Intrinsically disordered C-terminal segments of voltage-activated potassium channels: a possible fishing rod-like mechanism for channel binding to scaffold proteins

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1. INTRODUCTION

Voltage-activated potassium channels are allosteric pore-forming proteins that undergo conformational transitions between closed and open states—a process that underlies many fundamental biological processes (Bezanilla, 2000; Sigworth, 1994; Yellen, 1998). Kv channels are modular proteins composed of several domains including a ball-and-chain inactivation domain, a tetramerization (T1) domain, membrane-spanning voltage-sensor and pore domains and an intracellular C-terminal segment. Detailed structure–function studies of the N-terminal and membrane-embedded domains have clearly established their roles in different aspects of channel function (Yellen, 1998). Information on the C-terminal segments of Kv channels adjacent to the PDZ-binding motif is intrinsically disordered. Phylogenetic analysis of the Kv channel family reveals a cluster of channel sequences belonging to three out of the four main channel families, for which an association is demonstrated between the presence of the consensus terminal PDZ-binding motif and the intrinsically disordered nature of the immediately adjacent C-terminal segment. Our observations, combined with a structural analogy to the N-terminal intra-molecular ball-and-chain mechanism for Kv channel inactivation, suggest that the C-terminal disordered segments of these channel families encode an inter-molecular fishing rod-like mechanism for K+ channel binding to scaffold proteins.

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2. RESULTS AND DISCUSSION

2.1 The C-terminal tail segment of the Kv channel family is intrinsically disordered

The C-terminal segment of the prototypical Shaker Kv channel family exhibits sequence characteristics that are unusual for a folded protein domain. Inspection of the sequence reveals enrichment in hydrophilic amino acids, depletion of hydrophobic ones and repetitive strings of glutamines. These primary sequence characteristics suggest that the Shaker's C-terminal segment is unstructured and belongs to the recently defined class of intrinsically disordered protein sequences. As pointed out by Uversky et al. (2000), intrinsically disordered protein segments occupy a well-defined region in the mean net charge-mean hydrophobicity phase space diagram that is distinct from that of folded protein sequences. As can be seen in Figure 1A, the mean net charge of the channel with scaffold proteins like the PSD-95 protein, an interaction that underlies channel clustering (Kim et al., 1995; Tejedor et al., 1997; Tiffany et al., 2000; Zito et al., 1997).

Structural information on the C-terminal segments of the Kv channel family is also scarce. Apparently, only part of the C-terminal tail domain of the prototypical Shaker Kv channel presents compact density in cryo-electron microscopy analysis (Sokolova et al., 2003). In addition, the C-terminal domain of the Kv 1.2 channel, a Shaker-homologue, was not observed in X-ray crystallography analysis (Long et al., 2005). These observations reflect the flexible nature of these tail domains and further raise the possibility that the C-terminal domains of the Kv channel family belong to the recently defined intrinsically disordered protein class (Dunker et al., 2001; Fink, 2005; Romero et al., 2001; Tompa, 2002; Uversky, 2002; Wright and Dyson, 1999). Protein segments that belong to this group exhibit unusual sequence characteristics that oppose folding and are, therefore, unstructured or intrinsically disordered under native physiological conditions.

In this study we set out to examine whether the C-terminal segments of the Kv channel family immediately adjacent to the terminal PDZ-binding motif indeed exhibit the sequence characteristics of intrinsically disordered protein segments. We show this to be the case and further suggest, based on phylogenetic inference analysis, a mechanistic implication for this observation.
and mean hydrophobicity values calculated for the entire intracellular C-terminal sequence of the Shaker channel (residues 493 till end), do indeed lie within the intrinsically disordered protein space (black circle). Since, however, the segment in question may include both ordered and disordered stretches, an averaged assessment of these parameters for the whole C-terminal segment could present a biased view. Accordingly, we used the FoldIndex server (Prilusky et al., 2005) to predict the borders of those intrinsically disordered regions within the C-terminal Kv channel segment. This server is directly based on the mean net charge-hydrophobicity boundary condition of Uversky et al. (2000) and uses a sliding window approach for predicting intrinsically disordered segments and their boundaries. The use of this server allowed us to determine the presence or absence of intrinsically disordered segments immediately adjacent to the terminal PDZ-binding motif of the Kv channel. Prediction results for the entire sequence of the Shaker channel are shown in Figure 1B. Two major intrinsically disordered protein segments are clearly predicted at the N- and C-termini of the channel (grey peaks). Interestingly, as first noted by Dunker et al. (2001), the predicted N-terminal intrinsically disordered segment corresponds to the inactivation ball-and-chain domain (Hoshi et al., 1990). Immediately past the end of the pore domain, the sequence is predicted to be intrinsically disordered. This observation regarding the C-terminal structural state of the Shaker channel, although obvious upon inspection of the C-terminal channel sequence, is stated here with support from rigorous bioinformatics analysis. The lack of structure at the corresponding region of the Kv 1.2 channel as revealed by X-ray crystallography analysis (Long et al., 2005) lends substantial credence to this conclusion.

Is our observation regarding the unfolded nature of the Shaker C-terminal segment adjacent to the terminal PDZ-binding motif general, in the context of the entire Kv channel family? To answer this question, we constructed a dataset of 70 Kv channel sequences and examined the unfoldability profiles of the C-terminal tails. We found that 80% of all Kv channels contain intrinsically disordered C-terminal segments immediately adjacent to the terminal PDZ-binding motif (when present) (Table 1S in Supplementary Material). This is in striking contrast to the proportions of intrinsically disordered protein segments in eukaryotes (35%) (Fink, 2005). The mean net charge and mean hydrophobicity values for each of the terminal segments of the Kv channel sequences, identified using the FoldIndex server, were calculated and plotted on Uversky’s phase plane (Uversky et al., 2000) (Fig. 2). As can be seen, most of the terminal segments of the Kv channel tails adjacent to the PDZ-binding motif do indeed lie within the intrinsically disordered protein space. Such channel sequences were assigned a character state U (‘unfolded’) to indicate the structural state of their most terminal tail segment. The high frequency of intrinsic disordered predicted at the tail domain of the Kv dataset should be considered when attempting crystallization of the C-terminal segments. A total of 16 Kv channel sequences contained at their C-terminal tip stretches of amino acids predicted to be ordered. These terminal channel segments lie within the folded region of the Uversky phase space diagram and probably reflect low complexity regions with residual structure (see Methods in Supplementary Material). These segments were assigned the character state F (‘folded’).
indicate the transition range boundaries for which prediction accuracy is calculated and plotted on the Uversky phase space diagram. The dashed lines 70 Kv channel C-terminal segments detected using the FoldIndex server were intrinsically disordered. Mean net charge and mean hydrophobicity values of U for sequences involved in Kv channel. Arrows indicate the mean net charge and hydrophobicity values (Oldfield et al., 2005). The grey data point indicates the values for the Shaker Kv channel. Arrows indicate the mean net charge and hydrophobicity values for sequences involved in U to F evolutionary transitions (see Section 2.2).

2.2 The unfolded nature of Kv tail segments is associated with the presence of a PDZ-binding motif

We next considered the possibility that the property of unfoldability of the C-terminal segment of the Kv channel family is associated with the presence of a PDZ-binding motif at its end sequence. We examined the Kv sequences for the presence (+) or absence (−) of the consensus PDZ-binding motif (See Methods in Supplementary Material) and conducted a phylogenetic analysis of the entire dataset (Fig. 3). For each of the sequences at the leaves of the phylogenetic tree we marked the character state of the C-terminal tail segment adjacent to the PDZ-binding motif: U+ and F− character states that imply an association between the two tail domain properties in red; U− and F+ character states that imply no association in blue. The phylogenetic analysis reveals three major branches, A, B and C, evolving from a common ancestor (red circle at the center of the phylogenetic tree). As evident upon inspection of the phylogeny, out of the three dominant tree branches, only branch A, comprising sequences belonging to the major Kv 1, 3 and 4 families, exhibits a high proportion (60%) of sequences which are predicted to be intrinsically disordered at their C-terminal tails and to contain the PDZ-binding motif (U+ tail domain character state). The two other branches, one comprised of sequences belonging to the other major Kv 2 family along with its silent Kv 5, 6, 8 and 9 subunits (branch B), and the other comprised primarily of the KvLQT (Kv 7) subfamily members (branch C), exhibit only a few U+ sequences (20 and 11%, respectively). The observed differences in character-state frequencies between cluster A and either clusters B or C are statistically significant (Table 2S in Supplementary Material). The observations above suggest that for branch A sequences belonging to Kv families 1, 3 and 4, an apparent association exists between the presence of a PDZ-binding motif at the end sequence and the property of unfoldability of the C-terminal segment adjacent to this motif.

We were concerned, however, that this apparent association may be a result of dependent inheritance by descent from one or a few common ancestors. Imagine for example that the character state of the ancestral (now extinct) sequence from which all sequences evolved is U+. Given this notion, it is no wonder that the majority of evolutionary-related sequences in branch A exhibit U+ tail domain outcomes. To address this issue of sequence dependence we followed Ridley’s method (Ridley, 1983) and reconstructed the character states of the C-terminal tails of the putative, now extinct, ancestors (internal nodes in the phylogenetic tree). This procedure serves two important purposes: first, it would allow us to compute the character state of the ancestral central (red) node of the phylogeny. Second and most important, as pointed out by Ridley (1983), since the evolutionary change along one individual branch is independent of the change in other branches (to a first approximation), one way to test whether the two tail domain characters have evolved in a correlated fashion is simply to correlate the changes in the two tail domain character states throughout the phylogeny. Following character state reconstruction we can, therefore, count the number of independent evolutionary transitions along the phylogenetic branches leading to any of the four possible U+, U−, F+ and F− tail domain outcomes.

Phylogenetic reconstruction was performed using the maximum parsimony method (see Methods in Supplementary Material). The results of the reconstruction analysis are informative. First, the analysis reveals nine independent transitions in branch A that were unequivocally detected. Eight out of the nine independent transitions are to the U+ and F− character states that reflect association between the two tail-domain properties and one to the F+ character state (see Text 1S in Supplementary Material for further discussion on character state transitions). A two-sided Fischer’s exact test applied to the 2 × 2 contingency table of all transition counts revealed significant association between the two characters (p = 0.047). To evaluate the reliability of the p-value obtained, we applied power analysis. Such analysis indicated that given a sample size of nine (the number of independent evolutionary transitions detected within cluster A), a significance level of 0.05 and medium effect size, the probability of finding significant association is only 0.14. This outcome strengthens our argument of association, as even though the theoretical odds are against association, we nonetheless detect a significant association between the two characters. Second, the computed character state of the original node from which branches A, B and C evolved is U−. This striking outcome further suggests that the high prevalence of the U+ character state for branch A sequences is not a mere reflection of a common ancestor but reflects co-evolution within this branch.

2.3 Intrinsically disordered tail segments of Kv channels suggest a fishing rod-like mechanism for channel clustering

Co-variation of the two tail-domain properties during evolution might reflect functional association. We argue that, in addition to the presence of the PDZ-binding motif at the end of the Kv channel sequence, the intrinsically disordered C-terminal segment immediately preceding it is another important determinant in mediating the interaction of the channel with the PSD-95 scaffold protein. A hint
as to a possible mechanism for channel-scaffold protein interaction, mediated by the channel’s C terminus, is given by the interesting observation that the N-terminal inactivation domain is also predicted to be ‘unfolded’ or ‘folded’ in the FoldIndex analysis, respectively. The ‘+’ and ‘−’ signs refer to C-terminal sequences that contain or lack the consensus PDZ-binding motif, respectively (see Methods in Supplementary Material). Sequences with C-terminal tail domain outcomes of either U+ or F− (that imply an association between the two tail domain properties) are indicated in red whereas those with the U− and F+ tail domain outcomes that imply no association appear in blue. The numbers adjacent to the nodes marked with black circles indicate the different Kv channel family branches (Kv1–Kv9). The four major Kv channel families are indicated by red numbers.

as to a possible mechanism for channel-scaffold protein interaction, mediated by the channel’s C terminus, is given by the interesting observation that the N-terminal inactivation domain is also predicted to be intrinsically disordered (Fig. 1B). Detailed functional and structural analyses revealed an intra-molecular ball-and-chain mechanism for channel inactivation (Fig. 4A) (Hoshi et al., 1990). According to this mechanism, an N-terminal inactivation ball, tethered to a long extended flexible chain, reaches the open intracellular activation gate and binds in the hydrophobic binding pocket of the pore’s central cavity, thus clogging potassium currents through the channel (Zhou et al., 2001). From a structural perspective one may envisage that intrinsically disordered C-terminal tail segments of certain Kv channels encode an inter-molecular ball-and-chain mechanism for channel binding to scaffold proteins (Fig. 4B). The membrane-embedded Kv channel is anchored at unique membrane sites by binding to a scaffold protein using a fishing rod-like sequence that contains an extended string (an intrinsically disordered chain) and a hook (the PDZ-binding motif or ball) at its tip. From a functional perspective, the two N- and C-terminal intrinsically disordered segments of the Shaker Kv channel exemplify two distinct roles described for such segments: whereas the N-terminal intrinsically disordered inactivation segment serves as an entropic clock that modulates the kinetics of inactivation (Dunker et al., 2001), disorder at the C terminus provides the
characteristics (Dunker et al., 2000) will add novel dimensions to understanding complex processes such as synapse assembly, maintenance and function.

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REFERENCES

3 CONCLUDING REMARKS
In a recent article, Neduva et al. (2005) have indicated that short conserved sequence motifs tend to occur in low complexity unstructured regions. In accordance with this finding we here provide preliminary evidence for functional association between intrinsic disorder in Kv channels C-terminal segments and the presence of the PDZ protein–protein interaction motif. Therefore, no less important for binding to scaffold proteins than the PDZ-binding motif is the disordered chain to which it is attached. The observation that the C-termini of most channel sequences belonging to the fourth major Kv 2 family (and for the subfamilies 5–9) do not contain a canonical PDZ-binding motif, yet are predicted to be intrinsically disordered, is interesting. It is possible that for these Kv families tail domain unfoldability is associated with a different activity or that channel–scaffold protein interaction is mediated by another, yet to be determined mechanism. No matter what their role(s) might be, describing intrinsically disordered domains, with their unique characteristics (Dunker et al., 2001) will add novel dimensions to orientational freedom for searching and successfully connecting to the scaffold protein partner. This later mechanism has been described in the literature as ‘fly casting’ (Shoemaker et al., 2000) and ‘protein fishing’ (Evans and Owen, 2002) for proteins involved in DNA recognition and endocytosis, respectively.

Fig. 4. Inter-molecular fishing rod-like mechanism for Kv channel binding to scaffold proteins. (A) Schematic model for the ball-and-chain mechanism for K+ channel inactivation. An open potassium channel pore inactivates upon binding of a chain-tethered N-terminal peptide ball to its receptor site at the pore’s inner cavity. The membrane-embedded part corresponding to the channel’s voltage-sensor and pore domains while the rectangular shape is the T1 domain. (B) Schematic model for a ‘protein fishing’ mechanism for K+ channel binding to scaffold protein. A voltage-gated K+ channel interacts with a scaffold protein, such as the PSD-95 protein, for example, upon binding of the C-terminal peptide hook (the PDZ-binding motif) tethered to an extended chain to the PDZ domain(s) of PSD-95. The moon-like, box and rectangular shapes represent the PDZ, SH3 and guanylate kinase domains of the PSD-95 protein, respectively.