Mapping the Binding Site on Ankyrin for the Voltage-dependent Sodium Channel from Brain*

Yogambal Srinivasan, Mark Lewallen, and Kimon J. Angelides‡

From the Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas 77030

Erythrocyte ankyrin is a member of a family of proteins that mediate the linkage between membrane proteins and the underlying spectrin-actin-based cytoskeleton. Ankyrin has been shown to interact with a variety of integral membrane proteins such as the anion exchanger, the Na⁺K⁺-ATPase, and the voltage-dependent sodium channel (NaCh) in brain. To understand how ankyrin interacts with these proteins and maintains its specificity and high affinity for the voltage-dependent NaCh, we have mapped the binding site on ankyrin for the NaCh by examining the binding of purified ankyrin subfragments, prepared by proteolytic cleavage, to the purified rat brain NaCh incorporated into liposomes. ¹²⁵I-Labeled ankyrin and the radiolaabeled 89- and 43-kDa fragments of ankyrin bind to the NaCh with high affinities and with Kᵦ values of 34, 22, and 63 nM, respectively, and have stoichiometries of approximately 1 mol/mol NaCh. The 72-kDa spectrin binding domain is inactive and does not bind to the NaCh. Dissection of ankyrin reveals that the 43-kDa domain retains all the binding properties of native ankyrin to the NaCh. Analysis of the primary structure reveals that the NaCh binding site is confined to a domain of ankyrin consisting entirely of the 11 terminal 33-amino acid repeats and is distinct from the ankyrin domains that interact with spectrin and the Na⁺K⁺-ATPase.

The voltage-gated sodium channel (NaCh) is a 260-kDa transmembrane glycoprotein which mediates the rapid increase in sodium permeability associated with the depolarizing phase of the action potential. In myelinated nerve, segregation and maintenance of high NaCh density at the nodes of Ranvier is crucial for saltatory conduction along the axon (1). Measurement of the lateral mobility of NaChs at specific sites along the axon by fluorescence photobleach recovery suggests that NaChs are immobilitylized at these sites (2). What factors contribute to the segregation and immobilization of NaChs at specific sites along the axon is a question of considerable relevance to understanding their physiological function.

Recently we have reported that ankyrin co-purifies and binds with high affinity to purified NaChs from rat brain (3). Direct binding of ¹²⁵I-labeled ankyrin to the reconstituted NaCh shows that ankyrin binds tightly to the channel with 1:1 mol stoichiometry, an interaction which depends on the cytoplasmic domain of the NaCh. Most importantly, the interaction of ankyrin with NaChs is selective since neither the neuronal γ-aminobutyric acid receptor nor the dihydropyridine sensitive Ca²⁺ channel, both of which have different distributions on the neuron, immunoprecipitate or bind brain ankyrin. The idea that NaChs are restrained by cytoskeletal linkages is consistent with recent immunofluorescent and electron microscopic studies in rat sciatic nerve which have shown that a specialized form of erythrocyte ankyrin is localized at the nodes of Ranvier, whereas the "brain" ankyrin isoform is uniformly distributed along the axon (4).

Erythrocyte and brain ankyrin are members of a family of proteins that associate with a variety of integral membrane proteins such as the anion transporter (5), the Na⁺K⁺-ATPase (6, 7), lymphocyte adhesion antigen PgP-1 (8), as well as the spectrin-actin cytoskeletal network (9, 10). Because ankyrin is able to interact and associate with a diverse set of membrane proteins, there is great interest in determining the common structural motifs that mediate these interactions. Although the cytoplasmic domain of the anion transporter is able to compete with the ankyrin-NaCh interaction (3), there is no primary sequence homology (10) between these two proteins. The primary sequence for several erythrocyte (11) and brain ankyrins (12) have recently been determined from cloning of the cDNAs. Ankyrins are proteins of molecular mass ~220 kDa folded into three independent domains which include an 89-kDa NH₂-terminal domain (13), followed by a 72-kDa domain (14), and a 55-kDa regulatory domain at the carboxyl terminus (15).

To approach the question as to how ankyrin might interact with a diverse set of membrane proteins and still maintain its specificity for the NaCh, we sought to define the binding site of ankyrin for the NaCh by examining the binding of ankyrin subfragments prepared by proteolytic cleavage. We show here that a 43-kDa subfragment derived from the NH₂-terminal 89-kDa fragment containing 11 of the 22 tandem 33-amino acid repeats of ankyrin is sufficient to bind to the NaCh and is distinct from the domains that interact with spectrin and the Na⁺K⁺-ATPase.

EXPERIMENTAL PROCEDURES

Materials and Methods—Frozen rat brains were obtained from Pel Freez. Lipids (DOPC, PS, and PE) were obtained from Avanti Polar Lipids. TLCK-treated α-chymotrypsin, Staphylococcus aureus V8 protease, and other chemicals were obtained from Sigma. [H]Saxitoxin (STX) (specific activity 30 Ci/mmol) was obtained from Amer sham Corp. All other chemicals were the highest commercial grade quality available.

Purification of the Sodium Channel—NaChs were purified from frozen rat brains by sequential chromatography on DEAE-Sephadex,
hydroxypatite, and wheat germ agglutinin (WGA)-Sepharose columns as described (16, 17). Two to three hundred μg of protein was routinely obtained from the WGA step. In order to completely deplete spectrin and ankyrin from preparations of purified NaCh the WGA-Sepharose column was washed with 300 ml of Buffer A containing 50 mM NaCl, 20 mM HEPES, pH 7.4, 1% (v/v) Triton X-100, and 0.025% phosphatidylcholine before elution with 0.5-10 mM acetylglucosamine. Fractions from the WGA-Sepharose column (5 μg) were analyzed by SDS-gel electrophoresis on 4-15% linear gradient polyacrylamide gels and stained by ammonium silver (18). The purified NaCh protein routinely appeared as a single broad band with a molecular mass of -260 kDa. [H]STX binding to the purified NaCh was determined as described (19) by rapid centrifugation on Sephadex G-50 fine column. The average specific activity from the eight preparations of purified NaCh used in this study was 2,200 ± 100 pmol. Both the purity and the [H]STX binding activity of the NaCh obtained from fresh rat brains were comparable. Therefore, frozen rat brains were routinely used.

Reconstitution of Purified Sodium Channels—Purified NaChs were reconstituted into unilamellar phospholipid vesicles using a DOPC:PE:PS lipid ratio of 50:30:15 and a protein:lipid ratio of 1:100. Triton X-100 was removed by gentle shaking overnight with Bio-Beads SM-2. Vesicles were dialyzed against bis-o smotic G A E L U T I O N buffer to remove residual N-acetylglucosamine. The protein concentration of the NaChs was typically 200–200 μg/ml.

Each preparation of reconstituted NaChs was assayed for [H]STX binding activity by adding vesicles to an incubation mixture of 18 μM [H]STX in 100 mM choline chloride, 5 mM KCl, 7.8 mM CaCl2, 1.3 mM MgSO4, 20 mM Tris/HCl, pH 7.4, containing 0.1% bovine serum albumin. Samples were mixed and incubated for 60 min at 4 °C and the vesicles rapidly collected by filtration on Whatman GF/B filters. Filters were rinsed three times with ice-cold buffer and the radioactivity bound to filters determined by liquid scintillation counting. Nonspecific binding was measured in the presence of 10 μM tetrodotoxin and subtracted from the total.

The fraction of reconstituted NaChs oriented with their cytoplasmic domains at the external surface was measured by disruption of the vesicles with 2% Triton X-100 and 0.25% phosphatidylcholine, pH 7.4, and retention with 18 mM [H]STX (17). Data from the eight preparations of reconstituted NaChs used in this study showed an average of 45 ± 7% of the channels were oriented with their cytoplasmic domains exposed.

Purification of Ankyrin—Ankyrin was purified from outdated human blood as described (5). Purified fractions from the sacrose gradient were collected and concentrated to ~4 mg/ml. Alternatively, fractions from the DE-52 column were concentrated using CM-30 ultrafilters which removed >95% of the ankyrin and spectrin associated with NaChs.

Purification of Ankyrin Proteolytic Subfragments—Three proteolytic fragments were obtained from purified ankyrin as described (13-20). An 89-kDa domain from the NH2-terminal sequence of ankyrin was obtained by a-chymotryptic digestion, a 43-kDa fragment comprising the COOH-terminal fragment of the 89-kDa domain, and a 62.5-kDa fragment (apparent molecular mass that moves at the dye front occasionally appeared on storage of purified NaCh) (19). For these reasons, the purification was carried out directly after purifications.

The 72-kDa spectrin-binding fragment of ankyrin was purified from spectrin-depleted inside-out vesicles by a-chymotryptic treatment (14, 21). Spectrin-depleted erythrocyte ghosts were prepared by incubating erythrocyte ghosts for 30 min at 37 °C in 0.6 M sodium phosphate, pH 7.5. Ghosts were washed once with 7.5 mM sodium phosphate buffer, pH 7.5, and resuspended in 200 μl of ice-cold sodium phosphate buffer containing 0.2 mM DTT. Vesicles were digested with 1 μg/ml TLCK a-chymotrypsin for 45 min at 4 °C, and the digestion was terminated by the addition of PMSF to a final concentration of 200 μM. The digested protein was concentrated to 200 μl of DE-52 cellulose equilibrated with 7.5 mM sodium phosphate, pH 7.5. The slurry was mixed for 15 min and poured into a column and washed with 300 μl of 7.5 mM sodium phosphate buffer with 0.2 mM DTT and 50 μg/ml PMSF. The column was washed with 75 mM KCl in phosphate buffer with 0.2 mM DTT and eluted with 200 mM KCl in the same buffer. Purification of the 72-kDa fragment was analyzed on silver-stained 4-15% linear gradient polyacrylamide gels (18).

Radiolabeling of Ankyrin and Ankyrin-derived Proteolytic Fragments—Ankyrin, the 89-kDa and the 43-kDa proteolytic fragments of ankyrin were radiolabeled with [3H]-labeled Bolton Hunter reagent as described (5, 21). [3H]-Labeled proteins were used within 1 week of their preparation.

Protein Determinations—Protein concentration was routinely determined by the method of Bradford (22). The protein concentration of reconstituted vesicles was determined by solubilizing vesicles with 2.5% Triton X-100 and determining protein by the Peterson assay (23).

Binding of [3H]-Ankyrin and Ankyrin-derived Proteolytic Fragments to NaCh Vesicles—Vesicles containing purified NaChs in binding buffer (0.4 M NaCl, 25 mM HEPES/Tris, 0.05% Triton X-100, 0.01% phosphatidylcholine, pH 7.4, containing 1 mM PMSF, 1 mM 1,10-phenanthroline, 1 mM o-iodoacetamide, 1 μM leupeptin, 1 μM pepstatin A, 1 μM aprotinin) were incubated with various concentrations of [3H]-labeled ankyrin or [3H]-labeled ankyrin subfragments for 6 h at 4 °C. [3H]-Labeled ankyrin or [3H]-labeled ankyrin subfragments for 6 h at 4 °C with gentle shaking. The vesicles were then rapidly filtered on GF/B glass filters presoaked in binding buffer containing 0.1% bovine serum albumin and washed once with 5 ml of binding buffer to remove unbound ankyrin. Alternatively, after equilibration, vesicles were separated from unbound ankyrin by centrifugation through Sephacryl S-300 mini-columns (2 ml) from which vesicles were obtained in the void volume. Although both assays yielded identical results, the GF/B filtration assays were convenient for the large number of samples tested and in recovery of [3H]-labeled ankyrin from the NaCh for further purification. To reconstitute NaCh-ankyrin complexes, after filtration, 1.0 ml of Buffer C containing 6% Triton X-100 was added directly to filters, and proteoliposomes were solubilized for 20 min on ice. The supernatant was removed and adsorbed to 0.5 ml of packed WGA-Sepharose 4B for 12 h at 4 °C. The supernatant was removed, the gel washed twice with 1 ml of Buffer C, and the NaCh-ankryn complexes subsequently eluted with 1 ml of Buffer C containing 50 mM N-acetylglucosamine. Assays were performed in duplicate and aliquots were counted in a γ-counter.

RESULTS AND DISCUSSION

NaChs were purified from rat brain to high purity and on analytical polyacrylamide gels purified NaChs consisted of a single diffusely staining band of molecular mass 260 kDa (Fig. 1, lane A). In NaChs used from the final stage of purification, >85% of the ankyrin and spectrin associated with NaChs
were removed. In all preparations used, as before (3), neither the 36- nor 33-kDa polypeptides, which have been reported to accompany some preparations of NaChs (16), were observed in our preparations. After reconstitution of NaChs into liposomes, \[^{3}H\]STX binding showed that ~50% of the NaCh had their cytoplasmic surface oriented toward the outside of the vesicle, accessible to ankyrin binding. Ankyrin was purified to homogeneity from erythrocytes and on 4-15% silver-stained linear gradient SDS-polyacrylamide gels had a molecular mass of 220 kDa with no evidence of low molecular mass breakdown products (Fig. 1, lane B). In some preparations a faster migrating band ~195 kDa beneath the 220-kDa ankyrin band could be discerned on silver-stained gels, which may correspond to the calpain-derived 195-kDa fragment (15).

**Fig. 1.** Characterization of purified rat brain NaCh (lane A) and erythrocyte ankyrin (lane B). NaCh (5 μg) and ankyrin (20 μg) were electrophoresed on 4-15% linear gradient SDS-polyacrylamide gels and stained with ammonical silver (18). The specific activity of this preparation was 2,200 pmol of \[^{3}H\]STX/mg of protein.

When NaCh vesicles were incubated with varying concentrations of \(^{125}\)I-labeled ankyrin, ankyrin bound in a concentration-dependent and -saturable manner (Fig. 2). The interaction was specific since \(^{125}\)I-ankyrin could be displaced by unlabeled ankyrin. As described earlier (3), control experiments in which vesicles were used without NaCh protein or where the cytoplasmic surface of the NaCh was removed by proteolysis did not bind \(^{125}\)I-ankyrin or ankyrin subfragments. Analysis of the binding isotherm by Scatchard analysis shows that \(^{125}\)I-labeled ankyrin binds to the NaCh with a K_d of 34 nM and a capacity of 1.3 mol of ankyrin/mol of NaCh after correction for the orientation of NaChs in vesicles determined by \[^{3}H\]STX binding. Although the equilibrium binding measured in these experiments is consistent with earlier measurements, the slightly lower binding affinity of ankyrin for the NaCh observed in these experiments, compared with the previously published value of 20 nM (3), could possibly arise...
from differences in the addition of supplemental detergent and/or increases in ionic strength required to carry out these assays. Similar reductions in the binding of ankyrin to the anion exchanger in the presence of detergent or high ionic strength have been described (20). In addition, differences in lipid composition (DOPC versus the DOPC:PE:PS vesicles used here) or slight increase in density of NaChs reconstituted into vesicles used for this study could also contribute to the differences observed.

Although a calpain-like 195-kDa fragment of ankyrin lacking a portion of the COOH terminus accompanied some preparations of ankyrin, we did not observe any differences between ankyrin preparations in either the stoichiometry or binding affinities to the NaCh. This is likely related to the negligible role that the COOH terminus plays in mediating ankyrin binding- NaCh interactions (see below).

To understand the general role of ankyrin as a linker molecule between the cytoskeleton and several membrane proteins, we sought to dissect the domains of ankyrin that mediate its association with the NaCh. Mapping the domain structure of ankyrin by proteolytic cleavage and recent analysis of the primary structure of human erythrocyte ankyrin have shown that the molecule consists of three major domains: a 89-kDa NH2-terminal domain followed by a 62-kDa spectrin binding domain and a 55-kDa regulatory domain at the carboxyl terminus of the protein (Fig. 3). Among the three domains, the 89-kDa domain is unique in that together with some flanking sequences (11-15) it contains all of the 22 tandem 33-amino acid repeats of ankyrin. Previous work (20) has shown that this purified 89-kDa domain not only competes with ankyrin for binding to ankyrin-depleted inside-out vesicles but also binds with enhanced affinity to the reconstituted anion exchanger. In contrast the Na+K+-ATPase, a membrane protein that binds ankyrin with high affinity, has a very low affinity for the 89-kDa fragment (25). The question arises, then, as to which domain of ankyrin binds to the NaCh.

Each of the subfragments were tested for their ability to displace 125I-ankyrin from its interaction with the NaCh. As shown in Fig. 4, the 89-kDa fragment displaces 125I-labeled ankyrin from NaChs with a half-maximal displacement of about 20 nM. At equivalent concentrations, the 89 kDa was more effective than native ankyrin (Kd ≈ 50 nM) in these displacement assays. At all concentrations tested the 72-kDa internal domain did not compete for ankyrin binding to the NaCh, and this is consistent with our previous report using nitrocellulose binding assays which showed that the 72-kDa spectrin domain does not compete for NaCh-ankyrin interactions (3). In addition, the lack of competition of the 72-kDa spectrin domain to NaCh-containing vesicles confirms that the binding of ankyrin and the 89-kDa fragment is not due to residual spectrin associated with the NaCh.

Measurement of the direct binding of 125I-labeled 89-kDa ankyrin to NaChs reconstituted into vesicles shows that the 89-kDa fragment retains its binding activity and binds in a concentration-dependent manner with a Kd of 22 nM (Fig. 5) compared with the Kd of 34 nM of native ankyrin measured under similar conditions (Fig. 2). Both unlabeled ankyrin and 89-kDa protein compete effectively for binding of the 125I-labeled 89-kDa domain, indicating that both bind to the same or overlapping sites on NaCh. Scatchard analysis of 89-kDa fragment binding is consistent with a single class of high affinity sites on the NaCh with a maximum binding capacity of 1.1 mol of 89 kDa/mol of NaCh. Although there is evidence that the binding of 89-kDa ankyrin subfragment binds to erythrocyte membranes with either negative cooperativity or to two different affinity sites (20), Scatchard analysis of the binding of both ankyrin and the 89-kDa fragment to the erythrocyte membranes shows a single class of high affinity sites on the NaCh with a maximum binding capacity of 1.1 mol of 89 kDa/mol of NaCh.
Sodium Channel Binding Site on Ankyrin

FIG. 5. Direct binding of $^{125}\text{I}$-labeled 89-kDa domain of ankyrin to purified NaChs reconstituted into liposomes. Liposomes containing purified NaCh (1 pmol oriented with the cytoplasmic domain facing the medium) were incubated with increasing concentrations of $^{125}\text{I}$-labeled 89-kDa domain (specific activity 150,000 cpm/pmol) (△). Also shown are representative points in the presence of 500 nM ankyrin (○) or 500 nM unlabeled 89-kDa domain (Δ). Binding is shown as a function of bound 89-kDa domain versus total concentration of 89-kDa domain (left panel) or by the method of Scatchard (32) (right panel).

FIG. 6. Direct binding of the 43-kDa fragment of ankyrin containing 12 of the 22 tandem 33-amino acid repeats of ankyrin. The 43-kDa V8 proteolytic fragment was prepared as described under "Experimental Procedures." The preparation used for radiolabeling and used for this figure is shown in the left panel. Five μg of purified 43-kDa fragment were electrophoresed on 4-15% linear gradient polyacrylamide SDS gels and stained with ammoniacal silver. $^{125}\text{I}$-Labeled 43-kDa fragment (specific activity 78,000 cpm/pmol) was incubated at increasing concentrations with NaCh liposomes (1 pmol oriented with the cytoplasmic domain facing the medium) in the absence (●) and presence of 500 nM unlabeled ankyrin (○), 500 nM 89 kDa (▽), and 7.3 μM 43-kDa fragment (△) (middle panel). The data are expressed as a function of bound 43-kDa versus total 43-kDa concentration (middle panel) or according to Scatchard (32) (right panel).

NaCh show no evidence for such binding. Although there is some scatter in the data that is suggestive of two closely related sites that do not differ significantly in either affinity or capacity, the data are best fit to a single class of interacting sites with approximately equal capacities.

To further dissect regions of the 89-kDa NH$_2$-terminal domain that interact with NaChs, we prepared a 43-kDa fragment of the 89-kDa domain by digestion with V8 protease followed by purification by Mono S chromatography (Fig. 6). Analysis of the primary structure shows that the 43-kDa fragment consists entirely of 33-amino acid repeats containing repeats 12-22 (11). In displacement assays the 43-kDa fragment competes with native ankyrin for the NaCh interaction, and in direct binding the 43-kDa fragment binds to a single class of noninteracting sites on NaCh vesicles with a $K_d$ of 63 nM and a maximal capacity of 1.2 mol of 43-kDa fragment/mol of NaCh (Fig. 6). Unlabeled ankyrin, the 89-kDa domain, and 43-kDa fragment proteins all were able to displace the 43-kDa fragment (Fig. 6). Even though removal of the NH$_2$-terminal 403 amino acids from the 89-kDa domain yields a
fragment with a moderate reduction in the affinity, retention of binding to the NaCh suggests that the carboxyl terminal region, containing 11 of the 22 tandem 33-amino acid repeats of ankyrin is sufficient to mediate the interaction with the cytoplasmic domain of the NaCh and contains most if not all of the information for binding to the NaCh. Although the 43-kDa domain binds to the NaCh without requirement of other regions of the 89-kDa domain, the reduced affinity possibly reflects a need for the flanking sequences in order to fold a stable native ankyrin structure (15). Alternatively, it is possible that the high affinity interaction between ankyrin and the NaCh involves some of the nonrepeat domains of the NH₂ terminus of the 89-kDa domain or other multiple contact sites.

In each of the fragments that retained binding, the stoichiometry of NaCh-ankyrin complexes remained essentially unchanged. The stoichiometry was 1.3 mol of ankyrin bound per mol of NaCh using intact ankyrin, considering that 50% of the NaCh molecules are oriented with their cytoplasmic domains on the surface of the vesicles, accessible to ankyrin binding. The 89-kDa domain bound to NaCh vesicles with a stoichiometry of 1.1:1 and the 43-kDa fragment with an approximate stoichiometry of 1.2:1.

Ankyrin is a multifunctional protein whose primary sequence has revealed a structure which is uniquely suited to mediate the interaction between spectrin and several diverse membrane proteins. In this work we have localized the NaCh binding domain on ankyrin to the 43-kDa carboxyl terminal portion of the 22 tandem 33-amino acid repeat motif. Like the NaCh, both the 89- and 43-kDa domains of ankyrin also are sufficient to mediate the high affinity binding of ankyrin to the anion transporter (20, 25, 26). In contrast, the Na⁺K⁺-ATPase, which has a high affinity for ankyrin (6, 7), interacts weakly with the 89-kDa domain of ankyrin, requiring regions of the spectrin binding domain of ankyrin to reconstitute the high affinity interaction (25). Thus the interaction between ankyrin and the brain NaCh and the domains through which it interacts appear to be similar to those of the anion transporter. There are other similarities between these two membrane proteins. In previous work (3) we demonstrated that there is some structural homology in the cytoplasmic regions of these membrane proteins for the ankyrin binding site, because the cytoplasmic domain derived from the anion exchanger competed for NaCh-ankyrin interactions. The homology is possible at the level of secondary and tertiary structure, since there is no primary structural homology between these two proteins in their cytoplasmic domains. Taken together, these observations are consistent with the idea that both the NaCh and the anion exchanger evolved to interact with a similar domain of ankyrin, analogous to the lock and key of an antibody-antigen reaction. The Na⁺K⁺-ATPase, on the other hand, which interacts with ankyrin has differentially evolved and requires additional regions of ankyrin to confer high specificity.

In brain, two distinct isoforms of ankyrin have been detected. Based upon immunological differences (4) and from recent primary sequence analysis of cloned brain ankyrins (12), brain ankyrin contains the 22 tandem 33-amino acid repeats, each of which closely resemble the repeats of erythrocyte ankyrin. However, brain ankyrin is less active in displacing ankyrin from its anion exchanger binding site (26), indicating that association of ankyrin with the NaCh is not exclusively mediated by the 33-amino acid repeat and that additional specificity is likely built into some of the flanking sequences. Recent studies have also shown that the erythrocyte and brain ankyrins have differences in cellular expression and localization. In nerve, the erythrocyte form is expressed primarily in axons of neurons and at specialized cell domains such as the node of Ranvier, whereas brain ankyrin isoforms are present in both nerve and glial cells and are not found at nodes of Ranvier (4). The differential distribution of these ankyrin isoforms is consistent with the distribution of the NaCh in nerve and may be pertinent to the differential distribution of the NaCh and Na⁺K⁺-ATPase on the nerve cell surface. In nerve cells it is known that the NaCh is localized to axon hillocks and nodes of Ranvier together with some isoforms of the Na⁺K⁺-ATPase (27). An interesting issue is whether the erythrocyte and brain isoforms of ankyrin serve to mediate specific and separate interactions with the NaCh and the Na⁺K⁺-ATPase. The association of these membrane proteins with different domains on ankyrin could serve to spatially and functionally separate these two proteins within the same domain on the axon membrane.

The 22 tandem 33-amino acid repeat sequence is a highly conserved motif present in all ankyrins discovered so far and is also present in several proteins involved in cell differentiation such as the Drosophila Notch protein (28) and I2 protein of Caenorhabditis elegans (29). Recent work has localized this structural motif to transcription factors that bind to "GA"-rich DNA sequences and referred to as GA binding proteins (30). These repeats, termed ankyrin repeats, may constitute a new class of protein-protein interactions and may have a broad role as a molecular tether in binding proteins of several types, such as nuclear proteins that help regulate gene expression as well as cytoplasmic proteins that participate in cell signaling pathways (31). Most striking are the 33-amino acid repeats which are required to mediate the interaction of ankyrin and the voltage-dependent NaCh and anion exchanger. It is important of note that the Na⁺K⁺-ATPase does not interact with this domain and has diverged in its recognition site. Although the erythrocyte ankyrins and brain ankyrins are encoded by different genes on separate chromosomes (11, 12), the conserved NH₂ terminus comprising the tandem repeats suggests that all ankyrins have a basic functional role to perform, mediated by this region of the molecule. Within the motif itself, 15 amino acid residues are highly conserved and the remaining 18 are divergent. Since ankyrin has been demonstrated to bind to diverse membrane proteins such as the anion transporter, the Na⁺K⁺-ATPase, and the NaCh proteins, with no significant shared homologous domains, the interactions may be through the divergent amino acids in the motif rather than the conserved ones, where the conserved residues serve to mediate the initial low affinity interactions. These divergent amino acid residues may generate different structural elements (secondary or tertiary) capable of mediating the interaction of different types of membrane proteins. Although the 72-kDa and the COOH-terminal domain of ankyrin do not have a role in specifying the association of ankyrin with the NaCh, these domains have been shown to be important for interactions with spectrin (14) and the Na⁺K⁺-ATPase (25). For example, a spliced variant of ankyrin, ankyrin 2.2, has been shown to have a greater affinity for spectrin and for the anion exchanger (15). Recent cloning of the brain ankyrin isoforms have shown that the COOH-terminal domains distinguish these ankyrin isoforms from their erythrocyte counterpart and are regions that are likely to confer additional specificity to their distribution in neurons and their interactions with other membrane proteins.

REFERENCES

1. Waxman, S. G., and Ritchie, J. M. (1985) Science 228, 1502-1507.
Sodium Channel Binding Site on Ankyrin

2. Angelides, K. J., Elmer, L. W., Loftus, D., and Elson, E. (1988) J. Cell Biol. 106, 1911-1925
3. Srinivasan, Y., Elmer, L. W., Davis, J., Bennett, V., and Angelides, K. J. (1988) Nature 333, 177-180
4. Kordeli, E., Davis, J., Trapp, B., and Bennett, V. (1990) J. Cell Biol. 110, 1341-1352
5. Bennett, V., and Stenbuck, P. (1980) J. Biol. Chem. 255, 6424-6432
6. Nelson, W. J., and Veshnock, P. J. (1987) Nature 328, 533-535
7. Morrow, J. S., Cianci, C. D., Ardito, T., Mann, A. S., and Koshgarian, M. (1989) J. Cell Biol. 108, 455-465
8. Kalomiris, E. L., and Bourguignon, L. Y. (1988) J. Cell Biol. 196, 319-327
9. Bennett, V. (1990) Physiol. Rev. 70, 1029-1066
10. Morrow, J. S. (1989) Curr. Opin. Cell Biol. 1, 23-29
11. Lux, S. E., John, K. M., and Bennett, V. (1990) Nature 344, 36-42
12. Otto, E., Kunimoto, M., McLaughlin, T., and Bennett, V. (1991) J. Cell Biol. 114, 241-253
13. Bennett, V., and Stenbuck, P. (1980) J. Biol. Chem. 255, 2540-2548
14. Davis, J. Q., and Bennett, V. (1984) J. Biol. Chem. 259, 13550-13559
15. Hall, T. G., and Bennett, V. (1987) J. Biol. Chem. 262, 10537-10545
16. Hartshorne, R. P., and Catterall, W. A. (1984) J. Biol. Chem. 259, 1667-1675
17. Elmer, L. W., O'Brien, B., Nutter, T. J., and Angelides, K. J. (1985) Biochemistry 24, 8128-8137
18. Merrill, C. R., Dunau, M. L., and Goldman, D. (1981) Anal. Biochem. 110, 201-207
19. Levinson, S. R., Curatolo, C. J., Reed, J. K., and Raftery, M. (1979) Anal. Biochem. 99, 72-76
20. Davis, L., and Bennett, V. (1990) J. Biol. Chem. 265, 10589-10596
21. Bennett, V. (1983) Methods Enzymol. 96, 313-324
22. Bradford, M. M. (1976) Anal. Biochem. 72, 248-254
23. Peterson, G. L. (1977) Anal. Biochem. 83, 348-356
24. Elmer, L. W., Black, J. A., Waxman, S. G., and Angelides, K. J. (1990) Brain Res. 532, 222-231
25. Davis, J. Q., and Bennett, V. (1990) J. Biol. Chem. 265, 17252-17256
26. Levinson, S. R., Curatolo, C. J., Reed, J. K., and Raftery, M. (1979) Anal. Biochem. 99, 72-76
27. Ariyasu, R. G., Nichol, J. A., and Ellisman, M. H. (1985) J. Neurosci. 5, 2581-2596
28. Wharton, K., Johansen, K., Xu, T., and Artavanis-Tsakonas, S. (1985) Cell 43, 567-581
29. Greenwald, I. (1985) Cell 43, 583-590
30. Thompson, C. C., Brown, T. A., and McKnight, S. L. (1991) Science 253, 762-768
31. LaMarco, K. L., Thompson, C. C., Byers, B. P., Walton, E. M., and McKnight, S. L. (1991) Science 253, 789-792
32. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660-672