A β-Spectrin Isoform from Drosophila (βH) Is Similar in Size to Vertebrate Dystrophin

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Abstract. Spectrins are a major component of the membrane skeleton in many cell types where they are thought to contribute to cell form and membrane organization. Diversity among spectrin isoforms, especially their β subunits, is associated with diversity in cell shape and membrane architecture. Here we describe a spectrin isoform from Drosophila that consists of a conventional α spectrin subunit complexed with a novel high molecular weight β subunit (430 kD) that we term βH. The native αβH molecule binds actin filaments with high affinity and has a typical spectrin morphology except that it is longer than most other spectrin isoforms and includes two knoblike structures that are attributed to a unique domain of the βH subunit. βH is encoded by a different gene than the previously described Drosophila β-spectrin subunit but shows sequence similarity to β-spectrin as well as vertebrate dystrophin, a component of the membrane skeleton in muscle. By size and sequence similarity, dystrophin is more similar to this newly described β-spectrin isoform (βH) than to other members of the spectrin gene family such as α-spectrin and α-actinin.

The membrane skeleton of eukaryotic cells is made up of a specialized subset of cytoskeletal proteins that contribute to membrane structure and in some cases link the membrane to the transcellular cytoskeleton. Spectrins have been identified at the plasma membrane of many eukaryotic cell types and spectrin molecules can be categorized according to their structure, composition, and distribution (Moon and McMahon, 1987; Coleman et al., 1989). In most cases, the α and β subunits of spectrin associate laterally to form heterodimers that in turn associate head-to-head to form tetramers. In vertebrates, there are three major classes of spectrin molecules: erythroid, nonerythroid, and brush border (TW260/240). In chicken, these three classes all share a common α-spectrin subunit (Glennay et al., 1982a), whereas in mammals diversity is generated by multiple α- and β-spectrin subunits (Leto et al., 1988; Birkenmeier et al., 1988). Nonerythroid spectrins (also known as fodrins) have a broad tissue distribution both in vertebrates (Bennett, 1985) and in invertebrates (Pesacreta et al., 1989) and probably play a fundamental role at the plasma membrane. In contrast, the erythroid and brush border spectrins appear to be specialized isoforms since they have a restricted tissue distribution and substantial size differences in their β subunits when compared to nonerythroid spectrin.

The function of the membrane skeleton is best characterized in the human erythrocyte where it is thought to be responsible for maintenance of cell shape and membrane stability (Bennett, 1985; Elgsaeter et al., 1986). Defects in the protein components of the membrane skeleton (including spectrin, ankyrin, and band 4.1; Davies and Lux, 1989) have been correlated with anemias in humans and mice that are accompanied by membrane fragility and alterations in cell shape. Less is known about membrane skeleton function in nonerythroid cells, but, in skeletal muscle, defects in the protein dystrophin have been identified as the cause of Duchenne and Becker muscular dystrophies (Hoffman et al., 1987). Dystrophin is similar in some aspects of its sequence to spectrin and is normally found associated with the plasma membrane (Koenig et al., 1988; Arahata et al., 1988). Therefore, dystrophin is thought to play an important mechanical or structural role at the sarcolemma, analogous to the role of spectrin in the erythrocyte (Mandel, 1989; Monaco, 1989).

We have purified and partially characterized a spectrin from Drosophila that is structurally similar to other spectrins except that its β subunit is extraordinarily large (430 kD). We named this high molecular mass subunit βH. We have cloned a partial cDNA corresponding to βH (mRNA = 13 kb) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kb) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kb) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kb) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kb) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kb) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to βH (mRNA = 13 kD) and found that its predicted protein sequence is similar to the previously described Drosophila β-spectrin (Dubreuil et al., 1987).
trophin that may occupy a similar niche in the invertebrate membrane skeleton.

Materials and Methods

Maintenance of Cell Lines and Protein Purification

Ko cells were grown in large spinner flasks in M3 medium containing serum or in D2 medium without serum (Sang, 1981). Cells were harvested at a density of 10^7/ml by centrifugation. Spectrin purification was carried out as previously described (Dubreuil et al., 1987) but with the following modifications. The soluble fraction of cell lysates was applied to a DEAE Sephadex column in buffer with 0.15 M KCl and eluted with a single step increase to 0.35 M KCl. After Sepharose 4B and phosphocellulose chromatography, the two \( \alpha \) spectrin-containing fractions were separately pooled and concentrated by DEAE Sephadex chromatography as above.

SDS-PAGE, Nitrocellulose Blots and Immunoprecipitation

Protein samples were processed and separated on polyacrylamide gels containing 5% acrylamide and 0.08% bisacrylamide as previously described (Dubreuil et al., 1987). Denaturing gels of Coomassie blue-stained gels were done with a GS300 apparatus (Hoefer Scientific Instruments, San Francisco, CA).

For blotting experiments, proteins were transferred to nitrocellulose overnight at 12 V/cm as previously described (Dubreuil et al., 1987). Afterward, blots were stained with Ponceau S to monitor the efficiency of transfer and then blocked in TBS-Tween with 5% newborn calf serum (10 mM Tris-HCl, pH 7.5; 0.15 M KCl, 0.1% Tween 20). Antibodies used included rabbit anti-Drosophila \( \beta \)-spectrin (antibody 89; Byers et al., 1989), anti-Drosophila \( \alpha \)-spectrin (antibody 905; Byers et al., 1987), an antibody that reacts with \( \alpha \)-spectrin and \( \beta \)-spectrin (675; Dubreuil et al., 1987; Byers et al., 1987) and an antidiystrophin antibody (Koenig and Kunkel, 1990). Incubations were carried out for 2 h at room temperature at the indicated dilutions except for antidiystrophin antibody that was incubated overnight at 4°C. Antibodies 89, 905, and 675 were detected with an alkaline phosphatase conjugated anti-sheep antibody (Cappel Laboratories, Malvern, PA) using 4-chloro-l-napthol as substrate.

Binding of the fusion protein to the blot was detected using the method of Olmsted (1981).

Results

Purification of Spectrin and Comparison with Nonerythroid Spectrins and Dystrophin

We identified two distinct populations of spectrin from cultured cells of Drosophila. One of these, conventional \( \alpha \beta \)-spectrin, was previously purified from a soluble fraction of S3 cell lysates by DEAE Sephadex chromatography, gel filtration, and phosphocellulose chromatography (Dubreuil et al., 1987). Using essentially the same purification steps, we found a second isofrom of spectrin that was more prevalent in Ko cells than in S3. The two spectrin isoforms were fractionated through anion exchange and gel filtration, but were separated during phosphocellulose chromatography (Fig. 1A). \( \alpha \beta \)-spectrin (Fig. 1A, lanes 1–3) eluted from phosphocellulose at a position distinct from a second peak of \( \alpha \)-spectrin that co-eluted with a high molecular mass polypeptide (Fig. 1A, lanes 6–8). Since \( \alpha \)-spectrin alone does not bind to phosphocellulose (Dubreuil et al., 1987), we assumed that it was associated with the high molecular mass protein that in turn bound to the column. Antiserum 675, directed against a constellation of high molecular mass actin binding proteins from Drosophila (Byers et al., 1987), was used to stain both total Ko cell proteins (Fig. 1B, lane 1) and purified \( \alpha \beta \)-spectrin (Fig. 1C, lane 2) that had been transferred to nitrocellulose from an SDS gel. The antiserum reacted primarily with \( \alpha \)-spectrin and the high molecular weight protein.

The high molecular weight polypeptide appeared to be a \( \beta \)-spectrin by the following criteria. (a) In stained SDS gels of purified \( \alpha \beta \)-spectrin the ratio of integrated Coomassie blue stain of the high molecular mass protein to \( \alpha \)-spectrin with 200 \( \mu \)g/ml actin in 0.15 M KCl and 2 mM MgCl\(_2\). After incubation for 90 min at room temperature, the actin–spectrin mixture was diluted with 57% glycerol in actin polymerization buffer (Dubreuil et al., 1987) to a final glycerol concentration of 40% and then sprayed and replicated as above. Control actin filaments were processed and examined as above but without spectrin.

Actin sedimentation assays were carried out as previously described (Dubreuil et al., 1987). 21 nM spectrin (or 42 nM free ends) was mixed with 200 \( \mu \)g/ml actin and 2 mM MgCl\(_2\) at a final KCl concentration of 0.11 M. After 60 min at room temperature the mixtures were sedimented in a airfuge (Beckman Instruments, Inc., Palo Alto, CA), at 150,000 g for 30 min. Parallel samples were processed without actin. Pellets and supernatants from 120 \( \mu \)l reactions were solubilized in equal volumes of SDS sample buffer and analyzed on Coomassie blue–stained SDS gels.

Isolation and Characterization of cDNA

\( \beta \)-cDNA was isolated in a previous screen of a fly head cDNA library (Byers et al., 1987). The 5-kb insert was excised from the lambda vector with Eco RI and subcloned into Bluescript. Because the insert contained an internal Eco RI site, the insert was subcloned as a 4-kb fragment and a 1-kb fragment for DNA sequencing. The complete 5-kb fragment was also subcloned and used to confirm the Eco RI junction and as the probe in Northern blots. Northern blots were prepared from Drosophila Ko cell poly A+ RNA using standard methods (Sambrook et al., 1989).

DNA sequencing was carried out as previously described (Kiehart et al., 1989). Nested deletions of the above subclones were sequenced on both strands using single-stranded or double-stranded DNA. The overlap was confirmed by sequencing a third subclone that spanned the internal Eco RI site. Sequence data was assembled and analyzed using the CGC program (Devereux et al., 1984). Dotplot comparisons were done using the program Compare at a stringency of 20 identical matches/window of 100 residues with the all switch, and were displayed using the DotPlot program. GenBank was searched using the program TFASTA.
Figure 1. Purification and properties of two Drosophila spectrin isoforms. (A) Phosphocellulose separates spectrin into two populations. The soluble fraction of a Drosophila Kc0 cell lysate was chromatographed on DEAE Sephadex and Sepharose CL-4B before being applied to phosphocellulose. Successive phosphocellulose fractions were analyzed on a Coomassie blue-stained SDS gel. Spectrin was separated into two peaks containing αβ-spectrin (lanes 1–3) or α-spectrin together with a high molecular mass polypeptide (βH, lanes 6–8). Molecular mass markers are shown to the left in kilodaltons. (B) Interaction between the high molecular mass protein (βH) and α-spectrin. A Drosophila Kc0 cell homogenate was transferred to nitrocellulose and probed with the indicated antibodies. (Lane 1) Anti-α and βH (675); (lanes 2, 4, and 5) anti-α (905); (lane 3) anti-β (89); (lane 6) affinity-purified anti-β, (see text for details). Lanes 4 and 5 were also incubated with an α-spectrin fusion protein (pBS9AB, 0.1 and 10 μg/ml, respectively). (C) The high molecular mass protein is similar in size to dystrophin. Mouse muscle membranes (lane 1), purified αβ-spectrin (lane 2) and purified TW260/240 (lane 3) were transferred from an SDS gel to nitrocellulose and stained with either affinity-purified antidystrophin (lane 1) or antibody 675 (lanes 2 and 3). Antibody 675 recognizes both subunits of TW260/240.

was 1.7:1 indicating a mole ratio of 1.1:1 (assuming the molecular mass of βH to be 430 kD). (b) It reacted with an α spectrin fusion protein in a blot overlay assay designed to detect the subunit interactions between α- and β-spectrin (Byers et al., 1987; Fig. 1 B). Thus, in blot overlays of total Kc0 cell proteins, antibody against α-spectrin stained only α-spectrin in a control blot strip (Fig. 1 B, lane 2) but stained β-spectrin and the high molecular mass polypeptide, in addition to α-spectrin (Fig. 1 B, lanes 4 and 5) in strips preincubated with a purified α-spectrin fusion protein. (c) A monoclonal anti-α-spectrin antibody (M10-2; Dubreuil et al., 1987) that does not react with βH-spectrin on blots coprecipitated the high molecular mass polypeptide from soluble fractions of the purified protein (not shown). Coprecipitation was dependent on the α-spectrin-specific antibody and α-spectrin alone was immunoprecipitated in the presence of 0.1% SDS. (d) The predicted amino acid sequence of the high molecular mass polypeptide was 34% identical to the sequence of a previously identified Drosophila β- spectrin (described below). Based on these criteria, we designated the high molecular weight polypeptide as βH to distinguish it from the previously described Drosophila β-spectrin (Dubreuil et al., 1987; Byers et al., 1989).

The βH polypeptide differed from Drosophila β-spectrin in its immunological reactivity and its size. First, antibodies against the two β isoforms did not cross-react significantly on nitrocellulose blots of total Kc0 cell proteins (Fig. 1 B). Polyclonal antibody 675 (Fig. 1 B, lane 1) and affinity-purified βH-specific antibodies (Fig. 1 B, lane 6) recognized βH-spectrin but did not react with β-spectrin. Conversely, polyclonal antibody 89 (Fig. 1 B, lane 3) recognized β-spectrin (Byers et al., 1989) but did not recognize βH spectrin. Second, on SDS polyacrylamide gels, the Drosophila βH subunit (Fig. 1 C, lane 2) was approximately the same size as human dystrophin (Fig. 1 C, lane 1; 427 kD). For comparison, the TW260 subunit of chicken brush border spectrin migrated at an intermediate position between βH- and α-spectrin (Fig. 1 C, lane 3) with an Ms of 350 kD. Its size was estimated from a standard curve using proteins of known sequence as standards (myosin, α-spectrin, and dystrophin) and is much larger than previous estimates (260 kD; Glenney et al., 1982b).
Structure of αβ₃⁻Spectrin

The αβ₃ molecule appeared as two intertwined strands by electron microscopy of rotary replicated specimens (Fig. 2), but αβ₃-spectrin was considerably longer (contour length of 248 ± 12 nm versus 192 nm) and more loosely intertwined than αβ-spectrin from Drosophila and vertebrates (Dubreuil et al., 1987; Glenney et al., 1982b; Bennett et al., 1982). By these criteria, αβ₃-spectrin resembled TW260/240, the spectrin isoform found in the chicken brush border membrane (Glenney et al., 1982b; Coleman et al., 1989). The αβ₃ molecule was distinguished from TW260/240 by the presence of two large globular structures, or knobs, that were often found connected to the intertwined strands near the center of the tetramer. In addition, αβ₃ molecules were often found in complexes of two or more tetramers (Fig. 2, bottom right) associated with one another in the region of the knobs. The orientation of tetramers in these complexes ranged from parallel to a cross pattern (not shown). Some molecules with the expected properties of αβ₃ heterodimers were also identified (Fig. 3). These molecules were partially double stranded with a single strand extension that often terminated in a knob structure. The average contour length of these putative dimers was 158 nm ± 10 (n = 13), which is greater than one half the tetramer length.

cDNA Cloning and Partial Sequence of β₃⁻Spectrin

A cDNA clone encoding part of the β₃ sequence was recovered from a fly head cDNA library in lambda gt11. Antibody 675, which recognizes β₃- and α-spectrin, identified a single β₃ cDNA among a large number of Drosophila α-spectrin cDNAs (Byers et al., 1987). This cDNA (lambda-β₃) did not cross hybridize with α-spectrin cDNAs (not shown) and a fusion protein product of the cDNA could be used to affinity purify β₃-specific antibodies from antisera 675 (Fig. 1 B, lane 6). The cDNA probe detected a 13-kb transcript in Northern blots of Kc63 cells (Kiehart and Feghali, 1986). The cDNA probe hybridized with a 9-kb transcript in polytene chromosomes using the β₃ cDNA probe (not shown). For comparison, the Drosophila β spectrin gene is located at position 16C5 and the α-spectrin gene is located at position 62B (Byers et al., 1987, 1989).

The cDNA sequence of the 5-kb clone lambda-β₃ included a single long open reading frame of 1,645 amino acids. The methionine codon at position 18 is preceded by a short nonrepetitive sequence midway through the β₃ subunit. The gene for β₃-spectrin was localized to chromosomal position 63C,D by in situ hybridization to polytene chromosomes (Byers et al., 1987, 1989). DNA sequence comparison indicated that like β-spectrin, the β₃ polypeptide could be subdivided into repetitive and nonrepetitive segments (Fig. 5 A). The most striking similarity between the two β-spectrin isoforms occurred at their amino termini (bracket). The same region was also highly conserved between β₃-spectrin and dystrophin (Fig. 5 C, bracket). The major line of similarity between β₃- and β-spectrin extended from the amino terminal region through the repetitive region of the β₃ chain, which suggests that the two repetitive regions had a common evolutionary origin. This line of similarity was interrupted by a short nonrepetitive sequence midway through the β₃ se-
Figure 4. Interaction of spectrin with F-actin. (A) Purified *Drosophila* spectrins were sedimented in the presence (lanes 1, 2, 5, and 6) and absence (lanes 3, 4, 7, and 8) of F-actin. Pellets and supernatants were solubilized in SDS sample buffer and analyzed on Coomassie blue-stained SDS gels. (Lanes 1-4) \( \alpha \beta \)-Spectrin. (Lanes 5-8) \( \alpha \beta \)-Spectrin. The actin visible in lane 7 was a carry over from lane 6 during gel loading. Molecular mass standards are shown to the left in kilodaltons. (B) F-actin complexes with \( \alpha \beta \)-spectrin were rotary replicated and viewed by electron microscopy (see text for details). Sp, \( \alpha \beta \)-spectrin; Act, filamentous actin.

The repetitive regions of both \( \beta \) and \( \beta_n \) showed a periodicity of \( \sim \)106 residues and were related to the repetitive domains of *Drosophila* \( \alpha \)-spectrin as well (Fig. 5 B; Dubreuil et al., 1989). In contrast, there was only limited similarity between the repetitive region of \( \beta_n \) and the spectrin-like repeats of dystrophin (Koenig et al., 1988; Fig. 5 C).

A sequence register of \( \beta_n \)-spectrin segments was assembled using the repeat boundaries that were determined in dot-plots (Fig. 6). Segment 1 closely resembled the putative ac-
Figure 5. Dotplot comparisons of β₈-spectrin with other spectrins and dystrophin. The predicted polypeptide sequence of the 5-kb β₈-spectrin cDNA fragment was compared to the corresponding region of Drosophila β-spectrin (Byers et al., 1989) to full-length Drosophila α-spectrin (Dubreuil et al., 1989) and to dystrophin (Koenig et al., 1988) at a stringency of 20 identical matches/100 residue window. Brackets mark the conserved amino-terminal actin-binding region shared by β-spectrins and dystrophin. Arrows in A mark the line of greatest similarity between the two proteins. Each sequence is shown diagrammatically along the plot axes.

Figure 6. The amino acid sequence of β₈-spectrin. The partial polypeptide sequence of β₈ was aligned in a register of repetitive segments and compared to the consensus of Drosophila α-spectrin repeats. Amino acid positions are indicated to the right and segment numbers (underlined residues) in each repetitive segment is shown in parentheses. The alignment of nonrepetitive segments 1 and 7 within the register is arbitrary. An eight residue sequence was removed from segment 3 to the left. The number of consensus matches and compared to the consensus of u-spectrin repetitive segments (Dubreuil et al., 1989) to full-length Drosophila α-spectrin (Dubreuil et al., 1989) and most were 106 residues in length. The α-spectrin consensus consists of 54 positions in the 106
residue repeat unit that are conserved in at least 9 of the 18 α-spectrin repeats. On average, the βn-segments included 28 of the 54 consensus residues (α-spectrin repeats include an average of 37 of the 54 consensus residues). Segments 2 and 3 of βn also resembled the α-spectrin consensus, but were more closely related to segments 2 and 3 of β-spectrin and α-actinin (Byers et al., 1989) than to the α-spectrin repeats. Overall, the polypeptide sequences of βn-segments 1-14 and β-spectrin segments 1-13 (Byers et al., 1989; and our unpublished sequence) were 34% identical. βn-segment 7 was excluded from the comparison and additional gaps in the alignment were considered to be nonidentical positions.

By sequence alignments and dotplots (Fig. 5), segment 7 appeared to be an insertion in βn-spectrin relative to β-spectrin: it did not match the repeat consensus and appeared to be unique to βn. When this region of βn was used to search translated GenBank sequences, it showed homology to a sequence motif (Fig. 6, underlined sequence in segment 7) that was originally found to be shared by segment 10 of α-spectrin, certain nonreceptor protein kinases and phospholipase c (Lehto et al., 1988). Each αβn-spectrin tetramer thus includes four copies of this sequence, one in each α and β subunit. Similar sequences have also been found in a number of other cytoskeletal and membrane-associated proteins (Drubin et al., 1990).

Discussion

Membrane Skeleton Diversity Is Combinatorial

We have shown that Drosophila expresses an unusually large βn-spectrin subunit that interacts with α-spectrin to form a native molecule that resembles other nonerythroid spectrins. The αβn-spectrin molecule differs from the previously described αβ-spectrin from Drosophila in several ways, including length, actin-binding affinity and details of its structure. Indeed, the extraordinary size of the βn subunit is more similar to the size of dystrophin than to previously described spectrins.

Immunofluorescent localization of α-spectrin (present in both spectrin isoforms) within developing Drosophila embryos showed that spectrin is associated with the plasma membrane of nearly all cell types (Pesacreta et al., 1989). Using antibody 675 in Western blots, we have found that there is little or no βn detectable in fly heads (Byers et al., 1987), but it is abundant in the adult abdomen and thorax (our unpublished observation). We speculate that the two spectrin isoforms differ in function and may be targeted to different membrane anchors or may be differentially expressed. The additional polypeptide bands present in our purified αβ-spectrin fractions are likely to be overlapping βn-spectrin and its breakdown products since these bands react with antibody 675 and the lower molecular weight bands increase at the expense of βn during storage. However, we cannot exclude the presence of additional β-spectrin isoforms in Drosophila.

Variation in the β subunit modulates at least three properties of the spectrin tetramer. In chicken the protein 4.1 sensitivity of the spectrin-actin interaction, the interaction with the membrane through ankyrin, and the length of the spectrin tetramer all vary independently of the α subunit (Coleman et al., 1989; Moon and McMahon, 1987). Here we show that the length of the Drosophila spectrin tetramer depends on whether it includes a 430- or a 270-kD β subunit (protein 4.1 and ankyrin have not been identified in Drosophila). Glenney and Glenney (1984) first pointed out that an increase in the length of the β subunit could account for the difference in length between TW260/240 and other spectrins that share the same α subunit. We also find a correlation between β subunit mass and the length of the Drosophila spectrin tetramer, although there may be a domain in βn-spectrin that does not contribute to the end to end tetramer length. βn is 80 kD larger than the TW260 subunit, even though the lengths of αβn and TW260/240 are both ~250 nm.

The αβn-Spectrin Knobs May Represent a Carboxy-Terminal Domain of βn

If the length of the spectrin tetramer is controlled by a change in length of only the β subunit, then the dimer–dimer interface must be staggered in αβn-spectrin relative to αβ-spectrin (Fig. 7 A). A staggered interface may serve to promote interaction between like heterodimers and prevent the assembly of mixed tetramers containing both αβn and αβ heterodimers. The knobs in αβn appear to mark the termini of the longer subunit near the middle of the native molecule, suggesting that the αβn-spectrin dimer should be more than half of the length of the tetramer and that it should include a double stranded region (corresponding to the length of α-spectrin) and a single strand extension (corresponding to the carboxy-terminal extension of βn). TW260/240 (Fig. 7 B), the only other known spectrin in the size class of αβn, lacks the knob structures (Glenney et al., 1982b; Pearl et al., 1984; Coleman et al., 1989). Moreover, the molecular weight of TW260 is intermediate to that of βn and β-spectrins. We speculate that the TW260 subunit is greater in mass than other β subunits because it includes sequences that increase the length of spectrin, but it is smaller in mass than βn-spectrin because it lacks a knob at its carboxy terminus. This interpretation accounts for the presence of knobs in αβn but not TW260/240; for the difference in molecular weight between their β subunits; and for the similar length of the two native molecules. Each of these points is testable through sequence comparisons (once the sequence of TW260 becomes available) and through epitope mapping by electron microscopy. The shorter isoforms of αβ-spectrin (Fig. 7 C) also include knobs (Fig. 2; Dubreuil et al., 1987; Glenney et al., 1982c; Davis and Bennett, 1984; Tyler et al., 1979), but they are smaller than the αβn knobs and their mode of attachment to the strads of the spectrin tetramer is not apparent.

βn-Spectrin and Dystrophin Are Similar Proteins

Members of the ABP and spectrin superfamilies all share an amino-terminal nonrepetitive sequence motif that is thought to confer actin-binding activity (Byers et al., 1989; Davison et al., 1989; Noegel et al., 1989). α-Actinin and spectrin have been examined by electron microscopy and were shown to consist of antiparallel dimers (or tetramers in the case of spectrin) in which the actin-binding domain is displayed at each end of the elongated native molecule (Cohen et al., 1980; Wallraff et al., 1986). The rest of the sequence of each monomer consists primarily of sequence repeats that fall into one of two categories. Spectrins, α-actinins and dystrophin...
likely arose through the multiplication of an α helical ancestral segment (Davison et al., 1989; Dubreuil et al., 1989; Koenig et al., 1988) whereas Dictyostelium ABP-120 arose through multiplication of a cross-β sheet ancestor (Noegel et al., 1989). Thus, two or more classes of extended actin cross-linking proteins have evolved using a common amino terminal actin binding domain.

In addition to their sequence similarities, spectrin and dystrophin are thought to be functionally related. First, they are extended molecules that probably cross-link actin filaments at a much greater distance than proteins such as α-actinin and ABP-120. Second, the latter two proteins are found in the cytoplasm (Condeelis et al., 1988) whereas spectrin and dystrophin are largely confined to the membrane skeleton through their interactions with integral membrane proteins (Coleman et al., 1989; Campbell and Kahl, 1989). Through its interactions with the plasma membrane and other peripheral proteins of the erythrocyte membrane skeleton, spectrin appears to be responsible for some of the mechanical properties of the erythrocyte membrane (Elgsaeter et al., 1986). Nonerythroid spectrins and dystrophin are also thought to modify membrane properties (e.g., stability, elasticity, and shape) either at a global level, as in the erythrocyte, or in a polarized manner such as in epithelial cells and neurons (Lazarides et al., 1984; Nelson and Veshnock, 1987).

There are also significant differences between βn-spectrin and dystrophin. First, β-spectrins generally occur as complexes with α-spectrins. Dystrophin has yet to be purified under nondissociative conditions, but no evidence to date suggests that dystrophin interacts with another protein such as α-spectrin (Campbell and Kahl, 1989; Chang et al., 1989). There is an example of β-spectrin function independent of α-spectrin that may be analogous to the function of dystrophin. Bloch et al. (1989) have shown that α-spectrin is absent from acetylcholine receptor clusters at the neuromuscular junction and suggest that β-spectrin alone may be important for receptor clustering. Second, the consensus sequence of the dystrophin repetitive segments differs substantially from the consensus sequence of α-spectrin repeats (Koenig and Kunkel, 1990), although they probably arose from a common ancestor. If dystrophin evolved from a β-spectrin ancestor, then it has accumulated substantial changes in its sequence. Alternatively, dystrophin may represent a branch that occurred early in the evolution of spectrin-like proteins in which case the present similarities between dystrophin and βn-spectrin are the result of convergent evolution. If so, then it is interesting that two separate paths of evolution have produced proteins with remarkably similar features.

Given the similarities between βn-spectrin and human dystrophin, it seems likely that they occupy a similar niche in the eukaryotic membrane skeleton. βn-Spectrin may also be related to the recently described autosomal homologue of dystrophin (Love et al., 1989). In the future, sequence comparisons between the carboxy terminal region of βn-spectrin and dystrophins should illuminate the precise evolutionary relationship between these molecules. In any case, an understanding of the function of the αβn molecule may reveal important functional properties of the native dystrophin molecule.

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References

Arahata, K., S. Ishiura, T. Ishiguro, T. Tsukahara, Y. Suhara, C. Eguchi, T. Ishihara, I. Nonaka, E. Ozawa, and H. Sugita. 1988. Immunostaining of skeletal and cardiac muscle membrane with antibody against Duchenne muscular dystrophy peptide. Nature (Lond.), 333:861–863.

Bennett, V. 1985. The membrane skeleton of human erythrocytes and its implication for more complex cells. Annu. Rev. Biochem. 54:273–304.

Bennett, V., J. Davis, and W. E. Fowler. 1982. Brain spectrin, a membrane-associated protein related in structure and function to human erythrocyte spectrin. Nature (Lond.), 299:126–131.

Birkenmeier, C. S., E. C. McFarland-Starr, and J. E. Barker. 1988. Chromosomal location of three spectrin genes: relationship to the inherited hemolytic anemia of mouse and man. Proc. Natl. Acad. Sci. USA. 85:8121–8125.

Bloch, R. J., and J. S. Morrow. 1989. An unusual beta-spectrin associated with clustered acetylcholine receptors. J. Cell Biol. 108:481–493.

Byers, T. J., R. R. Dubreuil, D. Branton, D. P. Kiehart, and L. S. B. Goldstein. 1987. Drosophila spectrin. I. Conserved features of the alpha-subunit are revealed by analysis of cDNA clones and fusion proteins. J. Cell Biol. 105:2103–2110.

Byers, T. J., A. Husain-Chishti, R. R. Dubreuil, D. Branton, and L. S. B. Goldstein. 1989. Drosophila beta-spectrin: sequence similarity to the amino-terminal domain of alpha-actinin and dystrophin. J. Cell Biol. 109:1633–1641.

Campbell, K. P., and S. D. Kahl. 1989. Association of dystrophin and an integral membrane glycoprotein. Nature (Lond.). 338:259–262.

Cavener, D. R. 1987. Comparisons of the consensus sequence flanking translational sites in Drosophila and vertebrates. Nucleic Acids Res. 15:1353–1361.

Chang, H. W., E. Bock, and E. Bonilla. 1989. Dystrophin in electric organ of Torpedo californica homologous to that in human muscle. J. Biol. Chem. 264:20831–20834.

Cohen, C. M., J. M. Tyler, and D. Branton. 1980. Spectrin-actin associations...
studied by electron microscopy of shadowed preparations. Cell. 21:875-883.
Cotman, C. W., D. Fishkind, M. S. Mooseker, and J. S. Morrow. 1989. Functional diversity among spectrin isoforms. Cell Motil. Cytoskeleton. 12: 225-247.
Condeelis, J., A. Hall, A. Brennich, V. Warren, R. Hock, H. Bennett, and S. Ogihara. 1988. Hereditary disorders of the red cell membrane skeleton. Hum. Genet. Dis. 5:222-227.
Davis, J., and V. Bennett. 1984. Brain ankyrin. J. Biol. Chem. 259:13550-13559.
Davison, M. D., M. D. Baron, D. R. Critchley, and J. C. Wootton. 1989. Comparison of nonerythroid alpha-spectrin genes reveals strict homology among diverse species. Mol. Cell Biol. 8:1-9.
Drubin, D. G., J. Mulholland, Z. Zhu, and D. Botstein. 1990. Homology of the yeast actin-binding protein to signal transduction proteins and myosin-I. Nature (Lond.). 343:289-290.
Dubreuil, R., T. J. Byers, D. Branton, L. S. B. Goldstein, and D. P. Kiehart. 1987. Drosophila spectrin I. Characterization of the purified protein. J. Cell Biol. 105:2095-2102.
Dubreuil, R. R., T. J. Byers, A. L. Sillman, D. Bar-Zvi, L. S. B. Goldstein, and D. Branton. 1989. The complete sequence of Drosophila alpha spectrin: conservation of structural domains between alpha spectrins and alpha actin. J. Cell Biol. 109:2197-2206.
Elgersma, A., A. Stokke, A. Mikkelson, and D. Branton. 1986. The molecular basis of erythrocyte shape. Science (Wash. DC). 234:1217-1223.
Fryberg, E., M. Kelly, E. Ball, C. Fryberg, and M. C. Reedy. 1990. Molecular genetics of sarcomeric Drosophila alpha-actinin: mutant alleles disrupt Z-disc integrity and muscle insertions, but do not affect nonmuscle tissues. J. Cell Biol. 110:1999-2011.
Glenney, J. R., and P. Glenney. 1984. Comparison of spectrin isolated from erythrocytes and non-erythroid sources. Eur. J. Biochem. 144:529-539.
Glenney, J. R., P. Glenney, and K. Weber. 1982a. Erythroid spectrin, brain fodrin, and intestinal brush border proteins (TW-260/240) are related molecules containing a common calmodulin-binding subunit bound to a variant cell type-specific subunit. Proc. Natl. Acad. Sci. USA. 79:4002-4005.
Glenney, J. R., P. Glenney, M. Osborn, and K. Weber. 1982b. An F-actin and calmodulin-binding protein from isolated intestinal brush borders has a morphology related to spectrin. Cell. 28:843-854.
Glenney, J. R., P. Glenney, and K. Weber. 1982c. F-actin-binding and cross-linking properties of porcine brain fodrin, a spectrin-related molecule. J. Biol. Chem. 257:9781-9787.
Hoffman, E. P., R. H. Brown, Jr., and L. M. Kunkel. 1987. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell. 51:919-928.
Kiehart, D. P., and R. Feghali. 1986. Cytoplasmic myosin from Drosophila melanogaster. J. Cell Biol. 103:1517-1525.
Kiehart, D. P., M. S. Lutz, D. Chan, A. S. Ketchum, R. A. Laymon, B. Nguyen, and L. S. B. Goldstein. 1989. Identification of the gene for fly non-muscle myosin heavy chain: Drosophila myosin heavy chains are encoded by a gene family. EMBO (Eur. Mol. Biol. Organ.) J. 8:913-922.
Koenig, M., and L. M. Kunkel. 1990. Detailed analysis of the repeat domain of dystrophin reveals 4 potential hinge segments which may confer flexibility. J. Biol. Chem. 265:4560-4566.
Koenig, M., A. P. Monaco, and L. M. Kunkel. 1988. The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. Cell. 53:219-228.
Lazarides, E., W. J., Nelson, and T. Kasamatsu. 1984. Segregation of two spectrin forms in the chicken optic system: a mechanism for establishing restricted membrane-cytoskeleton domains in neurons. Cell. 36:269-278.
Leto, V. P., V. M. Wiesenius, P. Salven, and M. Saraste. 1988. Transforming and membrane proteins. Nature (Lond.). 334:388.
Lett, T. L., D. Fortuno-Erikson, D. Barton, T. L. Yang-Feng, U. Francke, A. S. Harris, J. S. Morrow, V. T. Marchesi, and E. J. Benzer. 1988. Comparison of nonerythroid alpha-spectrin genes reveals strict homology among diverse species. Mol. Cell Biol. 8:1-9.
Love, D. R., D. F. Hill, G. Dickson, N. K. Spurr, B. C. Byth, R. F. Marsden, F. S. Walsh, Y. H. Edwards, and K. E. Davies. 1989. An autosomal transcript in skeletal muscle with homology to dystrophin. Nature (Lond.). 339:55-58.
Mandel, J. L. 1989. The gene and its product. Nature (Lond.). 339:584-586.
Monaco, A. P. 1989. Dystrophin, the protein product of the Duchenne/Becker muscular dystrophy gene. Trends Biochem. Sci. 14:412-415.
Moon, R. T., and A. P. McMahon. 1987. Composition and expression of spectrin-based membrane skeletons in non-erythroid cells. Bioessays. 7:159-164.
Nelson, W. J., and P. J. Veshnock. 1987. Modulation of fodrin (membrane skeleton) stability by cell-cell contact in Madin-Darby Canine Kidney epithelial cells. J. Cell Biol. 104:1527-1537.
Noegel, A. A., S. Rapp, F. Lottspeich, M. Schleicher, and M. Stewart. 1989. The Dicyostelium gelation-factor shares a putative actin-binding site with alpha-actinins and dystrophin and also has a rod domain containing six 100-residue motifs that appear to have a cross-beta conformation. J. Cell Biol. 109:607-618.
Olmdsted, J. B. 1981. Affinity-purification of antibodies from diazotized paper blots of heterogeneous protein samples. J. Biol. Chem. 256:11955-11957.
Pearl, M., D. Fishkind, M. Mooseker, D. Keene, and T. Keller. 1984. Studies on the spectrin-like protein from the intestinal brush border, TW-260/240, and characterization of its interaction with the cytoskeleton and actin. J. Cell Biol. 98:66-78.
Pesacreta, T. C., T. J. Byers, R. Dubreuil, D. P. Kiehart, and D. Branton. 1989. Drosophila spectrin: the membrane skeleton during embryogenesis. J. Cell Biol. 108:1697-1709.
Rimm, D. L., J. H. Sinard, and T. D. Pollard. 1989. Location of the head-tail junction of myosin. J. Cell Biol. 108:1783-1789.
Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Sang, K. H. 1981. Drosophila cells and cell lines. Adv. Cell Biol. 1:125-182.
Tyler, J. M., W. R. Hargreaves, and D. Branton. 1979. Purification of two spectrin-binding proteins: biochemical and electron microscopic evidence for site-specific reassociation between spectrin and bands 2.1 and 4.1. Proc. Natl. Acad. Sci. USA. 76:5192-5196.
Walraff, E., M. Schleicher, M. Moderstizki, D. Rieger, G. Isenberg, and G. Gerisch. 1986. Selection of Dicyostelium mutants defective in cytoskeletal proteins: use of an antibody that binds to the ends of alpha-actinin rods. EMBO (Eur. Mol. Biol. Organ.) J. 5:561-57.