Biochimica et Biophysica Acta 1848 (2015) 951–957

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Testing the limits of rational design by engineering pH sensitivity into membrane-active peptides

Gregory Wiedman [a], William C. Wimley [b], Kalina Hristova [a,*]

[a] Department of Materials Science and Engineering, Johns Hopkins University, Baltimore, MD 21218, USA
[b] Department of Biochemistry, Tulane University, New Orleans, LA 70112, USA

ARTICLE INFO

Article history:
Received 26 September 2014
Received in revised form 22 December 2014
Accepted 24 December 2014
Available online 5 January 2015

Keywords:
Peptides
Membrane
pH sensitivity

ABSTRACT

In this work, we sought to rationally design membrane-active peptides that are triggered by low pH to form macromolecular-sized pores in lipid bilayers. Such peptides could have broad utility in biotechnology and in nanomedicine as cancer therapeutics or drug delivery vehicles that promote release of macromolecules from endosomes. Our approach to rational design was to combine the properties of a pH-independent peptide, MelP5, which forms large pores allowing passage of macromolecules, with the properties of two pH-dependent membrane-active peptides, pHlip and GALA. We created two hybrid sequences, MelP5_Δ4 and MelP5_Δ6, by using the distribution of acidic residues on pHlip and GALA as a guide to insert acidic amino acids into the amphipathic helix of MelP5. We show that the new peptides bind to lipid bilayers and acquire secondary structure in a pH-dependent manner. The peptides also destabilize bilayers in a pH-dependent manner, such that lipid vesicles release the small molecules ANTS/DPX at low pH only. Thus, we were successful in designing pH-triggered pore-forming peptides. However, no macromolecular release was observed under any conditions. Therefore, we abolished the unique macromolecular poration properties of MelP5 by introducing pH sensitivity into its sequence. We conclude that the properties of pHlip, GALA, and MelP5 are additive, but only partially so. We propose that this lack of additivity is a limitation in the rational design of novel membrane-active peptides, and that high-throughput approaches to discovery will be critical for continued progress in the field.

1. Introduction

Many potential applications of membrane-active peptides have been discussed in the literature [1–8]. Yet their utility has been limited, partly because many such peptides come from natural sources and have not been engineered with particular applications in mind [9,10]. We recently embarked on a project to synthetically evolve a potent pore-forming peptide [11]. We reported that an analog of the bee venom lytic peptide melittin, MelP5, possesses the unique ability to assemble into macromolecule-sized, equilibrium pores under conditions where natural membrane-active sequences are inactive [12]. Unlike the parent peptide melittin, which forms small, transient pores under most conditions [1,13,14], MelP5 forms pores that persist for hours and allow the passage of macromolecules across bilayers, including dextrans (10 kDa) and chymotrypsin (24 kDa), even at very low peptide concentrations [11,12].

A question arises if MelP5 can be engineered such that its unique macromolecular poration activity can be controlled by pH. Of particular interest would be the design of a peptide that is not membrane active at pH 7 but interacts with membranes and forms macromolecule-sized equilibrium pores at low pH. A peptide with this property would be useful for multiple biomedical applications. For example, it would be useful in the generic delivery of macromolecules into cells via endocytosis where endosomal acidification would lead to release of endocytosed macromolecular cargo into the cytosol. pH-sensitive membrane-active peptides could also be targeted to tumor cells, where membrane permeabilization would be triggered by the decreased pH that occurs in the vicinity of solid tumors [15]. While a peptide that forms macromolecular pores with pH-sensitive activity currently does not exist, there are known membrane-active peptides with pH-sensitive membrane binding, insertion, and permeabilization that guided the work described here. One such peptide, called pHLIP, is a monomeric random coil at neutral pH but inserts into membranes as a monomeric, membrane-spanning α-helix upon acidification, with an apparent pK_a of ~6.0 [16,17]. pHLIP does not cause membrane permeabilization. A compositionally similar peptide, called GALA, is likewise a random coil at neutral pH but assembles into highly active, but small, membrane-spanning pores with an apparent pK_a of ~5.5 [18,19].

Each of these pH-sensitive, membrane-active peptides has acidic glutamate and aspartate residues interspersed into an otherwise hydrophobic sequence (Fig. 1). Each folds into an α-helix in membranes when partitioning and folding become favorable at low pH. Similarly, MelP5 folds into an amphipathic helix when bound to membranes, although

* Corresponding author.
E-mail addresses: wwimley@tulane.edu (W.C. Wimley), kh@jhu.edu (K. Hristova).

http://dx.doi.org/10.1016/j.bbamem.2014.12.023
0005-2736/

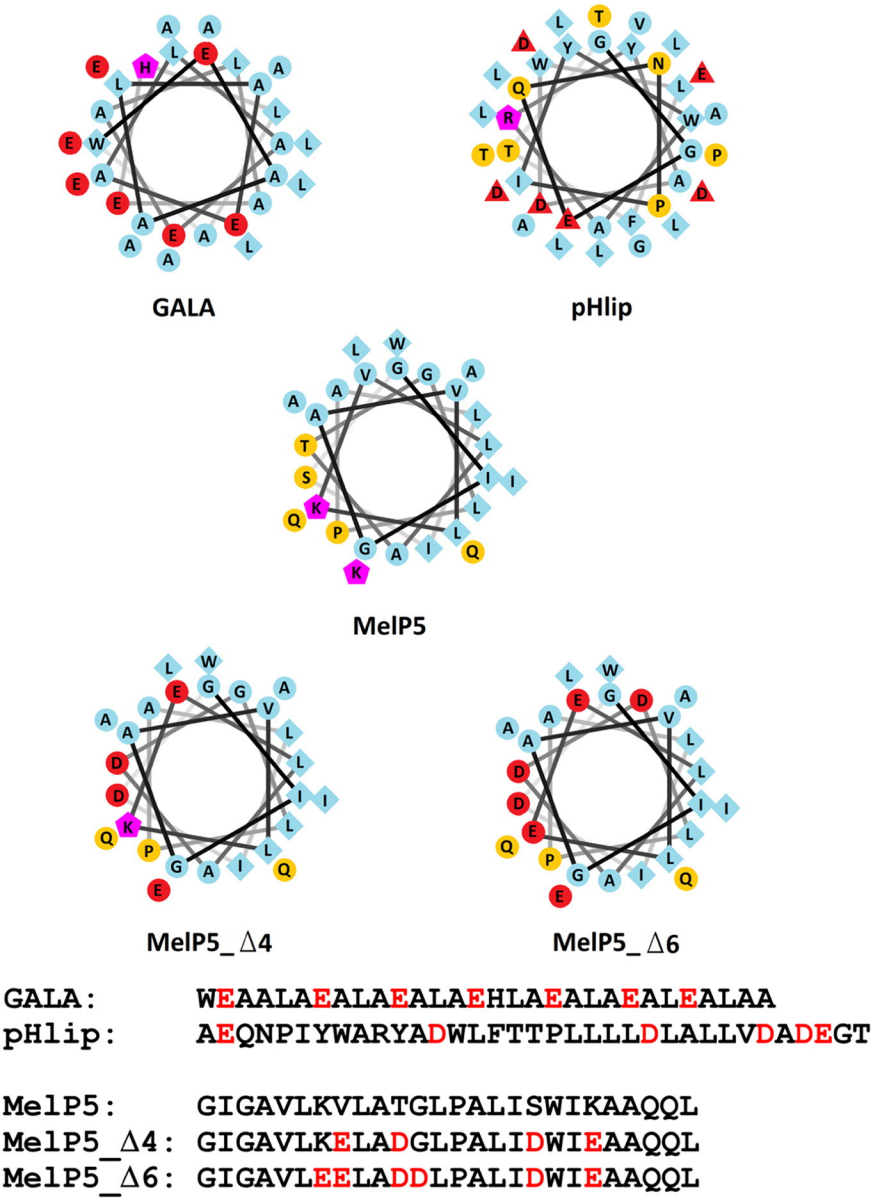

Fig. 1. Helical wheel projection diagrams of GALA, pHLIP, MelP5, MelP5_Δ4, and MelP5_Δ6. Residues colored blue are hydrophobic, yellow are hydrophilic, red are anionic at neutral pH, and purple are cationic at neutral and low pH. Below are the sequences of the five peptides. The red letters signify the acidic residues in GALA and pHlip, and the acidic residues inserted into the sequence of MelP5 to make MelP5_Δ4 (4 substitutions) and MelP5_Δ6 (6 substitutions).

MelP5 does so in a pH-independent manner. To approach our goal of designing pH-sensitive macromolecular pore-forming peptides, we sought to combine the macromolecular pore formation of MelP5 with the pH-sensitive membrane activity of pHLIP and GALA. Here we report on the rational design of two such peptides and on the investigation of their membrane activities over a pH range between 3 and 7.

2. Materials and Method

2.1. Reagents

1-Palmitoyl-2-oleoly-sn-3-glycero-phosphocholine (POPC) was purchased from Avanti Polar lipids as a lyophilized powder and dissolved in chloroform at 25 mg/ml for use. MelP5, MelP5_Δ4, and MelP5_Δ6 were synthesized by Biosynthesis, Inc. and were solubilized in methanol. Melittin was purchased as a lyophilized powder from Sigma-Aldrich and dissolved in methanol. Biotin-dextran-(10kD)-TAMRA and Avidin-Alexafluor488 were purchased from Invitrogen. All other reagents were purchased from Sigma-Aldrich.

2.2. Buffer Preparation

All buffers were made using millipore purified water. Sodium phosphate buffers were prepared at 10 mM with or without 100 mM potassium chloride at either pH 6 and pH 7. Sodium acetate buffers were prepared at 10 mM with or without 100 mM potassium chloride at pH 5, pH 4.5, pH 4, pH 3.5, and pH 3. Buffer for the ANTS/DPX experiment was made by adding 12.5 mM ANTS, 45 mM DPX, 5 mM HEPES, and 20 mM potassium chloride. Buffer for the FRET-leakage assay was made by adding 1 mg/ml of biotin-dextran (10kD)-TAMRA to either pH 7 sodium phosphate buffer or pH 4 sodium acetate buffer described above.