Bioorganic & Medicinal Chemistry 22 (2014) 3773–3780

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Interaction analysis of a ladder-shaped polycyclic ether and model transmembrane peptides in lipid bilayers by using Förster resonance energy transfer and polarized attenuated total reflection infrared spectroscopy

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ARTICLE INFO

Article history:
Received 25 February 2014
Revised 21 April 2014
Accepted 22 April 2014
Available online 9 May 2014

Keywords:
Polycyclic ether
Transmembrane peptide
Lipid bilayer
FRET
ATR-IR

ABSTRACT

Ladder-shaped polycyclic ethers (LSPs) are predicted to interact with membrane proteins; however, the underlying mechanism has not been satisfactorily elucidated. It has been hypothesized that LSPs possess non-specific affinity to α-helical segments of transmembrane proteins. To verify this hypothesis, we constructed a model LSP interaction system in a lipid bilayer. We prepared 5 types of α-helical peptides and reconstituted them in liposomes. The reconstitution and orientation of these peptides in the liposomes were examined using polarized attenuated total reflection infrared (ATR-IR) spectroscopy and gel filtration. The results revealed that 4 peptides were retained in liposomes, and 3 of them formed stable transmembrane structures. The interaction between the LSP and the peptides was investigated using Förster resonance energy transfer (FRET). In the lipid bilayer, the LSP strongly recognized the peptides that possessed aligned hydrogen donating groups with leucine caps. We propose that this leucine-capped 16-amino acid sequence is a potential LPS binding motif.

1. Introduction

Many secondary metabolites produced by marine organisms have unique molecular structures and possess remarkable bioactivities. Ladder-shaped polycyclic ethers (LSPs) among them, such as the brevetoxins[1,2] and ciguatoxins[3,4] produced by marine dinoflagellates, exhibit potent toxicity. These toxins are a known cause of fish or shellfish poisoning, and therefore, the molecular mechanism of toxicity onset needs to be clarified for further physiological studies and thus to prevent poisoning. A proposed target of these LSP toxins is channel proteins. Brevetoxin B, a red tide toxin, binds to voltage-sensitive sodium channel (VSSC),[5] preventing their inactivation. Similarly, ciguatoxin, the causative toxin in ciguatera fish poisoning, binds to VSSC and inhibits their inactivation. The dissociation constants (K_D) of these toxin to VSSC are in the nanomolar/sub-nanomolar range.[6,7] In addition to brevetoxins and ciguatoxins that bind to VSSCs, gambierol and its truncated analogues bind to voltage-gated potassium channels (VGPCs),[8–10] and maitotoxin causes calcium ion influx through an unidentified membrane-bound protein, but not on liposomes.[11] Interestingly, brevetoxins behave as maitotoxin antagonists,[12] and their binding to VSSC is inhibited by brevenal, a shorter LSP.[13] Recently, an artificial ladder-shaped heptacyclic polyether was found to inhibit maitotoxin-induced calcium ion influx.[14] These works suggest that the primary target molecules of LSPs are membrane proteins including ion channels. However, with the exception of yessotoxin (YTX),[15,16] their precise mode of interaction at the molecular level is yet to be satisfactorily elucidated. According to surface plasmon resonance (SPR) and saturation transfer difference NMR measurements, YTX interacts with a transmembrane domain of human glycophorin A (GpA).[17,18] Hydrogen bonds between α-protons of glycine residues, forming a glycine zipper,[19] and the vicinal ether

Abbreviations: ATR-IR, attenuated total reflection infrared; CD, circular dichroism; DMEQ-TAD, 4-[2-(3,4-dihydro-6,7-dimethoxy-4-methyl-3-oxo-2-quinoxalinyl)ethyl]-3H-1,2,4-triazole-3,5(4H)-dione; FRET, Förster resonance energy transfer; GpA, glycophorin A; LSP, ladder-shaped polycyclic ether; TFE, 2,2,2-trifluoroethanol; YTX, yessotoxin.

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http://dx.doi.org/10.1016/j.bmc.2014.04.044
0968-0896/

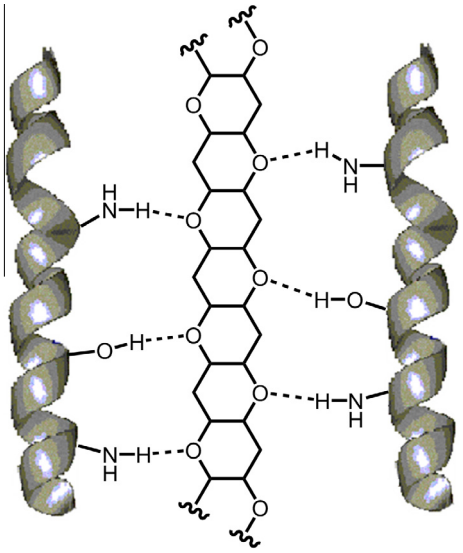

Figure 1. A hypothesized recognition model of a ladder-shaped polycyclic ether (LSP) and a membrane protein.

oxygen atoms of YTX are thought to be the key mediators of this interaction. On the basis that nearly identical distance between one pitch of an α-helix (5 Å) and the interval of the vicinal ether oxygen atoms at one side of LSP molecules (~5 Å), it is hypothesized that sequential hydrogen bonds exist between the vicinal ether oxygen atoms of LSPs and the protons of amino acid residues in the α-helical domains (Fig. 1).[20] These interaction analyses thus far described were performed in a solution phase with[21] or without[18,22] micelles. Therefore, investigation in a lipid bilayer system was desired to simulate real events in the biological systems. Here, we used YTX as a model LSP to construct an interaction model between YTX and the α-helical transmembrane peptides in liposomes to further elucidate the general recognition mechanism mediating LSP-membrane protein interactions. Although the rotational-echo double-resonance (REDOR) solid-phase NMR technique is one of the most powerful tools to observe intermolecular or distant intermolecular interaction in lipid bilayers,[23] stable isotope labelling of LSPs and peptides is laborious. For our analysis, we opted for FRET and ATR-IR techniques. The model interaction this study here reported establishes a method that can be used to elucidate the interaction mechanism of LSP and ion channel and other membrane proteins.

2. Results and discussion

2.1. Design and preparation of peptides

Initially, we predicted that hydrogen bonding and hydrophobicity are two of the most essential driving forces of the LSP-membrane protein interaction. On the basis of this speculation, we designed 5 α-helical peptides, as shown in Table 1. The leucine–lysine (peptide **1**) and leucine–serine (peptide **2**) repeat helical peptides are laterally amphiphilic, and their hydrophilic side chains with hydrogen donors (cyan in Table 1) aligned on one side of the helix, are predicted to interact with ether oxygen atoms of LSPs. Because the amino groups of the lysine repeats in peptide **1** make the α-helix too hydrophilic to be reconstituted in the lipid bilayer, peptide **1** was designed to have 2 helical segments. Similar to ion channels, this tandem helical structure was expected to segregate the hydrophilic side inside of the dimmer and thus to stabilize the α-helices in the lipid bilayer.[24] In contrast, peptide **2**, more hydrophobic and free of charged residues, was expected to be embedded in the lipid bilayer more comfortably. An additional 3 lysine residues were introduced at the both termini of the peptide in order hopefully to lock the helical segment between two sides of bilayer. Peptide **3** is a leucine–alanine repeat peptide, which has no hydrogen donors. This hydrophobic peptide is known to form a monomeric transmembrane structure.[25] As with peptide **2**, lysine and arginine residues were attached to both termini. The rest borrowed the sequence from a natural membrane proteins, namely the human GpA transmembrane domain (GpA-TM, peptide **4**), is known to interact with YTX in solution,[18] and as previously described, its recognition site is predicted to be the α-protons of the glycine residues within its sequence. Furthermore 3 glycine residues were replaced with serine in peptide **4**, with the expectation that it would have more efficient hydrogen bonding to ether oxygen atoms of YTX. The resultant peptide, peptide **5**, was named GpA-TM 3G/3S. GpA-TM 2G/2I (peptide **6**), which was subjected to SPR experiments in previous work,[18] was not used in this study. All peptides were prepared using solid-phase peptide synthesis (SPPS). In addition, native chemical ligation was used to connect the linker part at the middle of the sequence in the synthesis of peptide **1**.[26]

2.2. Reconstitution and secondary structural analysis

The secondary structures of these peptides were analysed using circular dichroism (CD) spectra attained in 2,2,2-trifluoroethanol (TFE) (Fig. S1). All spectra showed local minimums at 207 and 222 nm, indicating typical α-helical profiles. Therefore, the peptides all met the requirement at least for α-helix models. Next, we examined the reconstitution of the peptides in liposomes using egg-yolk phosphatidyl choline. The reconstitution method used depended on the behaviour of the peptide. A detergent removal method using dialysis[27] was suitable for the reconstitution of the hydrophobic peptides **3**, **4**, and **5**. We found that 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate (CHAPS) and *n*-octyl-β-D-glucopyranoside (OG) yielded the highest α-helix population in detergent screens for peptide **3**, and peptides **4** and **5**, respectively. In contrast, the thin-layer hydration method[28] was suitable for the reconstitution of the amphiphilic peptides **1** and **2**, because they were lost during a detergent removal step in the dialysis procedure.

Table 1
Amino acid sequences of α-helical model peptides

Peptide	N-CAP	1 5 10 15 20 25	C-CAP
1		KALKALAKLAKLWAKALAKLAKLAGGCGG	
		KALKALAKLAKLWAKALAKLAKLA	
2	CGGKKK	AALSALASLASLWASALASLASL	KKK
3	CKKK	AALAAALAAALWAALAAALAAA	RRR
4	CGEP	EITLIIFGVMAGVIGTILLISYGI	RRL
5	CGEP	EITLIIFSVMASVISTILLISYGI	RRL
6*	EP	EITLIIFIVMAIVIGTILLISYGI	RRL

*Compound **6** was not used in this study.