A β-Spectrin Isoform from *Drosophila* (β_H) 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 β_H. The native $\alpha\beta_H$ 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 β_H subunit. β_H 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 (β_H) 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 (Glenney et al., 1982*a*), 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 β_H to distinguish it from the previously described *Drosophila* β-spectrin (Dubreuil et al., 1987). We have also cloned a partial cDNA corresponding to β_H (mRNA = 13 kb) and found that its predicted protein sequence is similar to the previously determined amino terminal actin-binding region of *Drosophila* β-spectrin (Byers et al., 1989). Thus, the *Drosophila* membrane skeleton is diversified through the assembly of a common α-spectrin subunit with either β-spectrin or the unusually large β_H subunit. In vertebrates, additional membrane skeleton diversity is achieved by the expression of spectrinlike proteins such as dystrophin and its autosomal homologue (Love et al., 1989). Based on the similarity in size and sequence between β_H-spectrin and dystrophin, we speculate that β_H-spectrin is a homologue of vertebrate dys-

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trophin that may occupy a similar niche in the invertebrate membrane skeleton.

Materials and Methods

Maintenance of Cell Lines and Protein Purification

Kc_0 cells were grown in large spinner flasks in M3 medium containing serum or in D22 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 α 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 8% acrylamide and 0.08% bisacrylamide as previously described (Dubreuil et al., 1987). Densitometric scans 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, pH 7.5; 0.15 M KCl, 0.1% Tween 20). Antisera used included rabbit anti-*Drosophila* β-spectrin (antibody 89; Byers et al., 1989), anti-*Drosophila* α-spectrin (antibody 905; Byers et al., 1987), an antibody that reacts with α-spectrin and β_H-spectrin (675; Dubreuil et al., 1987; Byers et al., 1987) and an antidystrophin antibody (Koenig and Kunkel, 1990). Incubations were carried out for 2 h at room temperature at the indicated dilutions except for antidystrophin antibody that was incubated overnight at 4°C. Antibodies 89, 905, and 675 were detected with an alkaline phosphatase conjugated anti-rabbit secondary antibody as previously described (Dubreuil et al., 1987). Antidystrophin was detected with a peroxidase-conjugated anti-sheep antibody (Cappel Laboratories, Malvern, PA) using 4-chloro-1-napthol as substrate.

Some blot strips were incubated with a carboxy terminal α-spectrin fusion protein (cDNA clone 9; Byers et al., 1987) expressed in the Bluescript (Stratagene, La Jolla, CA) cloning vector. This fusion protein includes a binding site for β-spectrin (Byers et al., 1987). The fusion protein was purified by ammonium sulfate precipitation and gel filtration under nondenaturing conditions. Binding of the fusion protein to the blot was detected with anti-α spectrin antibody, as described above.

To affinity purify β_H-specific antibodies, a 4-kb Eco RI fragment of the B_H-spectrin cDNA (see below) was cloned and expressed in pUCX (Rimm et al., 1989). Bacterial lysates containing the induced fusion protein were fractionated on SDS gels and transferred to nitrocellulose. After incubation of the blot with antiserum 675, affinity pure antibodies were recovered by the method of Olmsted (1981).

For immunoprecipitation, 2 μl of monoclonal antibody M10-2 ascites fluid (Dubreuil et al., 1987) or an irrelevant monoclonal antibody was mixed with 20 μl of protein A Sepharose (Sigma Chemical Co., St. Louis, MO) in RIPA buffer (10 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 0.05% NaN_3, 1% Triton X-100). After 1 h at 0°C, the beads were rinsed in excess RIPA buffer, pelleted at low speed, and resuspended in 100 μl RIPA buffer with 1 μg of spectrin in the presence or absence of 0.1% SDS. After 90 min, the beads were rinsed 4× in excess RIPA buffer. Bound proteins were solubilized in SDS sample buffer and analyzed on silver stained gels and on nitrocellulose blots probed with antibody 675 as above.

Electron Microscopy and Actin Cosedimentation

Parallel samples of both spectrin isoforms were diluted directly from the final DEAE eluate to a final concentration of 10–20 μg/ml. Samples were sprayed onto mica in 48% glycerol, 35 mM KCl, 1 mM Tris-HCl, pH 7.5, and were rotary replicated as previously described (Dubreuil et al., 1987) and viewed in a Phillips 301 electron microscope at 80 kV.

Actin-spectrin complexes were prepared by mixing 30 μg/ml spectrin with 200 μg/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 μg/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 an 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 μl reactions were solubilized in equal volumes of SDS sample buffer and analyzed on Coomassie blue-stained SDS gels.

Isolation and Characterization of cDNA

β_H 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* Kc_0 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 GCG 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.

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 isoform of spectrin that was more prevalent in Kc_0 cells than in S3. The two spectrin isoforms cofractionated through anion exchange and gel filtration, but were separated during phosphocellulose chromatography (Fig. 1 *A*). $\alpha\beta$-spectrin (Fig. 1 *A*, lanes *1–3*) eluted from phosphocellulose at a position distinct from a second peak of α-spectrin that co-eluted with a high molecular mass polypeptide (Fig. 1 *A*, lanes *6–8*). Since α-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 Kc_0 proteins (Fig. 1 *B*, lane *1*) and purified $\alpha\beta_H$-spectrin (Fig. 1 *C*, lane *2*) that had been transferred to nitrocellulose from an SDS gel. The antiserum reacted primarily with α-spectrin and the high molecular weight protein.

The high molecular weight polypeptide appeared to be a β-spectrin by the following criteria. (*a*) In stained SDS gels of purified $\alpha\beta_H$ spectrin the ratio of integrated Coomassie blue stain of the high molecular mass protein to α-spectrin

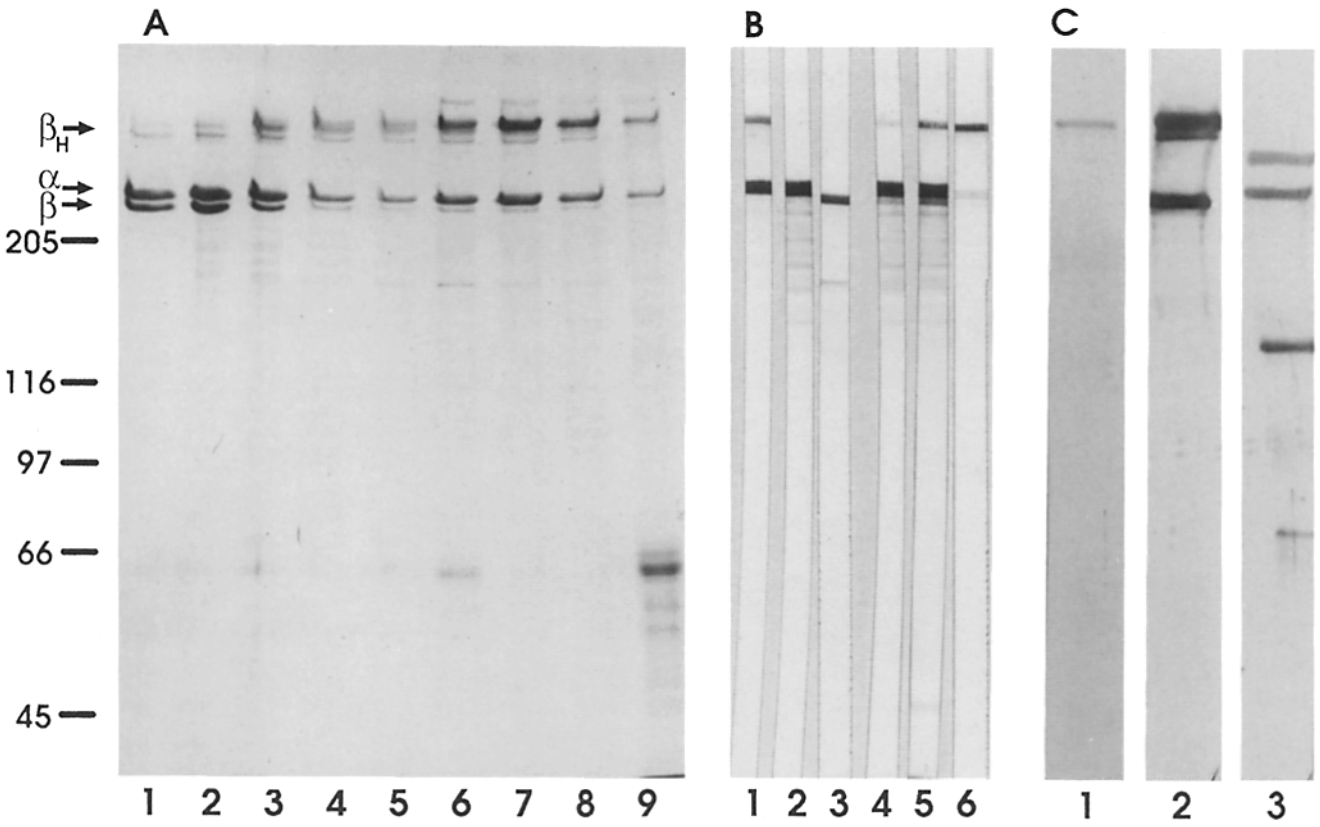
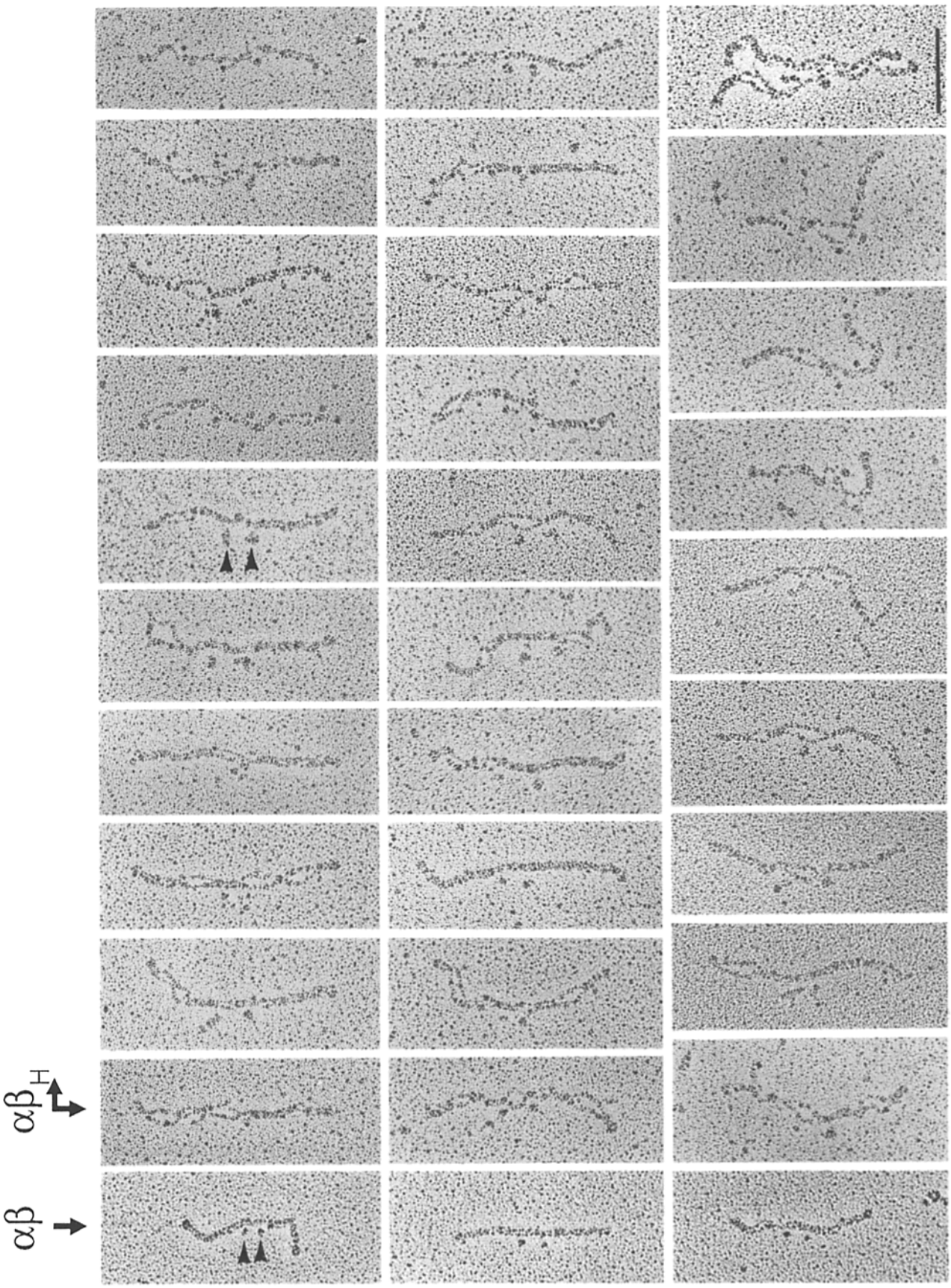


Figure 1. Purification and properties of two *Drosophila* spectrin isoforms. (*A*) Phosphocellulose separates spectrin into two populations. The soluble fraction of a *Drosophila* Kc_0 cell lysate was chromatographed on DEAE Sephadex and Sepharose CL-4B before being applied to phosphocellulose. Successive phosphocellulose fractions were analyzed on a Coomassie blue–stained SDS gel. Spectrin was separated into two peaks containing $\alpha\beta$-spectrin (lanes *1–3*) or α-spectrin together with a high molecular mass polypeptide (β_H, lanes *6–8*). Molecular mass markers are shown to the left in kilodaltons. (*B*) Interaction between the high molecular mass protein (β_H) and α-spectrin. A *Drosophila* Kc_0 cell homogenate was transferred to nitrocellulose and probed with the indicated antibodies. (Lane *1*) Anti-α and β_H (675); (lanes *2*, *4*, and *5*) anti-α (905); (lane *3*) anti-β (89); (lane *6*) affinity-purified anti-β_H (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 $\alpha\beta_H$-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 β_H 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 Kc_0 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 β_H-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 β_H to distinguish it from the previously described *Drosophila* β-spectrin (Dubreuil et al., 1987; Byers et al., 1989).

The β_H 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 Kc_0 cell proteins (Fig. 1 *B*). Polyclonal antibody 675 (Fig. 1 *B*, lane *1*) and affinity-purified β_H-specific antibodies (Fig. 1 *B*, lane *6*) recognized β_H-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 β_H spectrin. Second, on SDS polyacrylamide gels, the *Drosophila* β_H 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 β_H- and α-spectrin (Fig. 1 *C*, lane *3*) with an M_r 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., 1982*b*).


αβH
αβ

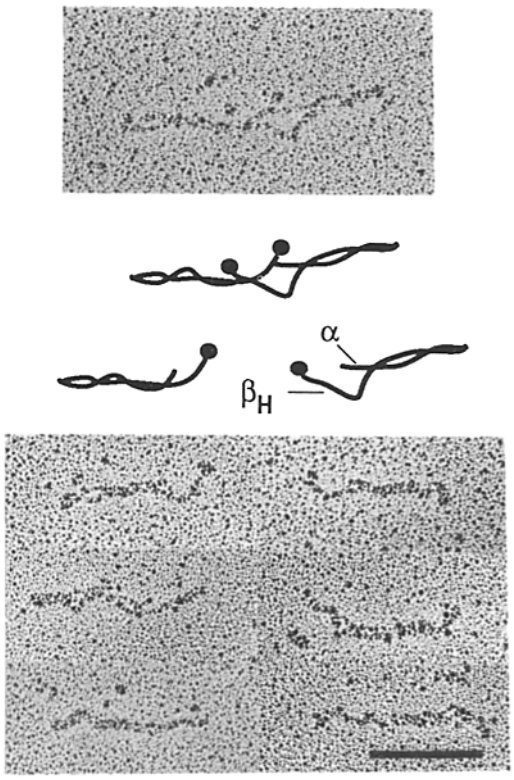


Figure 3. (*Upper*) A representative $\alpha\beta_H$-spectrin tetramer was traced and dissected to show the expected structure of $\alpha\beta_H$ heterodimers. (*Lower*) Putative heterodimers of $\alpha\beta_H$-spectrin were identified by electron microscopy as in Fig. 2. Bar, 100 nm.

Structure of $\alpha\beta_H$-Spectrin

The $\alpha\beta_H$ molecule appeared as two intertwined strands by electron microscopy of rotary replicated specimens (Fig. 2), but $\alpha\beta_H$-spectrin was considerably longer (contour length of 248 ± 12 nm versus 192 nm) and more loosely intertwined than $\alpha\beta$-spectrin from *Drosophila* and vertebrates (Dubreuil et al., 1987; Glenney et al., 1982*b*; Bennett et al., 1982). By these criteria, $\alpha\beta_H$-spectrin resembled TW260/240, the spectrin isoform found in the chicken brush border (Glenney et al., 1982*b*; Coleman et al., 1989). The $\alpha\beta_H$ 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, $\alpha\beta_H$ 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 $\alpha\beta_H$ 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.

The β_H polypeptide was initially detected in a fraction of actin-binding proteins obtained during the purification of myosin from *Drosophila* S3 cells (Kiehart and Feghali, 1986). A direct interaction between purified $\alpha\beta_H$-spectrin and F-actin was demonstrated in cosedimentation assays with purified proteins (Fig. 4 *A*). Most of the $\alpha\beta_H$-spectrin sedimented in the presence of F-actin (Fig. 4 *A*, lanes *1* and *2*), but not in the absence of actin (Fig. 4 *A*, lanes *3* and *4*). As previously shown (Dubreuil et al., 1987), $\alpha\beta$-spectrin also sedimented with F-actin (Fig. 4 *A*, lanes *5* and *6*) but not in the absence of actin (Fig. 4 *A*, lanes *7* and *8*). Under identical buffer conditions and at the same molar concentration of protein, $\alpha\beta_H$ cosedimented more efficiently than $\alpha\beta$-spectrin. The interaction between $\alpha\beta_H$-spectrin and actin was also viewed by electron microscopy of rotary-replicated specimens (Fig. 4 *B*). The actin binding sites on $\alpha\beta_H$ appeared to be at the ends of the molecule and some cross-linked actin filaments were observed.

cDNA Cloning and Partial Sequence of β_H-Spectrin

A cDNA clone encoding part of the β_H sequence was recovered from a fly head cDNA library in lambda gt11. Antibody 675, which recognizes β_H- and α-spectrin, identified a single β_H cDNA among a large number of *Drosophila* α-spectrin cDNAs (Byers et al., 1987). This cDNA (lambda-β_H) did not cross hybridize with α-spectrin cDNAs (not shown) and a fusion protein product of the cDNA could be used to affinity purify β_H-specific antibodies from antiserum 675 (Fig. 1 *B*, lane *6*). The cDNA probe detected a 13-kb transcript in Northern blots of Kc_0 cell poly A^+ RNA (not shown). The unusually large size of this transcript is consistent with the unusually large size of the β_H subunit. The gene for β_H-spectrin was localized to chromosomal position 63C,D by in situ hybridization to polytene chromosomes using the β_H cDNA probe (not shown). For comparison, the *Drosophila* β spectrin gene is located at position 16C1-4 and the α-spectrin gene is located at position 62B (Byers et al., 1987, 1989).

The cDNA sequence of the 5-kb clone lambda-β_H included a single long open reading frame of 1,645 amino acids. The methionine codon at position 18 is preceded by the nucleotide sequence "CAAC," which is a commonly used start sequence in Drosophila (Cavener, 1987). Comparison of the predicted protein sequence to the sequence of *Drosophila* β spectrin (Byers et al., 1989) in dotplots indicated that like β-spectrin, the β_H 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 β_H-spectrin and dystrophin (Fig. 5 *C*, *bracket*). The major line of similarity between β_H- and β-spectrin extended from the amino terminal region through the repetitive region of the β_H 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 β_H se-

Figure 2. Electron microscopy of purified *Drosophila* spectrin. Purified $\alpha\beta$-spectrin (*far left*) or $\alpha\beta_H$-spectrin was rotary replicated with platinum/carbon and viewed by electron microscopy. Arrows mark the position of knob structures in both spectrin isoforms. Bar, 100 nm.

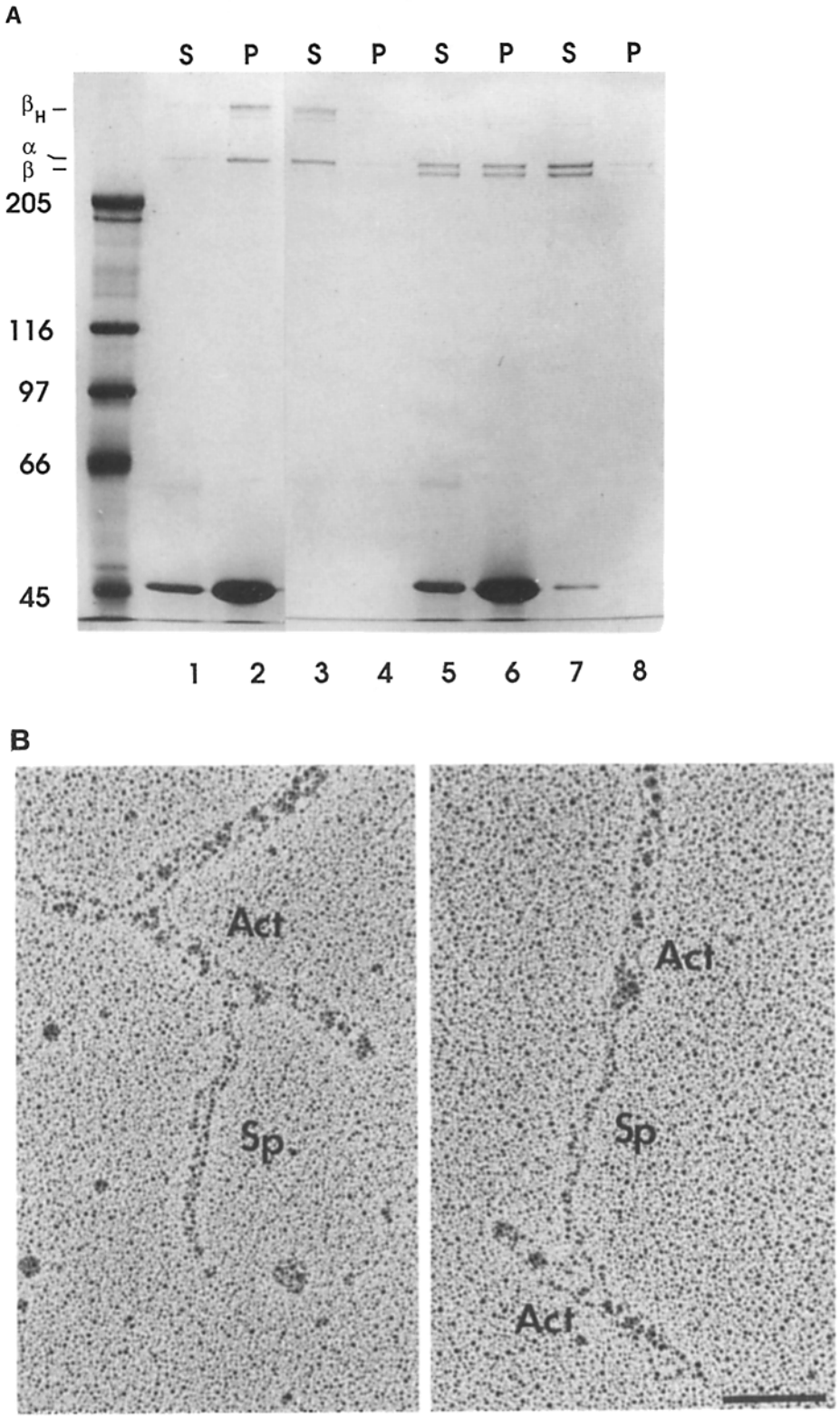


Figure 4. Interaction of spectrin with F-actin. (*A*) Purified *Drosophila* spectrins were sedimented in the presence (lanes *1*, *2*, *5*, and *6*) and absence (lanes *3*, *4*, *7*, and *8*) of F-actin. Pellets and supernatants were solubilized in SDS sample buffer and analyzed on Coomassie blue-stained SDS gels. (Lanes *1–4*) $\alpha\beta_H$-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 $\alpha\beta_H$-spectrin were rotary replicated and viewed by electron microscopy (see text for details). *Sp*, $\alpha\beta_H$-spectrin; *Act*, filamentous actin.

quence (segment 7, see below). The repetitive regions of both β and β_H showed a periodicity of ~106 residues and were related to the repetitive domains of *Drosophila* α-spectrin as well (Fig. 5 *B*; Dubreuil et al., 1989). In contrast, there was only limited similarity between the repetitive region of β_H and the spectrin-like repeats of dystrophin (Koenig et al., 1988; Fig. 5 *C*).

A sequence register of β_H-spectrin segments was assembled using the repeat boundaries that were determined in dotplots (Fig. 6). Segment 1 closely resembled the putative ac-

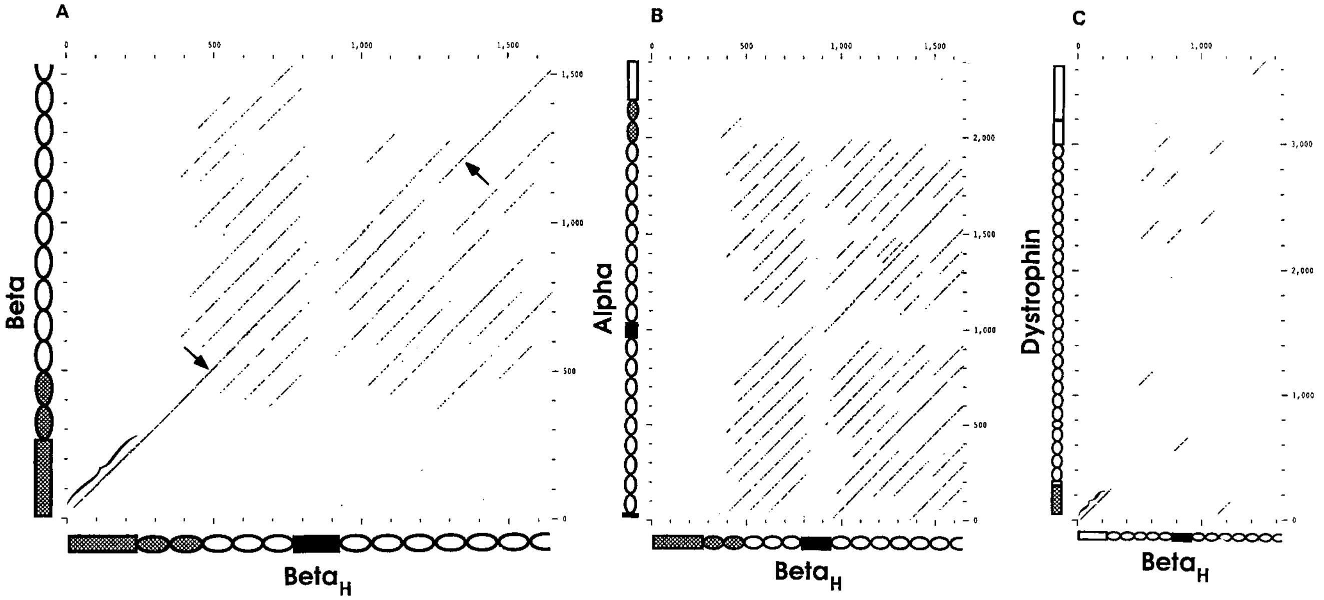


Figure 5. Dotplot comparisons of β_H-spectrin with other spectrins and dystrophin. The predicted polypeptide sequence of the 5-kb β_H-spectrin cDNA fragment was compared to the corresponding region of *Drosophila* β-spectrin (Byers et al., 1989) to full-length *Drosophila* α-spectrin (Dubreuil et al., 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.

tin binding domain of spectrin and other members of the spectrin superfamily (57% identity to *Drosophila* β-spectrin, Byers et al., 1989; 45% identity to human dystrophin, Koenig et al., 1988; and 49% identity to *Drosophila* α-actinin, Fyrberg et al., 1990). Segments 4–6 and 8–14 of β_H all resembled the consensus of α-spectrin repetitive segments (Dubreuil et al., 1989) and most were 106 residues in length. The α-spectrin consensus consists of 54 positions in the 106

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         10        20        30        40        50        60        70        80        90        100       110       120

1                                                                                                          LQTPTPSNRNSANMTQRDGIIK  22
   FENERIKTLQEERLHIQKKTFTKWMNSFLIKAKMEVEDLFTDLADGIKLLKLLEIISSEKLGKPNSGRMRVHKIENVNKSLAFLHTKVRLESIGAEDIVDGNPRLILGLIWTIILRFQI  141
   QEIEIDVDEENESSEKRSAKDALLLWCQRKTHGYPGVNITDFTNSWRSGLGFNALIHSHRPDLFEYSTIVNSKNSNLDNLNHAFDTAANELGIPSLLDAEDIDSARPDEKSILTYVAS  259
                                                       *
2  YYHTFARMKNEQKSGKRIANIVGQLMDADRKKMQYEGLTTNLLSWIRQKTLELEQRDLPNSLEGIQRELLAFKEYRTIEKPPKYKERSEIEALYFTINTLLKALNQPPYNPQ  371  (11/112)
            *                  ◀LLRQEKLE
3  DGQLVNDIEKAWQILEYAEHHREVALRDEQLNYKFEKKSVLREGYLKE---MIQVLSDPRYLRQVDATLKKHEAISADILARVERFNDLTAMAEELDRENYHGKER        482  (27/111)
4  VRRREQEVMAKWRQLLELLENQRLNLSQMSNLMNLLREIASTTEAVRELQQQFASEDVGPHLLGVEELLQAHSLQELQVNTYGETLKRFNRQALPYKSSEHKDAAL        588  (19/106)
5  LAQRLADLEEAYSELLRRSAARRARLEEARNFHHFMEDYDNEESWLVDKQRICKTGITAKDLRAVLSLQQKHKALEDEIKSRKPKSGQMSTAGKRLIGEQHPRSSE        694  (29/106)
6  IQSRIDSLAEHWQALEALVELRRRQLEDAAEAYQFYTDANEAESWLNEKMALVNSRDYGNDEPSAQALLQRHRDLQGELNAYSGDILNLNQQADKLIKAGICTLEL        800  (36/106)

7  SAAEPELPEVEQEEWVNETRLVPKEVWEDEWVEKLEHKKVTETKMLPHVKSLFPFEGQGMKMDKGEVMLLKSKTNDDWWCVRKDNGVEGFVPANYVREVEPRPVAC
   IVPKAEKVKSLQKVKKTILVRQVVPVKRIKPVSVAPKPLVQRRTSTQSINENADS                                                      961

8  VEKRQQRINQTYDELQEMAQKRHALLEDSIHLFGFYRECDDFEKWMKEKERMIKSDEGEG----VDNAKRKFEKFITDLSAASKRVEEIDGAVDTFRRQGHSQLDK       1063  (29/102)
9  IIARQRQIHQIWQRLNNAKAQREKSLEGASSVELFNRTCDEAKVWMSEKMLQLDTAVITPDLRTVQALQRRHQNLERELAPVEDKVNRVTYLGNSVKNAYPAEKDN       1169  (27/106)
10 VNARQQEVQDMWQQVQQRGSDLRNRIESEVGQQVFNNSAKVLLAWIDSVKDQLNADESARDVETANNLLKKHNDLGDDIRAHDTEFVEVIQLGKQLSDGKPNMAE        1274  (24/105)
11 TVAVIERLKAEQDAIHRGWAEKQKWLLQCVDLQMFNREADKIDATTKSHEAFLEYNNLGASLDEVEAILKRHLDFEKSLMAQDKILKGFSDNADKLISNDHYDSKY       1380  (29/106)
12 IGDRRNQVLGKRKAVKDRAFERKRLLQASKDFHKFAAEADDLKVWLQDKTRIAGDENYRDLSNLPRKLQ-KHQAFERELRANEGQLRNVTKDGQALVQAGNRVPE       1484  (28/104)
13 VESRVADLNKRWKDLLTLSEDKGRKLEQAASQREHNRSLEDAKKKVDELDAALRSGDVGNDLRSCKDLINKQQILESEITIWDQKVAELVSTGDDMAHGGHFNAQN       1590  (27/106)
14 IEAGTKELQQRFKDLRDPTQRRRAKLEESLNYHKFVFELDSEFQWINEHLPAAKS                                                       1645  (14/55)
          10        20        30        40        50        60        70        80        90        100
       |  |      |      |  |       |     |  ||       |   |      |  |      |
   I..+...L...W..L.....-++.+L.-....QQF.+-.--.-.WI.-+-...T.--YG+-L..VQ.LI++H-.F-.-L.A.-.+I..L...A..LI....Y..-.   ALPHA CONSENSUS &
          I     I                   Y           L       S   F        L         I    L I       L    F        ALTERNATES
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Figure 6. The amino acid sequence of β_H-spectrin. The partial polypeptide sequence of β_H 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 to the left. The number of consensus matches (*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 (*arrowhead*) and short gaps were introduced in segments 3 and 8 to maximize their alignment with the consensus. The asterisks mark the highly conserved tryptophans at positions 12 and 45. A sequence in segment 7 (*underlined*) shows similarity to several other proteins including α-spectrin, phospholipase c and nonreceptor protein kinases (see text for details). These sequence data are available from EMBL/GenBank/DDBJ under accession X53992.

residue repeat unit that are conserved in at least 9 of the 18 α-spectrin repeats. On average, the β_H-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 β_H 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 β_H segments 1–14 and β-spectrin segments 1–13 (Byers et al., 1989; and our unpublished sequence) were 34% identical. β_H 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 β_H-spectrin relative to β-spectrin: it did not match the repeat consensus and appeared to be unique to β_H. When this region of β_H 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 $\alpha\beta_H$-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 β_H-spectrin subunit that interacts with α-spectrin to form a native molecule that resembles other nonerythroid spectrins. The $\alpha\beta_H$-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 β_H 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 β_H 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 β_H-spectrin and its breakdown products since these bands react with antibody 675 and the lower molecular weight bands increase at the expense of β_H 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 β_H-spectrin that does not contribute to the end to end tetramer length. β_H is 80 kD larger than the TW260 subunit, even though the lengths of $\alpha\beta_H$ and TW260/240 are both ~250 nm.

The $\alpha\beta_H$-Spectrin Knobs May Represent a Carboxy-Terminal Domain of β_H

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 $\alpha\beta_H$-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 $\alpha\beta_H$ and αβ heterodimers. The knobs in $\alpha\beta_H$ appear to mark the termini of the longer subunit near the middle of the native molecule, suggesting that the $\alpha\beta_H$-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 β_H). TW260/240 (Fig. 7 *B*), the only other known spectrin in the size class of $\alpha\beta_H$, lacks the knob structures (Glenney et al., 1982*b*; Pearl et al., 1984; Coleman et al., 1989). Moreover, the molecular weight of TW260 is intermediate to that of β_H 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 β_H-spectrin because it lacks a knob at its carboxy terminus. This interpretation accounts for the presence of knobs in $\alpha\beta_H$ 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., 1982*c*; Davis and Bennett, 1984; Tyler et al., 1979), but they are smaller than the $\alpha\beta_H$ knobs and their mode of attachment to the strands of the spectrin tetramer is not apparent.

β_H-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

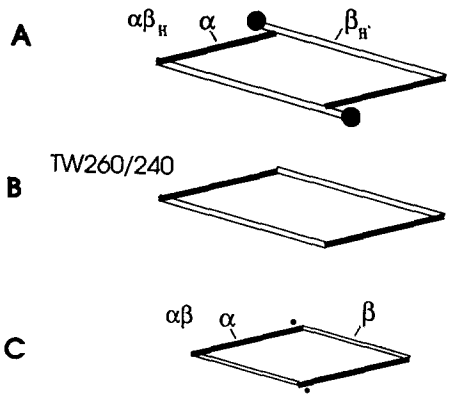

Figure 7. Length and structure of the spectrin tetramer. The difference in length between TW260/240 (*B*) and conventional αβ-spectrins (*C*) can be accounted for by simple length changes in the β subunit (see Glenney and Glenney, 1984). The large knobs in $\alpha\beta_H$-spectrin (*A*) provide further evidence to suggest that tetramer length is a function of β subunit length. The knobs that decorate the central region of each $\alpha\beta_H$ tetramer appear to mark a carboxy-terminal domain of β_H that extends beyond the α-spectrin binding site. In contrast, the carboxy terminus of TW260 is presumed to lie close to the α binding site so that the end points of the α and β subunits in TW260/240 cannot be resolved by electron microscopy. The αβ isoforms of spectrin also include knobs but their means of attachment to the strands of tetramer is not discernible.

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 with 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 β_H-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 β_H-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 β_H-spectrin and human dystrophin, it seems likely that they occupy a similar niche in the eukaryotic membrane skeleton. β_H-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 β_H-spectrin and dystrophins should illuminate the precise evolutionary relationship between these molecules. In any case, an understanding of the function of the $\alpha\beta_H$ molecule may reveal important functional properties of the native dystrophin molecule.

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