. OmpT (35.3 kDa) is a 10-stranded β-barrel (Fig. 2) that operates as an endopeptidase, cleaving between consecutive basic residues [40,41]. OmpT differs structurally from the other OMPs studied here, not only in that the β-barrel is larger, but also in that approximately 50% of the barrel is extra-membrane. Thus, these three OMPs provide an excellent platform for systematic investigation of the utility of different APols for stabilisation of OMPs for analysis using ESI-IMS–MS.

## 2. Materials and methods

### 2.1. OMP expression and purification

OMPs were overexpressed in BL21 (DE3) *Escherichia coli* cells in 500 mL LB culture and the bacteria harvested by centrifugation. OmpT and PagP were labelled with a $His_6$ tag at the N- or C-terminus, respectively. tOmpA was expressed without an affinity tag. Cell pellets were resuspended in 50 mM Tris·HCl pH 8.0 containing 5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethanesulphonylfluoride (PMSF), 2 mM benzamidine and lysed by sonication. The lysate was pelleted by centrifugation (25,000 × *g*, 20 min, 4 °C), resuspended in 50 mM Tris·HCl pH 8.0 containing 5 mM EDTA, 2% (v/v) Triton X-100 (1 h to fully resuspend and solubilise residual membrane) and pelleted as above. Inclusion bodies were washed twice by resuspension in 50 mM Tris·HCl pH 8.0 and centrifugation as described above.

All OMPs were resuspended in 10 mM Tris·HCl pH 8.0 containing 250 mM NaCl, 6 M guanidine HCl (Gu·HCl). PagP and OmpT were initially purified by $Ni^{2+}$-NTA affinity chromatography. Solubilised PagP or OmpT was loaded onto a 5 mL HisTrap column (GE Healthcare, Little Chalfont, Bucks, UK). The column was washed with 10 mM Tris·HCl pH 8.0 containing 250 mM NaCl, 6 M Gu·HCl, 20 mM imidazole and PagP or OmpT was eluted with 10 mM Tris·HCl pH 8.0, containing 250 mM NaCl, 6 M Gu·HCl, 250 mM imidazole.