Bioorganic & Medicinal Chemistry 23 (2015) 1413–1420

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Aminobenzoic acid incorporated octapeptides for cation transport

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ARTICLE INFO

Article history:
Received 4 December 2014
Revised 16 February 2015
Accepted 17 February 2015
Available online 24 February 2015

Keywords:
Peptides
Pores
Ion transporters
Carriers
Channel

ABSTRACT

Robust oligopeptides that mimic natural ion channels are attractive for use as molecular switches or model systems to study ion transport. Herein, we report octapeptides derived from aminobenzoic acid and L/D amino acids. Two of the alanine containing peptides were found to be most active and the peptide containing *p*-aminobenzoic acid was found to be most active (2.4 times its *m*-analog). Experimental studies indicate the peptides do not transport halides and transport alkali metal ions.

1. Introduction

Proteins that selectively transport ions across cell membranes are essential for controlling cell volume/pH and regulating cellular signal transduction.[1] Altered functioning of these proteins is known to be associated with diseases such as cancer, cystic fibrosis and cardiac arrhythmia.[1–3] The limitations associated with the stability and characterization of large proteins, inspires researchers to develop oligopeptides capable of ion-selective membrane transport.[4–6] Non-toxic low molecular weight peptidic ion transporters are potentially useful for pharmaceutical applications as well as attractive model systems for gaining mechanistic insights into membrane transport through larger proteins. A variety of the small oligopeptide ion transporters are derived from cyclic peptides comprising alternating D- and L-amino acids.[7,8] These peptides have been used as sensors[9,10] and antibacterial agents.[11,12] Cyclic peptides with β or γ amino acids have also been reported.[13–15]

Acyclic peptides containing –Gly_3-Pro-Gly_3– units which are readily accessible in comparison to cyclic peptides have been developed and reported to be anion-selective.[16–18] Acyclic pentadecapeptide Gramicidin A comprising D- and L-amino acids is known to specifically transport alkali metal cations via formation of a dimeric pore in the cell membrane.[19] The Gramicidin A channel does not allow transport of anions such as halides. The membrane affinity of Gramicidin A is attributed to the presence of the multiple tryptophan units in the peptide backbone. We have recently demonstrated that acyclic octapeptides synthesized from L/D alanine and *m*-aminobenzoic acid (ABA) transport ions across the lipid bilayer more efficiently than their cyclic analog.[20] Herein, we report the ion transport activity of octapeptides incorporated with aromatic aminobenzoic acid units (Fig. 1). Octapeptide **2** containing *p*-aminobenzoic acid and alanine is found to be most active. Vesicle-based assays indicate that the most active octapeptide **2** preferentially transports alkali metal ions and not halides.

2. Results and discussion

Octapeptides **1–5** contain two aminobenzoic acid units in a scaffold of alternating L- and D-amino acids. The octapeptides vary in the position of the aromatic units, the substitution pattern of the aromatic units or the nature of the L-amino acids in the scaffold. Octapeptide **1** was synthesized as reported earlier.[20] Peptide **2** was synthesized in solution as outlined in Scheme 1. Peptides **3–5** were also synthesized in solution, following a slightly modified synthetic route.[21]

To study ion transport, pH sensitive 8-hydroxypyrene-1,3,6-trisulfonic acid, trisodium salt (HPTS) dye was encapsulated in vesicles.[22,23] The vesicles were prepared in HEPES/NaCl buffer at pH 7.2 by multiple extrusions through a 0.1 μM polycarbonate membrane and external dye was removed by size exclusion chromatography.[24] At the beginning of the experiment, peptide dissolved in DMSO was added to the vesicles, following which NaOH (0.5 N) was added to introduce a pH gradient of 0.6 units (Fig. 2a). HPTS has different excitation maxima in its protonated (HPTSOH) and deprotonated state ($HPTSO^-$). To gauge ion

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http://dx.doi.org/10.1016/j.bmc.2015.02.031
0968-0896/

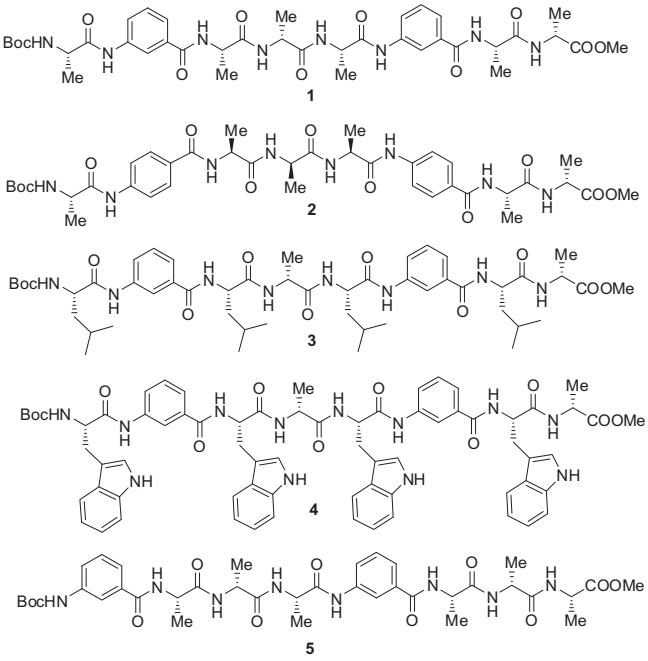
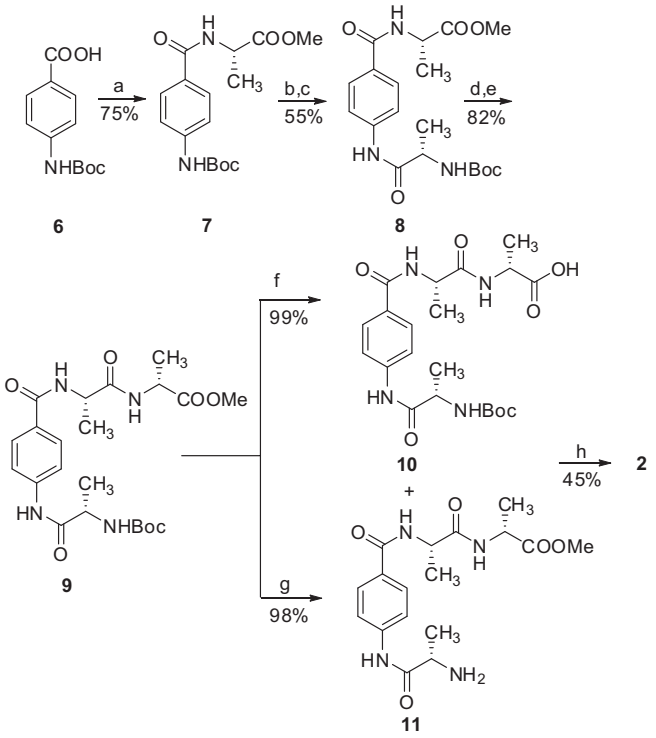


Figure 1. Octapeptides containing ABA units synthesized.



Scheme 1. Synthesis of peptide **2**. Reagents and conditions: (a) L-Ala-OMe, HBTU, HOBT, CH_2Cl_2, DIEA, 15 h, 0 °C–rt; (b) TFA, CH_2Cl_2, 2 h, 0 °C–rt; (c) Boc-L-Ala-OH, HBTU, HOBT, CH_2Cl_2, DIEA, 24 h, 0 °C–rt; (d) LiOH, H_2O, MeOH, 4 h, rt; (e) D-Ala-OMe, HBTU, HOBT, CH_2Cl_2, DIEA, 10 h, 0 °C–rt, (f) LiOH, H_2O, THF, 3 h, rt; (g) TFA, DCM, 0 °C–rt, 3 h. (h) HCTU, DIEA, THF, 8 h, 0 °C–rt.

transport, the emission of $HPTSO^-$ was monitored over the course of the experiment. Upon adding NaOH, a gradual increase in $HPTSO^-$ concentration was observed with peptides **1**–**5** (Fig. 2b). An increase in the concentration of $HPTSO^-$ indicates an increase in the internal pH of the vesicles upon addition of NaOH. Such an increase could be attributed to peptide mediated transport of Na^+ ions into the vesicles, which would lead to OH^- co-transport (symport) or exit of H^+ ions (antiport) from vesicles to maintain charge neutrality. Alternatively an increase in the internal pH could be due to peptide mediated OH^- transport into vesicles, which would lead to Na^+ symport or Cl^- antiport. The fluorescence intensity of $HPTSO^-$ in Figure 2b has been normalized with the final equilibrated intensity of the dye obtained upon lysing the vesicles using the non-ionic detergent Triton X. The HPTS assay indicated that all the peptides were transporting ions across the lipid bilayer. However, the rate of transport varied depending on the nature of the peptide.

The ion transport rates were quantified by fitting the curves to Eq. 1, where k, t, and I correspond to the rate constant, time and intensity of $HPTSO^-$, respectively.

$$I = Ae^{-kt} + B \tag{1}$$

Upon comparing the average k values over multiple experiments (Table 1), we determined that the sequence, nature of amino acids and aromatic substitution pattern of the peptides play a significant role in determining its activity.[25] Peptide **1** was found to be 1.5 times more active than peptide **5** which varies in the sequence of amino acids. Upon comparing peptides that vary only in the nature of the L-amino acids, it was observed that peptide **1** containing alanine was 1.2 and 1.5 times more active than its Leu and Trp analogs, respectively. Peptide **2** containing *p*-aminobenzoic acid was found to be the most active, its activity being 2.4 times more than its *m*-analog **1**.

A variety of natural and synthetic ion channels are known to possess Trp and Leu side chains to promote membrane insertion. It was surprising to observe that presence of these amino acids in our octapeptide scaffold lowered its activity (Table 1). Upon studying the effect of peptide on vesicle stability using dynamic light scattering (DLS), we observed an increase in vesicle size and dispersity after adding peptides **3**/**4** in contrast to the other peptides **1**, **2** and **5** (Figs. 3, S7 and S8).[26] The effect was more pronounced with peptide **4**, indicating that these peptides, might be affecting the structural integrity of vesicles and the larger aggregates might be due to vesicle fusion (Fig. 3b and c). Therefore, the availability of peptides for ion transport might be less leading to their lower activity. Such a disruption of vesicles was not observed for peptides **1**, **2** and **5** indicating that these peptides were not affecting the structural integrity of the vesicles and also not lysing the vesicles. Furthermore, the lipid solutions were found to turn turbid with peptides **3** and **4**, which was not the case with peptides **1**, **2** and **5**.

In order to gain insights into the mode of ion transport by the most active peptides **1** and **2**, the HPTS assay was carried out with different concentrations of these peptides (Fig. 4a and b). The observed k values for each concentration were determined by fitting the plot to Eq. 1. The k values were plotted against peptide concentration for these peptides (Fig. 4c and d). Eq. 2 illustrates the relationship between the observed k values and peptide concentration.[27] In this equation, n corresponds to the number of peptide units that interact with a single ion and K corresponds to the equilibrium for peptide aggregate–monomer dissociation. Since a background transport is observed with DMSO, the dependence of k with concentration is given by Eq. 3, where k_0 corresponds to rate constant with DMSO.[27]

$$k \propto K[\text{peptide}]^n \tag{2}$$

$$k = k_p K[\text{peptide}]^n + k_0 \tag{3}$$

The plots (Fig. 4c and d) were fitted using Eq. 3 and the n values were obtained. The n values were found to be close to 1 for peptides **1** and **2**.