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Research paper

Phospholipid membrane-interaction of a peptide from S4 segment of KvAP K^+ channel and the influence of the positive charges and an identified heptad repeat in its interaction with a S3 peptide

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ABSTRACT

In order to examine the ability of S3 and S4 segments of a Kv channel to interact with each other, two wild type short peptides derived from the S3 and S4 segments of KvAP channel were synthesized. Additionally, to evaluate the role of positive charges and an identified heptad repeat in the S4 segment, two S4 mutants of the same size as the S4 peptide, one with substitution of two leucine residues in the heptad repeat sequence by two alanine residues and in the other two arginine residues replaced by two glutamines residues were synthesized. Our results show that only the wild type S4 peptide, but not its mutants, self-assembled and permeabilized negatively charged phospholipid vesicles. The S3 peptide showed lesser affinity toward the same kind of lipid vesicles and localized onto its surface. However, the S3 peptide interacted only with S4 wild type peptide, but not with S4 mutants, and altered its localization onto the phospholipid membrane with increased resistance against the proteolytic enzyme, proteinase-k, in the presence of the S4 peptide. The results demonstrate that the selected, synthetic S3 and S4 segments possess the required amino acid sequences to interact with each other and show that the positive charges and the identified heptad repeat in S4 contribute to its assembly and interaction with S3 segment.

1. Introduction

Voltage-gated channel proteins sense the change in transmembrane (TM) electric field and response with a conformational change that allows ions to diffuse across the pore-forming structure. Voltage-gated potassium (Kv) channels are tetrameric membrane proteins that contain six TM segments per α-subunit. The first four TM segments i.e. S1–S4 form the voltage-sensing domain, whereas the fifth and sixth TM segments (S5–S6) assemble to form the pore domain [1]. Voltage-sensing domain is the voltmeter that reads the change in membrane voltage and regulates the pore. The S4 TM segment is highly conserved in Kv channels and also exists in each of the four domains of sodium and calcium channels [1]. The S4 segment has positively charged arginine or lysine residues appearing at every 3rd or 4th position in the hydrophobic stretch [1,2]. Based on the mutational analysis of highly conserved positively charged residues, S4 segment was assigned as a potent voltage-sensing element [1,3–5]. However, the results indicate that the S4 segment alone is not responsible for the distinct voltage dependent properties exhibited by the different members of Kv family. Additionally, S3 and S2 segments containing highly conserved negatively charged residues that interact electrostatically with the positively charged residues of S4 account for stabilizing highly charged S4 segment in the lipid bilayer [6,7]. These electrostatic local interactions between membrane embedded segments are likely to contribute in proper assembly and function of the voltage sensor domain in Kv channels [8,9]. Literature suggests that mutations which incorporate the neutralization or reversal of the charged residues in S4 and S3 segments disrupt the proper folding and assembly of the whole channel protein leading either to altered channel gating or retention of the whole protein in the endoplasmic reticulum [1,10–14].

There have been extensive studies to understand the functional role of S4 and S3 segments and the involvement of their key amino acids toward the ion channel activity of KvAP, a voltage-gated potassium channel, found in prokaryotic archaebacteria *Aeropyrum*

Abbreviations: TM, transmembrane; Kv, voltage-gated potassium channel; PBS, phosphate buffer saline (pH 7.4); CD, circular dichroism; Fmoc, *N*-(9-fluorenyl) methoxycarbonyl; HPLC, high performance liquid chromatography; NBD, 7-nitrobenz-2-oxa-1,3-diazole; PC, phosphatidylcholine; PG, phosphatidylglycerol; Rho, tetramethylrhodamine; LUVs, large unilamellar vesicles; TFE, trifluoroethanol.

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pernix. However, very few studies have been undertaken to investigate how these segments of KvAP could contribute in the membrane-interaction of the whole channel protein [15–19]. Therefore, it is not yet known: i) whether these segments possess the required amino acid sequence to interact with phospholipid membrane; ii) whether the small synthetic peptides derived from these two segments have the ability to interact with each other; iii) how the positively charged residues of S4 influences the interaction of S3 and S4 segments and iv) apart from the positively charged arginine residues is there any other sequence element in the S4 segment that can influence the interaction of S3 and S4 segment. Toward this end, the present study focuses on the phospholipid membrane-interactions of the synthetic S4 and S3 segments derived from KvAP and their mutual interaction in the membrane environment. We also examined the effect of charge neutralizing mutations in S4 peptide on its interaction with the synthetic S3 segment. For this purpose, a 24 residue (AA 115–138) S4 peptide and a 20-residue (AA 92–111) S3 peptide from the S4 and S3 segments of KvAP were synthesized. In order to look into the effect of positive charge neutralization, two arginine residues, Arg126 and Arg133, were replaced by two glutamine residues. It has been reported that charge neutralization mutations at the equivalent positions in shaker potassium channel altered the channel activation and disrupted the assembly of the whole channel respectively [1,13,14]. Furthermore, we identified a small heptad repeat like motif in the S4 segment. The heptad repeat is located in the amino acid region 115–125 of KvAP with all the 'a' (1st) and 'd' (4th) positions occupied by leucine residues. There is appreciable sequence homology in the 'a' and 'd' positions of this structural element within the homologous K^+ channel proteins. At least two 'a' positions and two 'd' positions contain hydrophobic amino acids namely leucine, isoleucine, valine and phenylalanine. To assess the role of this heptad repeat, an analog was designed in which leucine residues from one 'a' and one 'd' position were substituted by two alanine residues. Although there was no mention of this heptad repeat sequence in the literature to our knowledge, these residues of the S4 segment seem to interact with the S3 segment in the X-ray structure of KvAP [20].

It was observed that though both S4 and S3 synthetic segments bound to phospholipid vesicles, only S4 segment could self-assemble and induced membrane leakage therein. We also observed that these two peptides derived from the S3 and S4 segments interacted with each other in the presence of phospholipid vesicles. Substitution of positive charges as well as the replacement of leucine residues at the 'a' and 'd' positions by alanine residues influenced the interaction of S4 and S3 peptides. Results have been discussed with respect to the requirement of amino acids in S4 and S3 segments for membrane-interaction and membrane-assembly individually and their mutual interaction in the presence of phospholipid membrane.

2. Results

2.1. Design and synthesis of peptides derived from S4 and S3 segments of voltage-gated KvAP channel

To investigate whether the small S4 and S3 segments of KvAP channel possess the required amino acid sequence to interact with each other two wild types peptides, one each from S4 and S3 segments belonging to the amino acid regions 115–138 and 92–111 respectively were synthesized and characterized. Further, to understand the role of the conserved positively charged arginine residues and the identified heptad repeat sequence in the assembly of S4 peptide and its interaction with the S3 peptide, two analogs of the S4 peptide were designed and synthesized.

The multiple sequence alignment shows that S4 wild type peptide contains five positively charged conserved arginine (R) residues (Fig. 1A) [20]. In one of the S4 analogs, two leucine residues at the 'a' and 'd' positions of a heptad repeat were replaced by two alanine residues (Mut-1-S4). Since alanine is a hydrophobic amino acid and possesses a comparable helix propensity to leucine, it was chosen for replacing the two leucine residues so that the amphipathic character and helix propensity of the wild type peptide is appreciably maintained in its analog. To investigate the importance of the conserved positively charged arginine residues in the assembly and functional property of the S4 segment and its interaction with S3 segment, two arginine residues (at positions 126 and 133) were substituted by two uncharged glutamine (Q) residues (Mut-2-S4). Glutamine was chosen since it is polar and closer to arginine in size and hydropathy index [21,22]. The lengths of the two analogs were kept the same as the wild type peptide. The designations and amino acid sequences of the wild type S4 peptide and its analogs are shown in Fig. 1B.

Helical wheel projection of the S4 wild peptide and a mutant peptide is shown in Fig. 1C which indicates that 'a' and 'd' positions of KvAP S4 heptad repeat possess leucine residues.

A 20-residue peptide located in the amino acid region, 92–111, of the S3 segment was selected to look into its membrane-interaction and interaction with the synthetic S4 wild type and mutant peptides. The sequence of S3 peptide is shown in Fig. 1B.

2.2. Peptides derived from both S4 and S3 segments bound to phospholipid vesicles

The ability of the S4 and S3 synthetic peptides to bind to the membrane was detected by studying the binding of their NBD-labeled versions to phospholipid vesicles. Negatively charged PC/PG lipid vesicles were employed as model membrane, considering the nature of electrical charge of the bacterial membrane. The dependence of NBD-fluorescence on the dielectric constant of the medium has been employed widely to study the membrane-interaction of proteins and peptides by attaching the probe onto these molecules [23–26]. In PBS, NBD-labeled S4, Mut-1-S4, Mut-

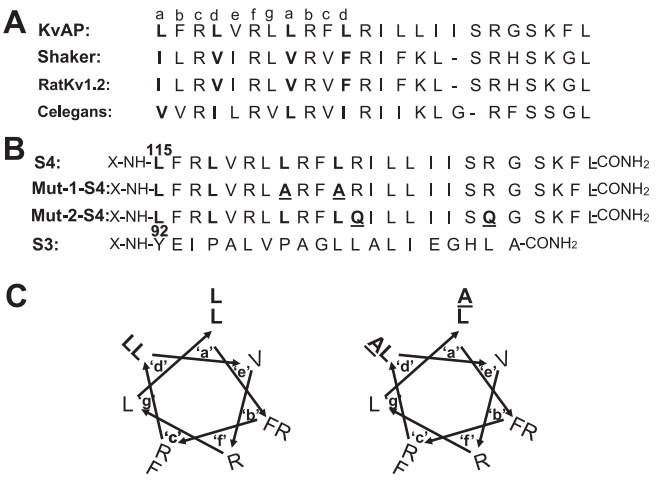

Fig. 1. *Panel A*, Multiple sequence alignment of the S4 segment of KvAP with other K^+ channels (AA sequence 115–138). KvAP, *Aeropyrum pernix* (GI:14601099); Shaker, *Drosophila melanogaster* (GI:24642914); RatKv1.2, *Rattus norvegicus* (GI:52000923); Celegans, *Caenorhabditis elegans* (GI:17559262). Amino acids at the 'a' and 'd' positions of the heptad are marked in bold letters. *Panel B*, Peptides designed from the S4 (AA sequence 115–138) and S3 (AA sequence 92–111) segment of KvAP potassium channel used in the study. Amino acids at 'a' and 'd' positions are marked as bold letters and mutated amino acid are marked as bold and underlined. (X = H, NBD and Rho). *Panel C*, Helical wheel projections of S4 and Mut-1-S4 (AA sequence 115–125).