Identification of Two Regions of $\beta_G$ Spectrin That Bind to Distinct Sites in Brain Membranes*

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Lydia H. Davis and Vann Bennett
From the Howard Hughes Medical Institute and Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

This study analyzed the complex interactions of intact spectrin with bovine brain membranes by evaluating membrane associations of defined regions of $\beta_G$ spectrin, the subunit responsible for high affinity membrane binding. Two regions of $\beta_G$ spectrin were expressed in bacteria and demonstrated to contain fully functional binding site(s) for a subset of spectrin-binding sites in brain membranes depleted of peripheral proteins. One region, located near the NH$_2$ terminus, was comprised of 106-residue repeats and required repeats 2-7 for full activity. The other binding domain was located at the COOH terminus, which is the most variable between $\beta_A$ and $\beta_B$ spectrins. This region is distinct from the 106-residue repeats, and contains a pleckstrin homology domain. NH$_2$-terminal $\beta$ spectrin polypeptides interacted with a membrane site(s) that recognized both brain and erythrocyte isoforms of spectrin, was inhibited by calcium/calmodulin, and was not blocked by the COOH-terminal polypeptide. The COOH-terminal region associated with a membrane site(s) that was specific for brain spectrin, was not inhibited by calcium/calmodulin, and was not blocked by the NH$_2$-terminal polypeptide. These observations demonstrate membrane association of spectrin with at least two independent sites, which differ with regard to regulation by calcium/calmodulin and in selectivity for spectrin isoforms.

Spectrin is a structural protein associated with the cytoplasmic surface of plasma membranes of most metazoan organisms including vertebrates and higher plants (reviewed by Bennett and Gilligan (1993)). Spectrin is present in especially high levels in postnatal vertebrate brain, and is expressed in both neurons and glial cells. Spectrin is a flexible rod-shaped protein 200 nm in length and contains two subunits, termed $\alpha$ and $\beta$, associated side-to-side and head-to-head to form heterotetramers. The ends of spectrin tetramers associate with actin filaments in vitro through interactions mediated by the actin-binding domain of the $\beta$ subunit. The organization of spectrin and actin has been resolved in erythrocyte membranes where these proteins form a polygonal network with 5-6 spectrin molecules associated with short actin filaments. The spectrin-actin network is linked to the plasma membrane of erythrocytes through association of spectrin with peripheral proteins ankyrin and protein 4.1, which both interact with the cytoplasmic domain of the anion exchanger.

Membrane interactions of spectrin are not well characterized in cells other than erythrocytes. The paradigm of the erythrocyte is not widely applicable in detail since ankyrin mediates contact between spectrin and a variety of proteins in addition to the anion exchanger (Bennett, 1992). Moreover, ankyrin linkages constitute only a portion of spectrin-binding sites in membranes. Spectrin associates directly with integral proteins in brain membranes, which bind to $\beta$ subunits of spectrin with K$_D$ values in the range of 5-50 nm (Steiner and Bennett, 1988). Certain of these ankyrin-independent sites for spectrin are under regulatory control by calcium, and are inhibited by calmodulin (Steiner et al., 1989), and by calpain cleavage of $\beta$ spectrin (Hu and Bennett, 1991).

The purpose of this study was to identify sites in $\beta_G$ spectrin$^1$ that mediate contacts with brain membranes. Two binding sites of $\beta_G$ spectrin were characterized which are located at NH$_2$-terminal and COOH-terminal regions, and interact with distinct binding sites in brain membranes. These findings provide direct evidence for contacts between spectrin and multiple membrane sites. This information will be important in designing experiments to precisely evaluate functions of individual spectrin-membrane interactions in vivo studies, and will provide reagents to isolate particular spectrin-binding proteins.

EXPERIMENTAL PROCEDURES

Materials—$^{125}$I-Labeled Bolton-Hunter reagent was from ICN. Diisopropyl fluorophosphate, leupeptin, pepstatin A, benzamidine hydrochloride, GTP, lysosome, phenylmethylsulfonyl fluoride, EDTA, EGTA, Tween 20, Triton X-100, and thioglycolic acid were from Sigma. Nitrocellulose paper and electrophoresis reagents were from Bio-Rad. Sucrose, ammonium sulfate, and urea were from Schwarz/Mann. Isoproxy-$\beta$-thiogalactopyranoside was from ICN. Deoxyribonuclease 1 was from U. S. Biochemical Corp. BactoAgar, yeast extract, and Bactopectone were from Difco Laboratories. NheI was from New England Biolabs and other restriction enzymes as well as bovine serum albumin were from Boehringer Mannheim. Taq polymerase was from Perkin Elmer. Human erythrocyte spectrin (Bennett, 1983), bovine brain spectrin (Bennett et al., 1986), and the $\beta$ subunit of brain spectrin (Bennett et al., 1986) were purified as described. Calmodulin was isolated from bovine brain as described (Gopalakrishna and Anderson, 1982). Brain membranes depleted of peripheral membrane proteins by extraction with sodium hydroxide were prepared from bovine brain tissue as described (Davis and Bennett, 1986).

Procedures—Spectrin and recombinant polypeptides were radiolabeled using Bolton-Hunter reagent as described (Bennett, 1983). Association of radiolabeled proteins with peripheral protein-depleted brain membranes was measured as described (Steiner and Bennett, 1986). Protein concentrations were determined by the methods of Bradford (1976) and Lowry et al. (1951) with bovine serum albumin as a standard. SDS-polyacrylamide gel electrophoresis and immunoblotting procedures were performed as described (Bennett and Davis, 1981). Molecular biology methods were performed essentially as described by Sambrook et al. (1989).

$^1$ Nomenclature for spectrins is based on Hu et al. (1992) and is as follows: $\beta_G$ spectrin refers to $\beta$ spectrin first characterized in erythrocytes and also expressed in a subset of neurons, skeletal muscle, and cardiac muscle; $\beta_B$ spectrin refers to the generally expressed spectrin present in most tissues except for mammalian erythrocytes; brain and erythrocyte spectrins refer to spectrin isolated from these tissues.
Ankyrin-independent Membrane Binding Sites for βG Spectrin

Bacterial Expression of βG Spectrin Polypeptides—The polymerase chain reaction was employed to amplify selected regions of cDNA encoding βG spectrin using CDNA contained in plasmids isolated by Hu et al. (1992). The expressed polypeptides have 3 additional residues at the NH₂ terminus, and no additional residues at the COOH terminus. Primers for the polymerase chain reaction, in addition to spectrin sequences, a stop codon at the 3' end and restriction enzyme sites at the 5' end for KpnI and at the 3' end for EcoRI or XhoI. Primers also contained 4 additional bases beyond the restriction sites to permit efficient digestion of the products by restriction enzymes. The amplified products were then ligated into a pET plasmid with a T7 promoter (Studier et al., 1990) purchased from Promega (pGEMEX). Digestion of this plasmid with KpnI deletes the region encoding a viral gene 10 protein that otherwise would be included on the amino termini of recombinant polypeptides. Ligated plasmids were used to transform JM109, a nonexpressor bacterial strain lacking the T7 polymerase. Plasmids with inserts were subsequently isolated and transfected into BL21, an Escherichia coli strain with a T7 polymerase under control of the lacZ promoter (Studier et al., 1990). BL21 is deficient in the 1on protease and lacks the ompT outer membrane protease. The BL21 strain employed in this study also contains a plasmid, pLysS, that confers resistance to chloramphenicol and encodes T7 lysozyme which inhibits the T7 RNA polymerase and reduces expression of the recombinant proteins.

Bacteria were isolated following culture and induction as described (Davis et al., 1991). Bacterial pellets were washed with 0.1 M NaCl, 10 mM sodium phosphate buffer, pH 7.4, resuspended in 50 mM sodium phosphate, 1 mM NaEDTA, 25% sucrose, 0.5 mg/ml lysozyme, pH 6.4, with protease inhibitors (10 μg/ml aprotinin and leupeptin, 0.5 mM disopropyl fluorophosphate, 5 mM benzamidine), and were incubated for 30 min in ice. MgCl₂ was added to a final concentration of 10 mM and DNase to 40 μg/ml and allowed to partially digest bacterial DNA during a 30-min incubation. Protein was extracted by the addition of 2 volumes of extraction buffer (200 mM NaCl, 2 mM NaEDTA, 1 mM diithiothreitol, 0.5 mM disopropyl fluorophosphate, 5 mM benzamidine), and were incubated for 30 min at 2°C with 10 μg/ml bovine brain membranes depleted of peripheral membrane proteins ("Methods") in a buffer containing 100 mM NaCl, 10 mM Hepes, 1 mM Na⁰EDTA, 1 mM Na⁰3, 5 mg/ml bovine serum albumin. 125I-Labeled proteins (5 nM; 1.5–3.5 × 10⁶ cpm/pmol) were then added in a final volume of 0.2 ml and the incubation continued for 120 min. Membrane-bound and free radiolabeled polypeptides were separated in 0.4-mL Microfuge tubes by sedimentation of membranes for 20 min at 5,000 × g through a barrier of 10% sucrose dissolved in assay buffer. The tubes were frozen, and tips, containing membrane-bound ligands, and tops, containing free ligand, were assayed for 125I. The data were corrected for nonspecific binding by either subtracting values obtained with heat-denatured protein in the case of spectrin, or values obtained with 640 μM unlabeled polypeptides for recombinant polypeptides. Data are presented as percent of control values determined in the absence of unlabeled polypeptides.

![Graph](image-url)
RESULTS

Identification of NH₂-terminal and COOH-terminal Regions of β₂ Spectrin Capable of Binding to Brain Membranes—Two regions corresponding to residues 376–1246 (containing complete repeats 2–8) and COOH-terminal residues 2060–2333 of β₂ spectrin were expressed in bacteria and evaluated for ability to bind to sites in brain membranes (Fig. 1). The rationale for selection of residues 376–1246 was that a calpain fragment of β₂ spectrin missing the NH₂-terminal 40 kDa was capable of binding to brain membranes, although with reduced affinity (Hu and Bennett, 1991). These observations suggested that at least one component of the binding site(s) would be located adjacent to the NH₂-terminal actin-binding domain. COOH-terminal residues were selected since the COOH-terminal domain is the most variable between β₂ and β₃ spectrins and is a candidate site to mediate β₂-specific interactions previously noted in brain membranes (Steiner and Bennett, 1988).

Expressed polypeptides were evaluated for proper folding by circular dichroism spectroscopy and sensitivity to protease digestion (data not shown). The circular dichroism spectrum of residues 376–1246 was consistent with equivalent or greater α helical content than native spectrin. The spectrum of the COOH-terminal domain, in contrast, had a much lower α helical content. Both NH₂-terminal and COOH-terminal polypeptides were relatively resistant to digestion with chymotrypsin under conditions where heat-denatured NH₂-terminal polypeptide was completely digested. A further examination of folding was not necessary since the polypeptides were fully functional in binding assays when compared to intact β spectrin (see below).

NH₂-terminal and COOH-terminal β₂ polypeptides were evaluated for activity in association with brain membranes depleted of ankyrin and other peripheral membrane proteins by alkaline extraction (Fig. 2). Association of radiolabeled polypeptides with brain membranes was displaced by unlabeled polypeptide in each case (Fig. 2). Half-maximal displacement occurred with 25 nM NH₂-terminal polypeptide, and 40 nM COOH-terminal polypeptide (Fig. 2). Binding data in these experiments were corrected for nonsaturable interactions by subtraction of values obtained with a 128-fold excess of unlabeled polypeptide. Brain spectrin, for comparison, exhibited 50% displacement at 10 nM, as reported previously (Steiner and Bennett, 1988).

Data from displacement experiments of NH₂-terminal and COOH-terminal polypeptides in Fig. 2 were used to calculate amounts of polypeptide bound at each concentration of unlabeled polypeptide, and these values are presented as Scatchard plots (Fig. 3). The plot for spectrin (Fig. 3A) includes a high affinity component with an apparent Kₚ of 2 nM, and a lower affinity portion with apparent Kₚ values ranging from 30 to 100 nM, as noted previously (Steiner and Bennett, 1988). The capacity of brain membranes for spectrin was in the range of 100 pmol/mg membrane protein, although this value is imprecise due to experimental limitations in obtaining binding data at high concentrations of spectrin. The plot for the NH₂-terminal polypeptide contains a class of sites with a Kₚ of 35 nM (Fig. 3B). The plot for the COOH-terminal polypeptide also could be fitted to a single class of sites with a Kₚ also of 35 nM (Fig. 3C). The capacity of brain membranes for both polypeptides was approximately 100 pmol/mg membrane protein.

Recombinant β spectrin polypeptides were evaluated for ability to displace binding of radiolabeled spectrin tetramer to brain membranes (Fig. 4). NH₂-terminal and COOH-terminal

![Fig. 3](image_url)

![Fig. 4](image_url)
polypeptides exhibited partial displacement of binding with 85
and 65% displacement, respectively, at concentrations of 600
nm. A combination of NH2-terminal and COOH-terminal polypep-
dides resulted in more complete displacement, with 92%
loss of binding at 600 nm. The NH2-terminal polypeptide was
more active, with half-maximal displacement at 40 nm, as com-
pared to 60 nm for the COOH-terminal polypeptide.

It was important in evaluating activities of recombinant β
spectrin polypeptides to compare them to isolated brain β spec-
trin in displacement assays (Fig. 5). An assumption in these
experiments was that β spectrin isolated from forebrain is pre-
dominately comprised of the β0 isofrom of spectrin. This as-
sumption is based on the fact that β0 is expressed in high levels
in forebrain and is the major component of brain spectrin (Davis
and Bennett, 1983; Hu et al., 1992). β0 Spectrin is ex-
pressed in only a minor subset of neurons in the forebrain
(Lambert and Bennetts, 1993), although cerebellum does ex-
press substantial levels (Niederer et al., 1986). Brain β spectrin
was nearly equivalent to the NH2-terminal recombinant polypep-
dide, and actually less active than COOH-terminal polypep-
dide in displacing binding of these polypeptides to brain mem-
branes (Fig. 5). This result indicates that the recombinant polypeptides contain fully functional binding site(s) for a subset of spectrin-binding proteins in brain membranes.

β Spectrin and recombinant β spectrin polypeptides exhib-
ited lower activity in binding assays than native spectrin tet-
ramer (Figs. 3–5). The reduced activity of isolated β spectrin
could result from the fact that native spectrin contains α as
well as β subunits, and potentially is multivalent with two β
subunits.

NH2-terminal and COOH-terminal β0 Polypeptides Bind to
Distinct Sites in Brain Membranes—Several different types of
experiments demonstrate that NH2-terminal and COOH-termi-
nal recombinant β0 polypeptides associate with distinct sites
and that these sites differ in specificity for spectrin isofroms as
well as regulation by calmodulin. Evidence for distinct sites is
provided by the lack of ability of unlabeled COOH-terminal polypeptide to displace binding of radiolabeled NH2-terminal polypeptide to membranes (Fig. 6). Similarly, unlabeled NH2-
terminal polypeptide lacks activity in displacing binding of
COOH-terminal polypeptide (Fig. 6).

Brain membrane sites for NH2-terminal and COOH-termi-
nal polypeptides differ with respect to specificity for brain and
erthrocyte spectrin. Binding of the NH2-terminal polypeptide
is displaced almost equivalently by spectrin isolated from hu-
an erythrocyte or bovine brain (Fig. 7). In contrast, binding of
the COOH-terminal polypeptide is displaced by brain spec-
trin, but not by erythrocyte spectrin (Fig. 7). The finding that
the membrane site(s) that recognize the NH2-terminal polypep-
dide do not distinguish between erythrocyte and brain spectrin
is not surprising in view of the high degree of sequence simi-
larity between βR and β0 spectrins in their NH2-terminal re-
gions (Hu et al., 1992). The lack of ability of brain membrane
sites that bind the COOH-terminal polypeptide to recognize
erthrocyte spectrin also is consistent with the complete diver-
gence of COOH-terminal sequences of β0 and the 246-kDa βR.$$
variant of spectrin expressed in erythrocytes.

Brain membrane sites that recognize NH2-terminal and COOH-terminal βS spectrin polypeptides also differ with respect to regulation by calmodulin. Binding of the NH2-terminal βS spectrin polypeptide was inhibited by 75% by calmodulin in the presence of calcium, while binding of the COOH-terminal polypeptide was unaffected (Fig. 8). Half-maximal inhibition of binding of the NH2-terminal polypeptide occurred at 150 nM calmodulin. The membrane site(s) recognized by the NH2-terminal polypeptide thus are responsible for the calmodulin-sensitive binding of brain spectrin noted previously in brain membranes (Steiner et al., 1989). Binding of the isolated NH2-terminal polypeptide is inhibited by lower concentrations of calmodulin than that of intact spectrin, which requires about 1 μM for half-maximal inhibition (Steiner et al., 1989). The increased sensitivity of binding of the NH2-terminal polypeptide to calmodulin compared to intact spectrin may reflect the fact that intact spectrin can associate with additional sites which are not regulated by calmodulin.

Membrane Binding Activity of the NH2-terminal βS Spectrin Polypeptide Involves Several 106-Residue Repeats—Purified recombinant polypeptides containing a complete set of spectrin repeats 2–8, 3–8, 4–8, and 5–8 (Fig. 9) were compared in terms of activity in displacing binding of radiolabeled NH2-terminal polypeptide 376–1246 (repeats 2–8) to brain membranes (Fig. 10). Repeat boundaries are based on the folding unit defined by Winograd and Branton (1991) for α spectrin. Deletion of residues 376–523, corresponding to a portion of repeat 1 and all of repeat 2, increased the concentration required for half-maximal inhibition from 55 nM in this experiment to 125 nM. Further deletion of residues 523–609, corresponding to most of repeat 3, caused a small increase in concentration required for 50% displacement to 150 nM. However, deletion of residues 609–739 corresponding to loss of repeat 4 resulted in a polypeptide with a greater than 10-fold increase in concentration required for 50% displacement (Fig. 10). These results suggest that repeats 2 and 3 contribute to activity but that repeat 4 is essential.

Activity of a polypeptide containing residues 1–777, corresponding to the actin-binding domain, repeats 1–4, and a portion of repeat 5, was evaluated to determine if repeats 2–4 were sufficient for binding and if the actin-binding domain or repeat 1 contributed significantly to membrane binding (Fig. 10). This polypeptide was about 5-fold less active than βS spectrin residues 376–1246 (Fig. 10). Reduced activity of this polypeptide is most likely not due to gross misfolding, since the polypeptide was not aggregated and was resistant to protease digestion (data not shown). The partial activity of the actin-binding domain plus repeats 1–4 indicates that repeat 4 is not sufficient for high-affinity binding, and that the actin-binding domain is not involved in membrane interactions of β spectrin measured in these assays.

Involvement of repeats COOH-terminal to repeats 2–4 was explored using constructs with various COOH-terminal deletions (Fig. 11). Deletion of residues 1037–1246 containing a portion of repeat 7, all of repeat 8, as well as part of repeat 9 had little effect on activity of polypeptides that retained repeats 2–6 (data not shown). Deletion of residues 1037–1246 did reduce by about 2-fold the activity of a smaller polypeptide retaining only repeats 4–6 (Fig. 11). These results indicate that, to a first approximation, residues 1037–1246 do not play a major role in association with the membrane site.

COOH-terminal deletions beyond residue 1037 significantly reduced activity in polypeptides that retained repeats 4 and 5. Deletion of residues 950–1037 corresponding to about 80% of repeat 7 resulted in a 4-fold loss of activity, while deletion of repeat 6 resulted in a greater than 10-fold loss of activity (Fig. 11). These results provide evidence for a role in membrane association of repeats 6 and 7 in addition to repeats 2–4.

Participation of multiple repeats in association of the NH2-
Ankyrin-independent Membrane Binding Sites for $\beta_G$ Spectrin

**FIG. 9.** Recombinant polypeptides derived from the NH$_2$-terminal region of $\beta_G$ spectrin. The location of these polypeptides within the sequence of $\beta_G$ spectrin is shown schematically in Panel A, and purified recombinant polypeptides resolved on a Coomassie Blue-stained SDS gel are in Panel B. Lane A, residues 1-777; lane B, residues 523-1246; lane C, residues 609-1246; lane D, residues 739-1246; lane E, residues 376-1036; lane F, residues 609-1036; lane G, 609-847; lane H, 609-949. Repeat boundaries based on the folding unit defined for $\beta$ spectrin (Winograd and Branton, 1991) are approximate in the drawing, and are based on the following residues: repeat 1, 298-419; repeat 2, 420-523; repeat 3, 524-633; repeat 4, 634-739; repeat 5, 740-844; repeat 6, 845-953; repeat 7, 951-1057; repeat 8, 1058-1164; repeat 9, 1165-1270.

**A**

| REPEATS |
|------------------|
| 1-777, 1-4 |
| 523-1246, 3-8 |
| 609-1246, 4-8 |
| 739-1246, 5-8 |
| 376-1036, 2-6 |
| 523-1036, 3-6 |
| 609-1036, 4-6 |
| 609-949, 4-6 |
| 609-847, 4-5 |

**B**

*Measurements of association of spectrin with brain membranes depleted of peripheral proteins have suggested heterogeneity in ankyrin-independent binding sites for spectrin that vary in affinity for spectrin, and selectivity for spectrin isoforms (Steiner and Bennett, 1988; Steiner et al., 1989). This study approached the problem of dissecting the complex interactions of intact spectrin by evaluating associations of defined regions of one of the spectrin subunits. Two regions of $\beta_G$ spectrin were identified that associate with distinct and most likely independent membrane sites. The NH$_2$-terminal region requires spectrin repeats 2-7 for full activity, interacts with a membrane site(s) that recognizes both brain and erythrocyte isoforms of spectrin, and is inhibited by calcium/calmodulin. The COOH-terminal region, which is comprised of nonrepeat sequence, associates with membrane sites that are specific for the general isoform of spectrin, and are not inhibited by calcium/calmodulin. These observations with expressed $\beta$ spectrin polypeptides provide strong evidence for association of spectrin with multiple membrane sites, and provide the tools for future characterization of spectrin binding sites.

The two regions of $\beta_G$ spectrin implicated in membrane interactions are separated from each other by about 60 nm along the folded spectrin molecule, and presumably could associate simultaneously with both attachment sites. It will be important to determine if the membrane-binding sites for spectrin are co-expressed in the same cells and if they are co-localized in the same membrane domains. In any case, the potential for interactions of spectrin with both calmodulin-sensitive and calmodulin-insensitive contacts has interesting implications for regulation of spectrin-based structures in brain. Signals leading to elevation of calcium ion and dissociation of one class of spectrin interactions would have no effect on the calmodulin-insensitive contacts. This difference in regulation would allow specific assembly-disassembly events to occur either in particular cells, cell domains, or with specialized molecules associated with the same spectrin, depending on the degree of overlap in expression of the two types of sites.

The COOH-terminal domain of $\beta_G$ spectrin was selected for these experiments based on several features that suggest potential for protein interactions. A globular knob most likely corresponding to the COOH-terminal domain has been visualized in the midregion of spectrin tetramers (Dubreuil et al., 1990) and is in an excellent location to mediate interactions between spectrin and other structures. The sequence of the COOH-terminal domain diverges from the 106-residue repeats that comprise the major portion of both $\alpha$ and $\beta$ subunits of spectrin, and is the most variable between $\beta$ spectrin isoforms (Winkelmann et al., 1990; Hu et al., 1992). The COOH-terminal domain thus is a likely candidate to mediate isoform-specific interactions of spectrins. Another interesting feature of the COOH-terminal domain is a 106-residue region (residues 2099-2305) with homology to sequences termed pleckstrin homology domains, which are present in a diverse group of proteins including signaling proteins ras-GAP, ras-GRF, son of sevenless, and pleckstrin, a major substrate for protein kinase C in platelets (Mayer et al., 1993; Haslam et al., 1993). Pleckstrin homology domains have been proposed, by analogy with SH2 and SH3 domains, to participate in protein interactions potentially regulated by phosphorylation, and to have a role in signal transduction. It will be of interest to determine if the membrane interactions of $\beta_G$ spectrin COOH-terminal domain detected in this study are mediated by the pleckstrin homology motif.
Repeats 2–7 required for full activity of the calmodulin-sensitive NH$_2$-terminal region extend approximately 20 nm along the length of $\beta_G$ spectrin (Figs. 10 and 11). Requirement of multiple repeats for high affinity binding could, in principle, result from participation of several repeat domains in correct folding of recombinant polypeptides. A conformational role for multiple repeats is not likely, however, since each 106-residue repeat of spectrin is believed to represent an independent foldable domain. The capacity of distal residues of spectrin are formed due to binding of spectrin. In support of this idea, many of the images of spectrin, visualized by electron microscopy exhibit a kink 20–30 nm from the ends of spectrin tetramers (Bennett et al., 1982; Dubreuil et al., 1990), in the position occupied by the portion of sequence implicated in membrane binding.

The capacity of brain membranes for recombinant $\beta$ spectrin polypeptides is comparable to amounts of native spectrin actually present in brain membranes. Binding capacities were in the range of 100 pmol/mg of membrane protein for membranes depleted of peripheral membrane proteins, which corresponds to about 20 pmol/mg of total, unextracted membranes. Spectrin, for comparison, is present in 30 pmol/mg of total brain membrane (Bennett et al., 1982). Candidates for spectrin-binding proteins currently include N-CAM180 (Pollerberg et al., 1987), and CD45, a membrane-spanning protein with a cytoplasmic domain containing tyrosine phosphatase activity (Lo-keshwar and Bourguignon, 1992). It should be possible with the recombinant $\beta$ spectrin polypeptides characterized in this study to evaluate these proteins for specific interactions as well as systematically search for heretofore unidentified spectrin-binding proteins.

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