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Air–water interfacial behavior of amphiphilic peptide analogs of synthetic chloride ion transporters

Elizabeth K. Elliott [d], Keith J. Stine [a,b], George W. Gokel [a,b,c,*]

[a] *Department of Chemistry & Biochemistry, Center for Nanoscience, University of Missouri-St. Louis, St. Louis, MO 63121, United States*
[b] *Department of Biology, Center for Nanoscience, University of Missouri-St. Louis, St. Louis, MO 63121, United States*
[c] *Department of Molecular Biology & Pharmacology, Washington University, St. Louis, MO 63130, United States*
[d] *Department of Chemistry, Washington University, St. Louis, MO 63130, United States*

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Abstract

A family of heptapeptide-based chloride transporters (called synthetic anion transporters, SATs) were designed to insert into phospholipid membrane bilayers and form pores. Many of these compounds have proved to be chloride selective transporters. The transporters were designed to incorporate hydrophilic heptapeptides that could serves as headgroups and hydrocarbon tails that could serve as hydrophobic membrane anchors. Insertion of the SAT molecules into a bilayer requires approach to and insertion at the aqueous-membrane surface. The studies reported here were conducted to model and understand this process by studying SAT behavior at the air–water interface. A Langmuir trough was used to obtain surface pressure–area isotherm data. These data for amphiphilic SATs were augmented by Brewster angle microscopy (BAM), molecular modeling, and calculations of the hydrophobicity parameter log *P*. The analyses showed that the heptapeptide (hydrophilic) module of the SAT molecule rested on the water surface while the dialkyl (hydrophobic) tails oriented themselves in the air, perpendicular to the water surface. Brewster angle microscopy visually confirmed a high order of molecular organization. Results from these studies are consistent with the previously proposed mechanism of SAT membrane insertion and pore formation.

Keywords: Chloride transporter; Langmuir trough; Brewster angle microscopy; Molecular monolayer; Amphiphile

1. Introduction

Transport of cations and anions through cellular phospholipid bilayer membranes is critical to maintain the vitality of most organisms [1]. The proteins that transport Na^+, K^+, Ca^{2+}, and Cl^-, are complex and quite different from each other. During recent years, we [2,3] and others [4–7] have developed synthetic models for ion channels that accomplish many of the functions of their natural counterparts, although they are typically less selective and/or efficient. We termed the family of Na^+-selective transporters that we developed "hydraphiles" [8]. These are typically *tris*(crown) compounds designed to span a bilayer and conduct a cation through the bilayer by forming a uni-molecular pore [9]. Although the hydraphiles were designed and synthesized before the first cation channel structure [10] was known, the similarity in structural features is striking.

The remarkable complexity [11] of the ClC family of Cl^- transporting proteins [12,13] has presented an equally daunting challenge. Our approach [14,15] to this problem was to prepare an amphiphilic peptide of the general form $(C_{18}H_{37})_2N$-$COCH_2OCH_2CO$-$(Gly)_3$-Pro-$(Gly)_3$-OR^1 that emulated a phospholipid monomer and incorporated four structural modules. A dialkylamine residue, typically *bis*(octadecyl)amine, mimicked the twin fatty acid chains typically present in a phospholipid [16]. The lipid's glyceryl unit (mid-polar regime) was approximated first by diglycolic acid [17]. This diacid possesses three oxygens in locations similar to those of glycerol and is readily converted from its anhydride form to the dialkylamide monoacid $R_2NCOCH_2OCH_2COOH$ in a single step. The third module is the peptide [18,19]. Our first studies used $\sim(Gly)_3$-Pro-$(Gly)_3\sim$ because the ClC family proteins have a conserved G-X-X-P unit within their presumed ion conduction pathway [20]. Finally, the peptide's C-terminal carboxyl was protected

* Corresponding author at: Department of Chemistry & Biochemistry, University of Missouri, One University Boulevard, St. Louis, MO 63121, United States. Tel.: +1 314 516 5321; fax: +1 314 516 5342.
E-mail address: gokelg@umsl.edu (G.W. Gokel).

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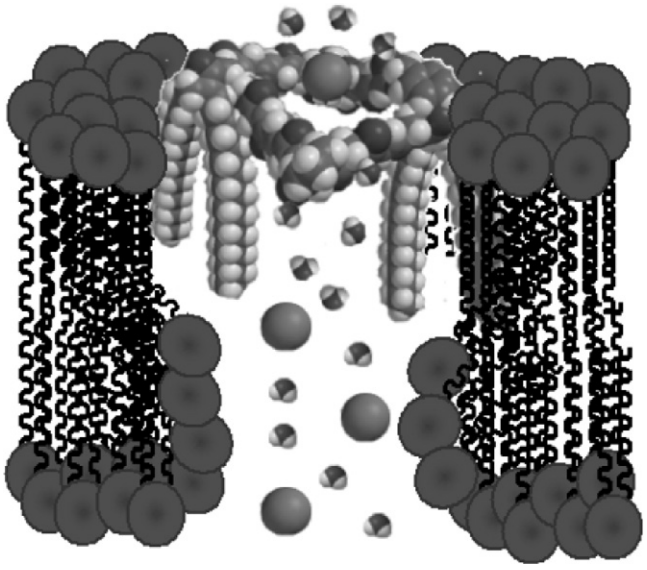

Fig. 1. Proposed mechanism by which two $(C_{18}H_{37})_2$N-$COCH_2OCH_2CO$-$(Gly)_3$-Pro-$(Gly)_3$-OCH_2Ph (SAT) molecules insert into the membrane bilayer to form a pore.

either as an ester or amide. The latter residue often served as an additional membrane anchor [21].

The first compound in this family, $(C_{18}H_{37})_2$N-$COCH_2OCH_2CO$-$(Gly)_3$-Pro-$(Gly)_3$-OCH_2Ph, mediated the release of Cl^- or carboxyfluorescein from phospholipid vesicles. Its proposed mechanism of membrane insertion is depicted in Fig. 1. Biophysical studies revealed that these synthetic anion transporters (SATs) were at least 10-fold selective for Cl^- over K^+ and that the pores they formed were typically dimers [22]. Despite extensive structural modifications in conjunction with transport studies, many questions remain. Two points are of critical interest to us. First, what part, module, or fragment of the SAT is hydrophilic and which portion(s) is hydrophobic? It seems obvious that when the dialkyl chains are octadecyl this module is hydrophobic. When the alkyl groups are hexyl and the C-terminal anchor is heptyl or decyl, the clarity fades. Second, when the SAT inserts into the bilayer, what portion, fragment, or module remains at or near the bilayer-aqueous interface? We therefore decided to assess the surface interactions of SATs and several close relatives by measurement of surface pressure–area isotherms in a Langmuir trough. This effort was augmented by observing the monolayers using a Brewster angle microscope (BAM).

A systematically varied family of compounds related to **1** was prepared, and their behavior at the air–water interface was examined. The hydrocarbon chains were held constant in compounds **1**–**5** to investigate the hydrophilicity of the heptapeptide pore-forming moiety. Surface pressure–area isotherms, BAM, molecular modeling, and the hydrophobicity parameter log *P* were utilized to investigate the surface behavior of synthetic anion transporters.

2. Results and discussion

2.1. Compounds studied

Five compounds were used in the present study. They are shown as **1**–**5** in Fig. 2. Compounds **1**–**5** possess twin octadecyl groups at the N-terminal end of the molecule. The peptide chain was shortened from ∼$(Gly)_3$-Pro-$(Gly)_3$∼ (**1**) to ∼$(Gly)_3$∼ in **2** and to a single glycine in **3**. Compounds **4** and **5** possess no amino acids and may be represented as $(C_{18}H_{37})_2$N-$COCH_2OCH_2COX$ in which X = OH (**4**) or OCH_2Ph (**5**).

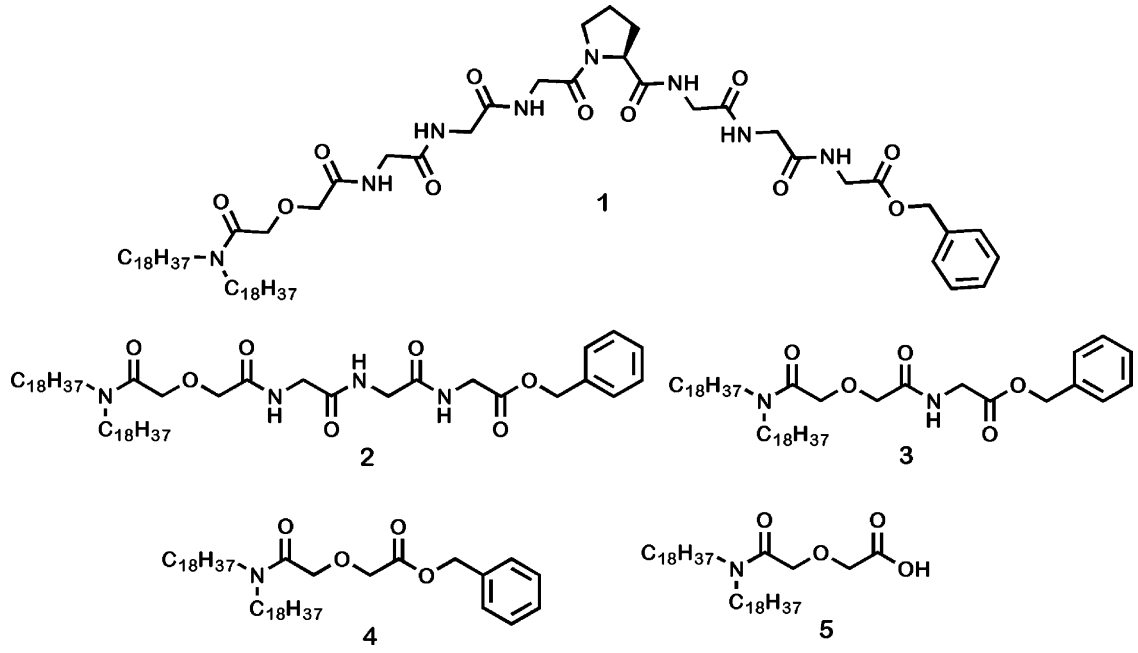

Fig. 2. Structures of Compounds 1–5.