Tryptophan Scanning Mutagenesis of the First Transmembrane Domain of the Innexin Shaking-B(Lethal)

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ABSTRACT The channel proteins of gap junctions are encoded by two distinct gene families, connexins, which are exclusive to chordates, and innexins/pannexins, which are found throughout the animal kingdom. Although the relationship between the primary structure and function of the vertebrate connexins has been relatively well studied, there are, to our knowledge, no structure-function analyses of invertebrate innexins. In the first such study, we have used tryptophan scanning to probe the first transmembrane domain (M1) of the Drosophila innexin Shaking-B(Lethal), which is a component of rectifying electrical synapses in the Giant Fiber escape neural circuit. Tryptophan was substituted sequentially for 16 amino acids within M1 of Shaking-B(Lethal). Tryptophan insertion at every fourth residue (H27, T31, L35, and S39) disrupted gap junction function. The distribution of these sites is consistent with helical secondary structure and identifies the face of M1 involved in helix-helix interactions. Tryptophan substitution at several sites in M1 altered channel properties in a variety of ways. Changes in sensitivity to transjunctional voltage (Vj) were common and one mutation (S39W) induced sensitivity to transmembrane voltage (Vm). In addition, several mutations induced hemichannel activity. These changes are similar to those observed after substitutions within the transmembrane domains of connexins.

INTRODUCTION

The innexin family of proteins constitutes gap junctions in arthropods and other prechordate animals (1). Chordate gap junctions are composed primarily of proteins of the connexin family; a small number of distantly related innexins, referred to as pannexins, are present in chordates but appear to function predominantly as single-cell, rather than intercellular channels (2,3). Although very little is known about the structure of innexin-based junctions, connexin-based junctions are well characterized (4). Each channel is composed of two hemichannels also known as connexons. A connexon is formed when six connexin proteins oligomerize forming a central pore. Connexins have four transmembrane domains (M1–M4), cytoplasmic amino-termini and carboxyl termini (NT and CT) and two extracellular loops (E1 and E2) (5,6). Three-dimensional structures of connexin-based gap junction channels confirm the dodecameric nature of the connexin protein complex and a α-helical secondary structure for the membrane-spanning domains (Cx43 (7); Cx26 (8); Cx26 (9)).

Arthropod gap junctions appear similar to connexin-based junctions in electron micrographs (3) with a slightly wider gap observed in invertebrates (10–12). In both cases, intercellular channels form in dense plaques at sites where adjacent cell membranes are held within a few nanometers of one another. Innexins are predicted to have the same membrane topology as connexins including four membrane-spanning domains and two extracellular loops. The extracellular loops are longer in innexins than connexins, with two, rather than three, conserved cysteines per loop (1,13). In oocyte expression studies, innexin-based gap junctions form heterotypically as well as homotypically, display a range of sensitivities to voltage, and are gated by protons (14,15), all properties shared by their connexin-based counterparts.

The shaking-B gene of Drosophila was the first innexin shown to encode functional gap junction channels (16) and is one of the best characterized members of this family. The gene encodes three proteins, ShakB(N), ShakB(N+16), and ShakB(L) (13), and is required in the GFS. The fly GFS (17), like that in other arthropods such as crayfish (18,19), is a neural circuit responsible for a stereotypical escape response. Classical studies in the crayfish showed that fast transmission is achieved by rectifying electrical synapses between the lateral giant interneurons and giant motorneurons (18). More recent studies in Drosophila have established that GFS synapses are assembled from shakB gene products (20). In particular, ShakB(N+16) is required presynaptically in the Giant Fiber interneurons and ShakB(L) is expressed in the postsynaptic tergotrochanteral muscle motorneurons. These proteins assemble rectifying heterotypic gap junctions suggesting that such junctions are the molecular basis of rectification at arthropod giant synapses (14).

We selected ShakB(L), which reliably forms both homotypic and heterotypic junctions in heterologous systems (14,16), for initial structure-function analysis of innexins. The first transmembrane domain provides an interesting

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Abbreviations used: GFS, giant fiber system; Gj, junctional conductance; Ij, transjunctional current; M1, first transmembrane domain; ShakB(L), Shaking-B(Lethal); ShakB(N), Shaking-B(Neural); ShakB(N+16), Shaking-B(Neural+16); Vj, transjunctional voltage; Vm, transmembrane voltage; WT, wild-type.

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target based on studies of proteins that are related in sequence (pannexins) or function (connexins). M1 contributes to the pore of pannexin- and connexin-based channels (9,21), although in both cases the pore is complex and composed of multiple domains. In connexins, the pore lining is formed in part by the amino terminus that folds into the cytoplasmic mouth of the pore, interacting with the cytoplasmic end of M1. Additional contributions to the pore occur at the cytoplasmic end, where the second transmembrane helix extends beyond the bilayer, and in the extracellular region, where the first extracellular loop lines the pore (9). The conduction pathway of pannexin channels includes residues at the extracellular end of M1 and residues in the carboxyl terminus. In a study involving accessibility of substituted cysteines in Panx1, adjacent sites in M1 (e.g., residues 58C–62C) and in the carboxyl terminus (e.g., residues 413C–426C) were reactive. These patterns are inconsistent with helical secondary structure and suggest that pannexin-based channels have structural features that are distinctly different than their connexin-based counterparts (21). The complexity of channels formed by connexins and pannexins highlights the importance of confirming the helicity of M1 and identifying the extent of its interactions with other domains in structure-function analyses.

In many membrane proteins, helical secondary structure of transmembrane domains is reflected in a pattern of residues involved in interhelical interactions. A diverse set of methods reveals critical amino acids at approximately four-residue intervals in transmembrane helices. For instance, in one of the first studies specifically aimed at identifying interactions between transmembrane segments, dimerization of glycoporphin A was disrupted by mutations induced at 3.9-residue intervals along the helix (22). In other proteins, sequence-specific interactions have been identified including a GxxxG or GxxxxxxG motif that places glycine at positions where helices interact (23), and a serine zipper motif that places serines at positions 7, 14, and 21 of one helix and 1, 8, and 15 of another (24). More recently, a consensus motif for interhelical associations in integrins identified a set of large and small interacting side chains located at four-residue intervals on associated helices (25).

Several mutagenic approaches have proved successful in studies of transmembrane domain interaction including alanine scanning (22), pairwise substitution (26), alanine insertion (27), and tryptophan scanning (28,29). When combined with functional analysis at the biochemical or biophysical level these provide information about the nature and importance of helix-helix interactions. Tryptophan scanning has become the most broadly applied mutagenic approach for analysis of interhelical interactions in membrane proteins with transport function. The approach was first applied to the MotB protein from Escherichia coli (30) and has since been applied to a number of membrane proteins including the acetylcholine receptor channel (29,31), potassium channels (32–34), GABA receptor channels (35), copper transporters (28), and hyperpolarization activated cyclic-nucleotide gated channels (36). In some of these studies tryptophan scanning has been specifically applied to provide insight into the secondary structure of transmembrane domains (33,37), engineer mutant membrane proteins with specific properties (38), and to compare the structure of similar transporters (28).

Tryptophan scanning was selected for this structure-function analysis of ShakB(L) to establish the secondary structure of M1 and to examine the extent of interactions between M1 and other domains. Tryptophan substitution at many sites altered channel properties, and we characterized several of the most interesting mutants.

**MATERIALS AND METHODS**

**Preparation of tryptophan mutants and cRNA**

*Drosophila shaking-B(letal)* was cloned into pSPICL2. Tryptophan mutants were created using the QuikChange or QuikChange Lightning mutagenesis kits (Agilent Technologies-Stratagene, Santa Clara, CA). Primers were designed using the QuikChange Primer Design Program (Agilent Technologies - Stratagene) and custom synthesized by Integrated DNA Technologies (Coralville, IA) in 25 mmole quantities with standard desalting. Mutations were confirmed by sequencing through the coding region (Roswell Park Cancer Institute DNA Sequencing Facility, Buffalo, NY). DNAs were linearized with Xhol and RNA prepared using a standard mMessage mMachine RNA kit (Applied Biosystems/Ambion, Austin, TX). RNA was purified with lithium chloride and quantified using gel electrophoresis and ethidium bromide staining through comparison to an RNA 250 control (Applied Biosystems/Ambion).

**Expression and recording from oocytes**

The technique of recording intercellular currents from paired *Xenopus* oocytes was carried out as described by Skerrett et al. (39). Oocytes were removed from ovulating *Xenopus laevis* females and the follicular layer partially removed with collagenase (Type 1A, Sigma-Aldrich, St. Louis, MO). The remaining follicular layer was removed using fine forceps before oocytes were preinjected with 0.5 ng of morpholino antisense oligonucleotide directed against *Xenopus* Cx38 (Gene Tools, Philomath, OR). Approximately 24 h after preinjection, oocytes were injected with 5 ng of cRNA. Injected oocytes were incubated at 18°C for 12–24 h and then stripped of their vitelline membranes and paired overnight in agar wells. Oocytes were cleaned and digested in Oocyte Ringers 2 (82.5 mM NaCl, 2 mM KCl, 1 mM MgCl2, 5 mM HEPES, pH 7.4) and maintained in modified Barth’s (MB) solution (88 mM NaCl, 1 mM KCl, 0.41 mM CaCl2, 0.82 mM MgSO4, 1 mM MgCl2, 0.33 mM Ca(NO3)2, 20 mM HEPES, pH 7.4) for injection, pairing, and recording.

To assess junctional conductance (Gj), paired oocytes were clamped at −20 mV using two Geneclamp Amplifiers (Molecular Devices, Sunnyvale, CA). One cell was then pulsed to +80 mV and +120 mV eliciting a 2 s trans-junctional current (Ij) in the partnered oocyte. Currents were measured at their maximal level, which occurred within the first 100 ms of the voltage pulse for WT channels and most mutants. Current was measured at the end of a 2 s voltage pulse for S39W, which induced currents that activated rather than inactivated upon application of Vj. For all mutants, a detailed characterization of junctional properties was obtained with a set of longer voltage steps applied in 10 mV increments to maximum VjS of ±100 mV.

For studies of sensitivity to Vm, paired oocytes were clamped at identical holding potentials ranging from -100 mV to +60 mV while a Vj was elicited as described previously. For studies of Vm sensitivity in single oocytes, the technique of recording intercellular currents from paired *Xenopus laevis* females and the follicular layer partially removed with collagenase (Type 1A, Sigma-Aldrich, St. Louis, MO). The remaining follicular layer was removed using fine forceps before oocytes were preinjected with 0.5 ng of morpholino antisense oligonucleotide directed against *Xenopus* Cx38 (Gene Tools, Philomath, OR). Approximately 24 h after preinjection, oocytes were injected with 5 ng of cRNA. Injected oocytes were incubated at 18°C for 12–24 h and then stripped of their vitelline membranes and paired overnight in agar wells. Oocytes were cleaned and digested in Oocyte Ringers 2 (82.5 mM NaCl, 2 mM KCl, 1 mM MgCl2, 5 mM HEPES, pH 7.4) and maintained in modified Barth’s (MB) solution (88 mM NaCl, 1 mM KCl, 0.41 mM CaCl2, 0.82 mM MgSO4, 1 mM MgCl2, 0.33 mM Ca(NO3)2, 20 mM HEPES, pH 7.4) for injection, pairing, and recording.

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oocytes, cells were impaled with their vitelline layers intact and voltage pulses were applied from a holding voltage of -40 mV. Oocytes were bathed in MB while data were acquired and analyzed using pClamp10 software (Molecular Devices, Sunnyvale, CA). In the initial screening of tryptophan mutants, each mutant was studied heterotypically in pairings with WT ShakB(L) to avoid additive effects of mutations on function. Pairings between WT-injected oocytes served as a positive control and provided the baseline conductance for each batch of cells. Pairings between WT-injected oocytes and oocytes injected only with antisense morpholino (oligo/ShakB(L)) served as a negative control. A set of experiments was considered only if pairings between WT and antisense-injected oocytes failed to induce measurable intercellular currents and pairings between WT resulted in averaged conductance above 25 μS. Such high conductance positive controls allowed us to differentiate nonfunctional mutants from those with reduced function. Furthermore, working with a saturating concentration of RNA (5 ng/oocyte, see Fig. 5) negated the effect of small variations in RNA quantification or injection on overall coupling levels.

**Statistical analysis**

To determine if tryptophan substitution significantly altered function, the conductance induced by each mutant was averaged. This average was then compared to the average conductance induced by WT ShakB(L) on the same day. A Student’s t-test was applied to determine if the mean value differed significantly (p < 0.05) from that of WT.

**RESULTS AND DISCUSSION**

**Properties of ShakB(L) junctions**

ShakB(L) expressed reliably and robustly in oocytes. Example traces are shown in Fig. 1A and the behavior is summarized in a conductance (Gj) versus voltage (Vj) relationship in Fig. 1B. In response to voltage pulses between 0 and ±80 mV, steady-state conductance was best fit by a one-state Boltzmann equation yielding the parameters $G_{\text{min}} = 0.422 \pm 0.008$ and $V_{1/2} = -38.5 \pm 1$ mV for relatively negative $V_j$ and $G_{\text{min}} = 0.467 \pm 0.007$, $V_{1/2} = 41.3 \pm 1$ mV for relatively positive $V_j$ (all values represent mean ± SD). $G_{\text{min}}$ represents the normalized minimum $G_j$, and $V_{1/2}$ represents the voltage at which conductance is halfway between its maximum and minimum values. The $G_j$ versus $V_j$ relationship deviated from a one-state Boltzmann equation at voltages beyond ±60 mV. These points are represented by open squares on the $G_j$ versus $V_j$ plot and were excluded from analysis.

The behavior of ShakB(L) expressed in oocytes was similar to that reported previously at $V_j$ between ±80 mV (14). Gating at higher $V_j$s has not previously been characterized. In our experiments, macroscopic gating events at ±100 mV deviated from the Boltzmann fit and were excluded from analysis. Although the values of $G_{\text{min}}$ represent a macroscopic residual conductance state also observed in single channel recordings of well-characterized connexin- and innexin-based junctional channels (40,41), in some gap junction channels, additional slow gating transitions have been shown to gate channels to a fully closed state similar to that induced by chemical gating (41,42). Further characterization of ShakB(L) will be necessary to characterize gating at high $V_j$.

**Analysis of tryptophan substitutions in the M1**

The first transmembrane domain of ShakB(L) was defined using the hydrophathy analysis program TMHMM 2.0 (43). Residues S21-T43 are predicted to lie within the boundaries of the plasma membrane. We targeted 16 consecutive residues for tryptophan scanning beginning with F24, avoiding membrane boundaries where large aromatic side chains have the potential to influence positioning of helices within the membrane (44). Twelve of the 16 mutants induced junctional currents of at least normal magnitude while four were compromised in function. As shown in Fig. 2A, the mutant H27W consistently failed to induce $G_j$, while T31W, L35W, and S39W were compromised in function. These residues...
were arranged on a helical net plot to determine whether the results were consistent with helical secondary structure (Fig. 2 B).

**Sensitivity to tryptophan insertion is consistent with helical secondary structure**

When tryptophan-sensitive sites were plotted on a helical net diagram (Fig. 2 B) the sensitive sites formed a diagonal stripe spanning four rotations of a helix. Because a membranous environment favors hydrogen-bonded secondary structure, transmembrane domains are expected to assume helical or \(\beta\)-sheet secondary structure (45). The periodicity and specificity of the disruptions in ShakB(L) provides strong evidence that M1 is helical, rather than \(\beta\)-sheet, and identifies a face of the M1 helix that interacts with another domain.

**Tryptophan mutants with interesting properties**

Scanning mutagenesis often leads to identification of mutants with interesting properties. In some cases it may even be selected as a screening method for creation of mutants for further analysis (38). In this study, we identified ShakB(L) mutants that displayed a range of altered properties including altered sensitivity to Vj, altered sensitivity to Vm, and altered hemichannel gating. Each of these behavioral changes reflects disruption of a gating mechanism inherent to gap junction function.

Only one mutant, H27W, consistently failed to induce coupling in oocytes and we did not further assess the basis for disruption of function. Because packing of transmembrane helices plays an important role in folding of membrane proteins (in (45)) mutations along the interacting faces of transmembrane helices are likely to prevent proper localization. The histidine is highly conserved among *Drosophila* innexins (46). However, the preceding three residues are also highly conserved but tolerant to tryptophan substitution, including a conserved arginine at position 25 (Fig. 2 A).

**Mutants with altered sensitivity to Vj**

Mutants with altered sensitivity to Vj were most often observed when long sets of voltage pulses were applied to mutant-expressing oocytes. Most mutants displayed at least subtle changes in Vj sensitivity, reflected by asymmetry in the currents recorded from heterotypic pairings with WT ShakB(L). In our initial screening we did not record the orientation of currents with respect to the mutant-expressing oocyte and did not quantify the changes in gating behavior.

**S39W displays altered sensitivity to Vj and Vm**

When tryptophan was substituted for serine at position 39 (S39W), a reduction in Gj was accompanied by a reverse-gating phenotype (Fig. 3 A) observed previously in macroscopic recordings of connexin-based channels (47,48). The term “reverse-gating” refers to, and emphasizes, the tendency for currents to activate rather than inactivate in
In studies of connexins, reverse-gating occurs in response to mutagenesis, particularly point mutations within the transmembrane domains (47,48). For instance, in Cx32, cysteine mutations in all four transmembrane domains induce reverse-gating and sensitive locations tend to be confined to a specific face of each helix (49). More detailed analysis of several of these sites suggested that amino acid side-chain properties such as length or branching are critical at specific locations. For example, in Cx32, replacing the methionine at position 34 with amino acids having shorter side chains (e.g., M34A, M34C, M34T, and M34S) induced reverse-gating; replacing M34 with leucine (M34L), an amino acid with a side chain comparable in length to that of methionine, had little effect on gating (48), while replacement with tryptophan (M34W) rendered the channels nonfunctional (49). These observations suggest that the reverse-gating phenotype correlates with disruption of interactions between helices in connexins. The changes observed after tryptophan substitution at S39 in ShakB(L) represent the first, to our knowledge, reported observation of reverse-gating in an innexin-based channel. The position of S39 on the interacting face of the M1 helix, along with H27, T31, and L35, suggests that disruption of helix-helix interactions can also induce reverse-gating of an innexin-based channel.

Gap junction channel properties were further assessed in ShakB(L)/S39W pairs by reversing the polarity of Vj. When this was done, the reverse-gating phenotype was maintained, however, the magnitude of the current differed. This suggested that the channels were sensitive to transmembrane holding potential (Vm) as well as Vj. A detailed analysis of Vm sensitivity was, therefore, carried out and the results are summarized in Fig. 4. When normalized to the Gj measured at -20 mV, S39W displayed steep sensitivity to Vm, decreasing greater than twofold at 20 mV and increasing by almost twofold at -60 mV. This is in contrast to WT ShakB(L), which is insensitive to Vm (16).

Analysis of ShakB(L)S39W represents the first report of a reverse-gating phenotype in an innexin-based junction, and suggests that a common mechanism may be disrupted by point mutations within the transmembrane domains of connexins and innexins. Plausibly, a disruption of interactions that stabilize the open state of the channel results in a partially closed conformation sensitive to the application of Vj. Because all channels with a reverse-gating phenotype tend to open in response to relatively positive Vjs (47,49,50) it is unlikely that disruption of a Vj gate is responsible for the phenomenon. In the case of S39W in ShakB(L), a high sensitivity to Vm occurred in addition to changes in Vj sensitivity. An altered hemichannel gating mechanism known as the loop gate (40,41) would have such an effect.

Three mutants induced large transmembrane currents in oocytes

Three tryptophan mutants (F24W, S37W, and F38W) induced higher Gj between paired oocytes than WT ShakB(L). These are denoted by asterisks on the bar graph in Fig. 2A reflecting that the increase was statistically significant. The relative position of these residues within M1 is displayed on the helical net plot in Fig. 5A. They are positioned close to the predicted cytoplasmic (F24)
and extracellular (S37 and F38) borders of the M1 helix. Fig. 5B shows that small variations in the RNA concentration cannot account for the large conductance increase induced by these mutants. The arrow in Fig. 5B represents the RNA concentration used for analysis of tryptophan mutants.

After injection of RNA encoding the mutants F24W, S37W, and F38W the health of oocytes was compromised. However, once oocytes were paired for gap junction experiments survival rates increased. To test whether these mutations induced membrane currents in oocytes, voltage pulses were applied to study currents across the plasma membrane in the range -160 mV to +80 mV. For studies of membrane currents, oocytes were maintained in a solution of MB containing 0.74 mM Ca$^{2+}$ and 1.82 mM Mg$^{2+}$. Attempts to transfer oocytes to typical solutions used for connexin hemichannel analysis, with extracellular calcium concentration at or <0.1 mM, caused the oocytes to swell and burst, prohibiting stable electrical recordings. Comparison of transmembrane currents induced by F24W, S37W, F38W, and WT ShakB(L) showed that expression of ShakB(L) does not induce transmembrane currents in oocytes, whereas all three tryptophan mutants induced large currents. As shown
in Fig. 6 A, the current versus voltage relationship for WT ShakB(L)-injected oocytes was similar to that of Cx38 antisense oligonucleotide-injected oocytes, whereas F38W induced large voltage-dependent currents. Fig. 6, B and C, show similar results for F24W and S37W, respectively, both of which induced large voltage-dependent membrane currents. Injecting oocytes with one-quarter the amount of RNA used in studies of paired oocytes induced smaller but significant currents across the plasma membrane. Because two of the sites (S37 and F38) are located at the extracellular end of M1, it is possible that tryptophan insertion induced hemichannel activity by disrupting interactions between the extracellular loops required to maintain the closed conformation of nonapposed channels (reviewed by (51)).

Given the correlation between induction of membrane currents and high Gj mediated by F24W, S37W, and F38W one could speculate that the presence of open hemichannels induces junctional formation, a phenomenon previously observed when Cx46 and Cx50 were expressed in oocytes (52). All three mutants tended to maintain unusual sensitivity to Vj at high conductances. Wilders and Jongsm (53) suggested that junctions that maintain voltage sensitivity at higher conductance could be explained by the presence of a higher number of small junctions, analogous to a situation where several high resistors replace low resistance units in a circuit that involves significant contributions from electrode series resistance and cellular input resistance. We often observed sensitivity to Vj at conductances in the 100 μS range for these mutants, which is uncharacteristic of junctions formed by ShakB(L) or other junctional proteins expressed in oocytes. An example trace recorded from a 100 μS oocyte pair expressing S37W is shown in Fig. 6 D. Overall, the results suggest that increased hemichannel function tends to induce the rapid formation of many small junctional plaques.

CONCLUSIONS

Tryptophan scanning was used to establish the helical nature of the first transmembrane domain of the innexin ShakB(L). One face of the helix, including residues H27, T31, L35, and S39, was sensitive to tryptophan insertion, representing a localized region of contact between M1 and another transmembrane domain. Further experiments are required to determine whether the interacting face of M1 is involved in inter- or intrasubunit interactions.

Very little is known about the role of transmembrane domain interactions in gap junction channels. In innexins, as in other membrane proteins, transmembrane domain interactions are likely to stabilize tertiary structure, regulate protein function, and maintain quaternary structure by mediating subunit oligomerization (in (45)). We observed a range of effects when helical interactions were disrupted in ShakB(L). In the case of H27W, a complete disruption in function was observed, while T31W, L35W, and S39W induced very low levels of coupling. Further experiments are required to determine whether these mutations disrupt the efficiency of gap junction formation and/or impair the function of channels in otherwise normal plaques. Three
of the mutants with impaired function (T31W, L35W, and S39W) displayed changes in sensitivity to Vj, and S39W displayed sensitivity to Vm not previously reported in studies of ShakB(L). These observations suggest that disruption of helical interactions in innexins affects mechanisms regulating channel gating in response to Vm and Vj but does not preclude the possibility that mechanisms of channel formation are also disturbed.

Although innexins and connexins represent unrelated protein families, they perform similar functions by assembling into large plaques to mediate electrical and chemical coupling of adjacent cells. Correlations between structure and function are of particular interest in cases where unrelated proteins perform similar functions. Connexin and innexin proteins have a common membrane topology and are expected to oligomerize similarly to form intercellular channels. To our knowledge, there are currently no published results of tryptophan scanning for connexins but in the crystal structure of a gap junction channel composed of Cx26, the first transmembrane domain is packed between the N-terminus, which folds into the cytoplasmic mouth of the channel, and the fourth transmembrane domain that lies parallel and adjacent to it. M1 also interacts closely with M2 near the middle of the membrane where the helices cross one another (9). Our results appear to be inconsistent with a similar tight packing arrangement for M1 of the innexin ShakB(L), because this domain interacts along only one helical face.

Several ShakB(L) mutants with interesting phenotypes were generated as a byproduct of tryptophan scanning. The mutant S39W displayed a reverse-gating phenotype often observed after amino acid substitution within the transmembrane domains of connexin-based channels. In addition, several mutants displayed altered sensitivity to Vj, whereas F24W, S37W, and F38W induced currents across the plasma membrane of nonapposed oocytes. Similar changes are commonly observed after amino acid substitution within the transmembrane domains of connexins (4) and suggest that channels formed by innexins and connexins may have similar regulatory mechanisms.

In summary, our results confirm that M1 of the innexin ShakB(L) is helical and identify a localized region of contact between M1 and another transmembrane domain. Amino acid substitution within M1 of ShakB(L) induces similar changes to those observed after mutagenesis of connexins. Some of the mutants with interesting properties are likely to prove useful in future studies, particularly those aimed at characterization of the mechanisms underlying rectification at electrical synapses in the GFS of Drosophila.

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