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Interaction of a peptide derived from C-terminus of human TRPA1 channel with model membranes mimicking the inner leaflet of the plasma membrane

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ABSTRACT

The transient receptor potential ankyrin 1 channel (TRPA1) belongs to the TRP cation channel superfamily that responds to a panoply of stimuli such as changes in temperature, calcium levels, reactive oxygen and nitrogen species and lipid mediators among others. The TRP superfamily has been implicated in diverse pathological states including neurodegenerative disorders, kidney diseases, inflammation, pain and cancer. The intracellular C-terminus is an important regulator of TRP channel activity. Studies with this and other TRP superfamily members have shown that the C-terminus association with lipid bilayer alters channel sensitivity and activation, especially interactions occurring through basic residues. Nevertheless, it is not yet clear how this process takes place and which regions in the C-terminus would be responsible for such membrane recognition. With that in mind, herein the first putative membrane interacting region of the C-terminus of human TRPA1, (corresponding to a 29 residue peptide, IAEVQKHASLKRIAMQVELHTSLEKKLPL) named H1 due to its potential helical character was chosen for studies of membrane interaction.
The affinity of H1 to lipid membranes, H1 structural changes occurring upon this interaction as well as effects of this interaction in lipid organization and integrity were investigated using a biophysical approach. Lipid models systems composed of zwitterionic and anionic lipids, namely those present in the lipid membrane inner leaflet, where H1 is prone to interact, where used. The study reveals a strong interaction and affinity of H1 as well as peptide structuration especially with membranes containing anionic lipids. Moreover, the interactions and peptide structure adoption are headgroup specific.

1. Introduction

The TRP superfamily comprises a large group of non-selective cation channels. They respond to diverse stimuli such as temperature, pressure, osmolarity, pH, and various chemical compounds including plant ingredients [1,2]. Moreover, TRP channel activity is modulated by calcium ions and lipid metabolites [3,4]. TRP ion channels are widely expressed in many different tissues and cell types, where they are implicated in diverse pathological states, including neurodegenerative disorders, kidney diseases, inflammation, pain and cancer [5,6]. TRP channels are organized into six families: classical (TRPC), vanilloid (TRPV), melastatin (TRPM), muclopins (TRPML), polycystin (TRPP), and ankyrin (TRPA). Similarly to voltage-gated potassium channels, TRP channels have four subunits, consisting of six transmembrane segments (S1–S6) each, a pore region loop between S5 and S6 segment, and long cytosolic N- and C-termini.

The transient receptor potential ankyrin 1 channel (TRPA1) is a nociceptor and polymodal ion channel activated by deep cooling (<17 °C), depolarizing voltages, calcium ions, environmental irritants (such as allyl isothiocyanate from wasabi), endogenous mediators involved in inflammation (including bradykinin), reactive oxygen and nitrogen species, and lipid metabolites (such as arachidonic acid) [7,8]. Furthermore, TRPA1 is regulated by phosphatidylinositol-4,5-

Abbreviations: ATR-FTIR, attenuated total reflection Fourier transform infrared; CD, circular dichroism; DMPA, dimyristoyl phosphatidic acid; DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidyglycerol; DMPS, dimyristoyl phosphatidylserine; DSC, differential scanning calorimetry; LUV, large unilamellar vesicle; MLV, multilamellar vesicle; NMR, nuclear magnetic resonance; P/L, peptide/lipid ratio; PI(4,5)P_2, L-α-phosphatidylinositol-4,5-bisphosphate; POPA, palmitoyl-oleoyl-phosphatidic acid; POPC, palmitoyl-oleoyl-phosphatidylcholine; POPE, palmitoyl-oleoyl-phosphatidylethanolamine; POPG, palmitoyl-oleoyl-phosphatidylglycerol; POPS, palmitoyl-oleoyl-phosphatidylserine; DPC, 1,2-di-0-dodecyl-*sn*-glycero-3-phosphocholine; SDS, sodium dodecyl sulfate; PWR, plasmon waveguide resonance; SUVs, small unilamellar vesicles

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bisphosphate (PIP_2), yet the PIP_2 binding sites have not been determined [9–11].

The specific binding of anionic lipids to certain protein domains is emerging as an important mechanism of channel regulation [12,13]. It is thought that distinct positively charged side chains from the protein interact with negatively charged lipid headgroups. Interactions between nontransmembrane protein domains and the lipid membrane were proposed to modulate ion channel activity [14–16]. The intracellular C-terminus is an important regulator of TRP channel activity [17–19]. Positively charged residues at the C-terminus of TRP channels have been shown to be putative PIP_2 interacting sites [20–22]. For the heat and capsaicin receptor TRPV1, it has been demonstrated that association of the C-terminus with the lipid bilayer alters channel sensitivity to thermal and chemical stimuli [23]. Besides phosphoinositides, several other negatively charged intracellular lipids, including phosphatidylglycerol, were reported to support TRPV1 activity [24].

Negatively charged molecules present in the inner leaflet of the cell membrane such as polyphosphates and phosphoinositides regulate TRPA1 from the cytoplasmic side, probably through interaction with positively charged domains in the C-terminus [25]. Electrophysiological studies show that basic amino acid residues in the C-terminus of the pain and wasabi receptor TRPA1 play a vital role in activation behavior and chemical sensitivity of the ion channel protein [25]. Therefore, it is likely that the basic residues in the C-terminus of TRPA1 may participate in channel–lipid interactions. Such polybasic clusters may be able to specifically recognize negatively charged phospholipids, and to associate with the membrane to control channel gating.

Following these thoughts, herein we propose to investigate the potential of the C-terminal region of the TRPA1 channel for membrane interaction. Since the C-terminal is extremely long (162 residues), we have made a search for potential membrane interacting sequences within this region. Taking into account that the interaction between the TRPA1 channel and the membrane are postulated to occur through positively charged amino acid residues, by the establishment of electrostatic interactions with anionic lipids, databases to predict antimicrobial peptide activity were used. Thus, we employed a web server-based method for predicting antibacterial peptides in a protein sequence to identify regions in the C-terminus of human TRPA1 with a high propensity for interaction with negatively charged phospholipids [26]. Indeed, antimicrobial peptides, despite their great diversity with respect to their amino acid sequence and secondary structure, they share as a common feature an affinity for negatively charged phospholipids [26]. An important property of antimicrobial peptides is their capability to distinguish bacterial from mammalian cells based on the differences in plasma membrane lipid composition [27]. In mammalian cells, the outer leaflet of the plasma membrane is composed of zwitterionic lipids, whereas substantial amounts of negatively charged phospholipids are present on the inner (cytosolic) leaflet of the plasma membrane [28,29]. In contrast, anionic lipids exposed on the outside of the bacterial membrane govern cell selectivity of antimicrobial peptides [30]. We have chosen the first membrane-interacting sequence in the C-terminus region of TRPA1 channel as potential site of interaction with the inner lipid leaflet of the membrane. This corresponds to a peptide of 29 amino acid residues (IAEVQKHASLKRIAMQVELHTSLEKKLPL) harboring 5 positively charged residues and 13 hydrophobic ones, named H1 due to its predicted helical propensity. To investigate the potential interaction of H1 with the cell membrane inner leaflet, we have employed lipid model systems containing anionic lipids (some of which present in the membrane inner leaflet like PIP_2, phosphatidylserine, phosphatidic acid). The interaction and structural changes induced by H1 in the lipid systems were monitored using several biophysical techniques. The possible functional role of Ca^{2+} in membrane association and structural characteristics of the peptide was studied. Results show that the peptide strongly binds to anionic lipids (dissociation constants in the nM range) present in the inner leaflet of eukaryotic plasma membranes. An enhanced affinity was observed in the case of PS. Calorimetry (differential scanning calorimetry) and plasmon waveguide resonance (PWR) reveal that peptide influence on lipid organization and packing is highly dependent on the lipid composition. Higher membrane affinity was observed in the presence of PS and PIP_2. As per the peptide structural changes upon lipid interaction, it was observed that H1 partially folds into β-sheet structure in the presence of anionic lipids and in a headgroup-specific manner. Therefore the first membrane-proximal cluster of positively charged residues in the cytoplasmic C-terminus of TRPA1 is implicated in protein–lipid interactions governed by electrostatic forces and lipid specificity akin to those found for other membrane-active peptides with cell membranes.

2. Materials and methods

2.1. Materials

All lipids were purchased from Avanti Polar Lipids (Alabaster, AL). Calcein was obtained from Sigma. The peptide (H1, IAEVQKHASLKRIAMQVELHTSLEKKLPL) was purchased from Selleckchem (Houston, TX) and used without further purification.

2.2. Preparation of MLVs and LUVs

Liposomes were prepared by dissolving the appropriate amounts of lipid into chloroform or a mixture of chloroform and methanol 2/1 (v/v), followed by solvent evaporation under nitrogen to deposit the lipid as a film on the wall of a test tube. Final traces of solvent were removed in a vacuum chamber attached to a liquid nitrogen trap during 3–4 h. Dried lipid films were hydrated with 10 mM Tris, 0.1 M NaCl, 2 mM EDTA, pH 7.6 (Tris buffer) (for DSC experiments) or with 10 mM phosphate buffer, pH 7.6 (for CD experiments) and thoroughly vortexed at a temperature superior to the phase transition temperature (Tm) of the lipid to obtain MLVs. To better mimic biological conditions, the peptide was added to the lipids after vesicle/liposome formation. To form LUVs, the MLV dispersion was subjected to five freeze/thawing cycles and passed 11 times through a mini-extruder equipped with two stacked 0.1 μm polycarbonate filters (Avanti, Alabaster, AL).

2.3. Calcein leakage

Calcein leakage assays are a quick and simple method to test lipid membrane integrity upon peptide action. While liposome encapsulated calcein is not fluorescent, perturbations in membrane integrity by peptides (e.g. pore formation) result in a fluorescence increase. Calcein-containing LUVs were prepared using the same protocol used to make regular LUVs, except for the hydration step of the lipid films performed in presence of 70 mM calcein. Free calcein was separated from calcein-containing LUVs using size exclusion column chromatography (Sephadex G-75) with Tris as elution buffer. The concentration of lipids was estimated using Rouser protocol [31]. For the assay, the lipid concentration was set at 1 μM and a P/L ratio of 1/50 and 1/25 used. All measurements were performed with a Perkin Elmer LS55 spectrometer (Buckinghamshire, UK). Data were collected every 1 s at room temperature using a $\lambda_{exc} = 485$ nm and $\lambda_{em} = 515$ nm with an emission and excitation slit of 2.5 nm in a cuvette of 2 mL. The fluorescence from calcein at 70 mM concentration was low due to self-quenching, but increased considerably upon dilution. The fluorescence intensity at equilibrium was measured after 2.5 h. At the end of the assay, complete leakage of LUVs was achieved by adding 100 μL of 10% Triton X-100 solution dissolving the lipid membrane without interfering with the fluorescence signal. The percentage of calcein release was calculated according to the following equation:

$$\%\text{Calcein leakage} = (F_t - F_o)/(F_f - F_o) * 100 \quad (1)$$