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Ankyrin and Synapsin: Spectrin-Binding Proteins Associated With Brain Membranes

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Brain membranes contain an actin-binding protein closely related in structure and function to erythrocyte spectrin. The proteins that attach brain spectrin to membranes are not established, but, by analogy with the erythrocyte membrane, may include ankyrin and protein 4.1. In support of this idea, proteins closely related to ankyrin and 4.1 have been purifed from brain and have been demonstrated to associate with brain spectrin. Brain ankyrin binds with high affinity to the spectrin beta subunit at the midregion of spectrin tetramers. Brain ankyrin also has binding sites for the cytoplasmic domain of the erythrocyte anion channel (band 3), as well as for tubulin. Ankyrins from brain and erythrocytes have a similar domain structure with protease-resistant domains of M_r = 72,000 that contain spectrin-binding activity, and domains of M_r = 95,000 (brain ankyrin) or 90,000 (erythrocyte ankyrin) that contain binding sites for both tubulin and the anion channel. Brain ankyrin is present at about 100 pmol/mg membrane protein, or about twice the number of copies of spectrin beta chains. Brain ankyrin thus is present in sufficient amounts to attach spectrin to membranes, and it has the potential to attach microtubules to membranes as well as to interconnect microtubules with spectrin-associated actin filaments.

Another spectrin-binding protein has been purified from brain membranes, and this protein cross-reacts with erythrocyte 4.1. Brain 4.1 is identical to the membrane protein synapsin, which is one of the brain's major substrates for cAMP-dependent and Ca/calmodulin-dependent protein kinases with equivalent physical properties, immunological cross-reaction, and peptide maps. Synapsin (4.1) is present at about 60 pmol/mg membrane protein, and thus is a logical candidate to regulate certain protein linkages involving spectrin.

Key words: spectrin, ankyrin, synapsin, membrane skeleton, tubulin, secretion

The human erythrocyte plasma membrane currently is the best understood cell membrane in terms of knowledge of the organization and associations of its protein constituents [reviewed in 1–4]. Mammalian erythrocytes contain on the cytoplasmic surface of their plasma membrane a two-dimensional meshwork of structural proteins that stabilizes the fragile lipid bilayer and determines the shape of these cells. The

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principal component of this supportive network is a protein named spectrin. Spectrin is a flexible rod-shaped molecule 200 nm in length composed of two subunits of M_r = 260,000 (alpha) and M_r = 225,000 (beta). The alpha and beta subunits are aligned side-to-side to form heterodimers and the dimers assemble by head-to-head association to form tetramers. Spectrin tetramers form the meshwork by binding of their ends to short actin filaments (12–15 actin molecules) and to a 78,000 M_r protein (band 4.1). It is likely that in erythrocyte tropomyosin also is complexed with actin filaments [5]. Spectrin is attached to the membrane by a high-affinity association of the beta subunit with a membrane-associated protein named ankyrin. Ankyrin, in turn, is attached to a membrane-spanning integral membrane protein that contains an anion transport channel (band 3) (Fig. 1).

The linkage of actin to spectrin and subsequently to the anion transporter via ankyrin provides the first detailed mechanism for attachment of actin to membrane proteins. Similarly, the spectrin-ankyrin-anion transporter association is the first example of the linkages of a membrane-spanning protein with cytoplasmic structural proteins. In the last few years, analogs of erythrocyte membrane proteins including spectrin, ankyrin, protein 4.1, and band 3 have been discovered, using antibodies as probes, to be significant components of plasma membranes in many cells and tissues [reviewed in 4, 6].

As a general rule, proteins in other tissues are products of different genes from their erythrocyte homologs and have substantial differences in antigenic sites and peptide maps. These observations raise the questions of what are the functions of erythrocyte proteins in a complex tissue such as brain, and how closely related are the immunoreactive analogs to erythrocyte proteins in terms of function and their organization. One possibility is that the relationship between erythroid and nonerythroid proteins is analogous to that of intermediate filament proteins such as desmin and vimentin, which have distinct antigenic sites and different peptide maps, but

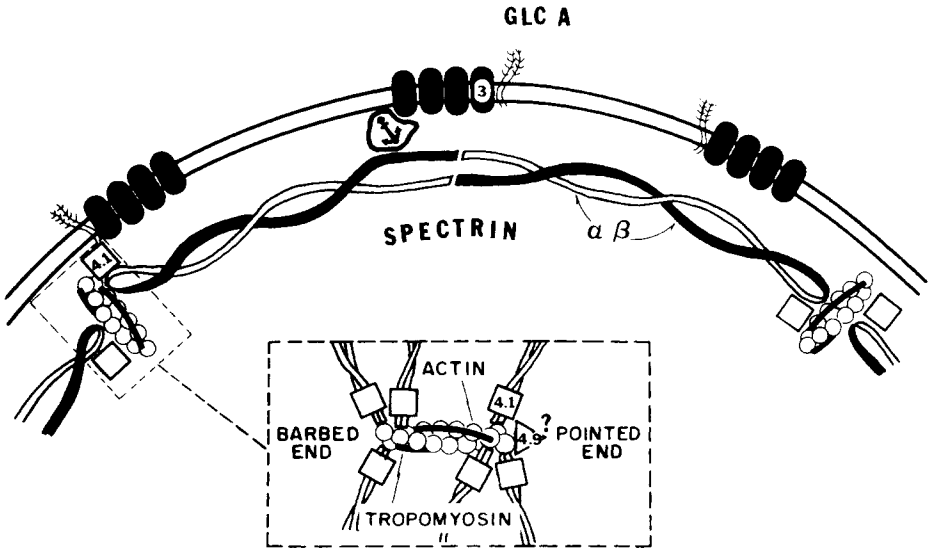

Fig. 1. Schematic model of the human erythrocyte membrane skeleton.

nevertheless form very similar structures and have substantial amino acid sequence homology [7]. Alternatively, it also is conceivable that erythrocyte proteins have become highly specialized in function and have diverged significantly from their analogs in other cells. To address the issue of extent of homology and as a first step toward understanding the functions of erythroid membrane proteins in brain, one approach is to purify these erythrocyte-related proteins from brain. Once pure proteins are available, their binding interaction can be evaluated and some insight can be obtained regarding nearest neighbors.

The purpose of this review is to briefly cover work on tissue spectrins and to summarize initial results from this laboratory involving purified brain forms of ankyrin and 4.1. These proteins, it turns out, are closely related but not identical in binding activities to the erythrocyte analogs. An additional complexity is that brain ankyrin and 4.1 interact with microtubules as well as the familiar erythrocyte proteins. Furthermore, brain 4.1 is identical to synapsin I and is associated with secretory vesicles as well as the plasma membrane. The functions of these proteins are likely to be more complex than a static membrane skeleton, and they may involve linkage of various structural proteins of the cytoplasmic matrix with plasma membranes as well as intracellular membrane systems.

TISSUE SPECTRINS

Spectrin was first noticed in brain during studies of axonally transported proteins, and it was localized by immunofluorescence to the plasma membrane of axons as well as cells of several nonnervous tissues [8]. The protein was initially named fodrin before the close relationships to erythrocyte spectrin were appreciated. Within a period of about a year many other laboratories independently discovered this membrane-associated protein that now is known to be closely related to spectrin. A high-molecular-weight protein with subunits of ~265,000 (alpha) and 260,000 M_r (beta) that constituted 3% of the total membrane protein and associated with actin filaments was isolated from brain [8–13]. The alpha subunit of this protein also binds to calmodulin in a Ca^{2+}-dependent manner, as determined by overlays of SDS-gels and adsorption to calmodulin affinity columns [9,12–16]. The protein cross-reacted with erythrocyte spectrin and was localized by immunofluorescence to the plasma membrane of many types of cells [8,17–19]. The brain protein and a similar actin-binding protein from intestinal epithelial cells have been visualized by low-angle rotary shadowing as flexible rods, about 200 nm in length, which are similar to those of erythrocyte spectrin [12,13,20,21].

The brain protein was identified as a form of spectrin [19–21] based on the following properties shared with mammalian erythrocyte spectrin: (a) ability to bind to ankyrin sites on erythrocyte membranes, (b) similar structure of a tetramer with the morphology of a 200 nM flexible rod, and (c) common antigenic sites in both alpha and beta subunits. An additional important feature in common between the brain protein and erythrocyte spectrin is the fact that functional hybrid molecules can be formed with the alpha subunit of brain and the beta subunit from erythrocyte spectrin [21]. The subunits of brain spectrin are most likely arranged the same way as those of erythrocyte spectrin, with laterally associated alpha and beta dimers attached by head-to-head linkage of each alpha chain with a beta chain [21]. Furthermore, the amino acid compositions of brain and erythrocyte spectrin are remarkably

similar [13,21]. Brain spectrin also binds to erythrocyte band 4.1 and its association with actin is promoted by band 4.1 [22,23]. The binding sites of brain spectrin for brain ankyrin have been localized to the beta subunit, and they have been visualized by electron microscopy to occupy positions in the midregion of tetramers, which are very similar to those of the erythrocyte system [24] (see below).

A major problem that delayed recognition of nonerythroid spectrin is the limited cross-reactivity between mammalian erythrocyte and tissue spectrins. These proteins also have distinct peptide maps [20,25] and thus are products of different genes. Furthermore, the spectrin family does not have a single, definitive feature such as actin-activated ATPase activity of the myosins. Mammalian erythrocyte spectrin also clearly differs from brain spectrin with respect to certain functions. The alpha chain of tissue and avian erythrocyte spectrin binds calmodulin in overlays of SDS-gels, while the alpha subunit of mammalian erythrocyte spectrin does not bind calmodulin under these conditions [16,26], presumably owing to a lower affinity. Another difference is the dimer-tetramer equilibrium. Human erythrocyte spectrin tetramers dissociate into dimers about 30°C at concentrations less than 0.1 μM, while brain spectrin tetramers are quite stable under these conditions. Finally, the subunits of erythrocyte spectrin appear, on the average, somewhat more loosely associated than those of brain spectrin as visualized by rotary shadowing with platinum.

It is likely that mammalian erythrocyte spectrins are the most divergent members of the spectrin family. Avian erythrocyte spectrin appears much closer to tissue spectrins in terms of cross-reactivity and calmodulin-binding, even when compared with mammalian tissues [18,26]. Thus the divergence of mammalian erythrocyte spectrin occurred relatively recently, most likely during evolution of nonnucleated erythrocytes. It will be important to obtain sequence data on tissue spectrins to determine if these proteins have a similar 106-residue repeating unit as observed for erythrocyte spectrin [27,28] and to evaluate the extent of homology between members of the spectrin family.

Spectrin proteins have been purified and characterized in several tissues other than brain, including the chicken intestinal brush border, where the protein has been referred to as TW 260/240 [11,29], and HeLa cells [30]. Immunoreactive polypeptides of M_r ~260,000 cross-reacting with brain or erythrocyte spectrin have been characterized by antigenicity and peptide maps in a number of tissues [12,25]. The alpha subunit of brain spectrin (ie, the subunit that hybridizes with erythrocyte spectrin beta subunit and binds calmodulin) is well conserved by the criterion of peptide mapping among different avian tissues including the intestine. The beta subunit (ie, the ankyrin binding subunit) also exhibits similar but not identical peptide maps among tissues with the exception of the TW 260/240 and muscle spectrin. Little is known at a biochemical level regarding the variant subunits in muscle and terminal web spectrins. It will be of interest to determine if these variants have an ankyrin-binding site and if they can hybridize with the alpha subunit of brain spectrin.

A striking feature of tissue spectrins is their localization at the plasma membrane. Such a plasma membrane distribution is exemplified in liver, where spectrin polypeptides are restricted to plasma membrane fractions isolated by cell fractionation procedures [20,21] and by immunofluorescence using frozen sections of liver (Fig. 2). Liver spectrin is uniformly distributed around the plasma membrane with no obvious preference for the sinusoidal or bile canalicular surfaces. How is spectrin attached to the membrane? Ankyrin is an obvious candidate for an attachment protein and has been purified and characterized from brain as discussed below.

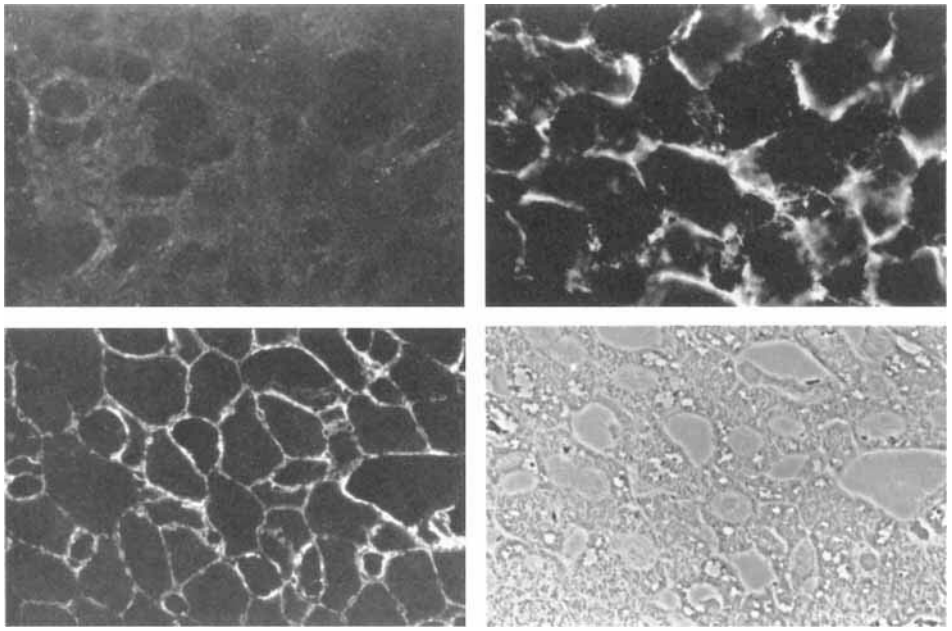

Fig. 2. Localization of spectrin in frozen sections of rat liver by immunofluorescence. Rat livers were perfused and frozen as described by Hubbard et al [63]. Sections of either 0.5 μM (top left, bottom panels) or 7 μM (top right panel) in thickness were incubated with either 20 μg/ml affinity-purified rabbit IgG against brain spectrin [21] (top right, bottom panels) or with 20 μg/ml preimmune IgG (top left panel), followed by rhodamine-labeled goat anti-rabbit IgG as described [63]. A phase contrast micrograph of the 0.5-μm section is in the lower right panel.

BRAIN ANKYRIN

The first evidence for ankyrin in cells other than erythrocytes was from radioimmunoassay [31], which revealed immunoreactivity in membrane fractions from brain, which contained the most, as well as from liver, testes, adipocytes, kidney, cultured fibroblasts and peripheral lymphocytes, neutrophils, and platelets. Ankyrin has been detected by immunoblots as polypeptides of M_r = 225,000 in lens membranes, M_r = 190,000 in liver plasma membranes, and two polypeptides of M_r = 220,000 and 210,000 in brain [32]. Brain ankyrin is associated tightly with membranes, and solubilization of ankyrin requires a combination of potassium iodide (0.8 M) and a small amount of Tween 20, a mild nonionic detergent [24]. Brain ankyrin has been purified in milligram amounts by procedures that include affinity chromatography on erythrocyte spectrin-agarose [24]. The purified protein contains two polypeptides of M_r = 220,000 and 210,000 that are nearly identical by peptide mapping [24]. The explanation for these closely related forms of ankyrin is not known, but they could result from either two genes or some posttranslational modification of a single gene product. In either case, these proteins may have some specialized activity, although functions of these polypeptides are identical in assays employed so far. Brain ankyrin is a monomer in dilute solution, and, as is the case with erythrocyte ankyrin, has an elevated frictional ratio of about 1.6. The basis for the asymmetry is not due to an extended rod-like shape, since rotary-shadowed ankyrin molecules are round and

relatively compact. The molecule may be flat and/or have an extended tail that was not visualized by low-angle rotary shadowing.

Association of brain ankyrin and brain spectrin has been characterized in detail, and it has close parallels with the binding between erythrocyte spectrin and ankyrin. The binding of brain ankyrin to brain spectrin tetramer has been visualized by electron microscopy and occurs at two sites symmetrically placed about 40 nm apart in the midregion of spectrin tetramers (Fig. 3), as has been observed with erythrocyte ankyrin and spectrin [33]. The subunit of brain spectrin and erythrocyte spectrin that contains the binding site for brain ankyrin has been identified as the lower M_r or beta subunits of both spectrins [24]. The beta subunit of erythrocyte spectrin is known to contain the binding site for erythrocyte ankyrin [34,35]. Another parallel between the ankyrin-binding subunits of brain and erythrocyte spectrin is that these subunits both associate with brain spectrin alpha subunit, while the brain alpha subunit does not bind to itself or the erythrocyte spectrin alpha subunit [21]. Binding of brain ankyrin to brain spectrin tetramer has been measured in solution, and it is a high-affinity interaction with half-maximal binding at 25 nM ankyrin (Fig. 4). The capacity of spectrin was about 2 mols of ankyrin per mol of spectrin tetramer, in agreement with the evidence from electron microscopy that each tetramer can bind two ankyrin molecules. Binding occurs with positive cooperativity with increasing concentrations of ankyrin. The basis for positive cooperativity could be interaction between adjacent ankyrin molecules bound to spectrin tetramers resulting in a cyclic complex of high avidity. Alternatively, binding of one ankyrin may induce a conformational change in spectrin tetramers, which is reflected in an increased affinity at the second site. In any event, the consequence of such positive cooperativity may be that spectrin tetramers would preferentially bind to regions of the membrane where ankyrin molecules were clustered.

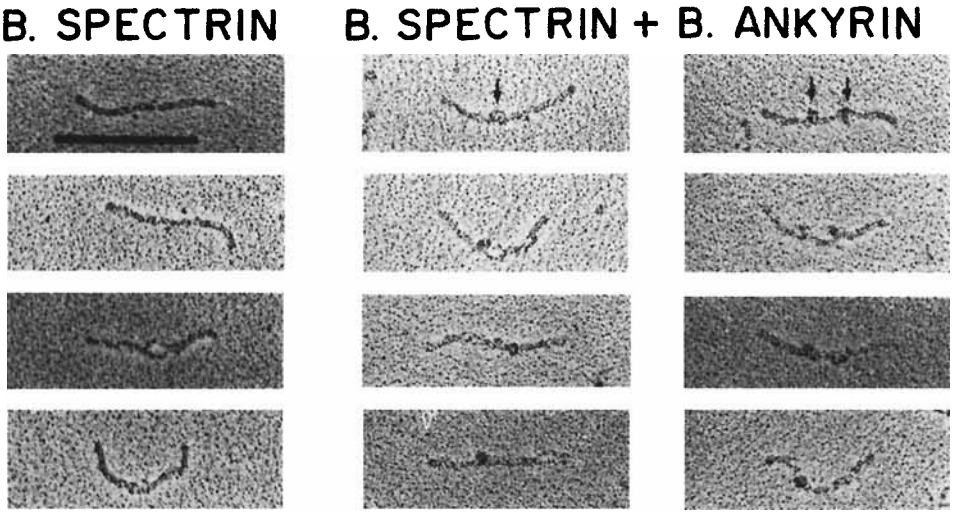

Fig. 3. Visualization of binding of brain ankyrin to brain spectrin by low-angle rotary shadowing. The data is from Davis and Bennett [24]. Bar = 200 nm. Arrows mark the position of ankyrin molecules in top panels.

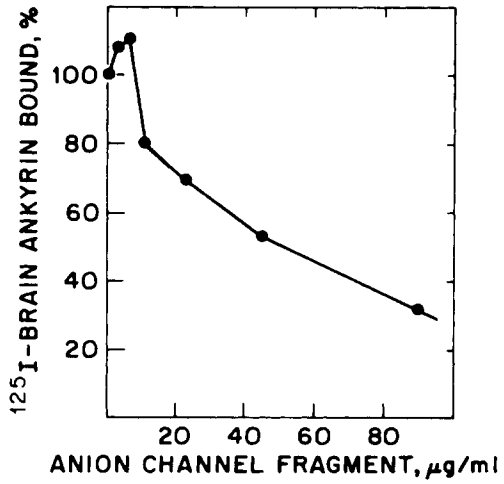

Fig. 4. Displacement of binding of ^{125}I-labeled brain ankyrin to brain membranes by the cytoplasmic domain of the erythrocyte anion transporter. Binding of brain ankyrin labeled with Bolton-Hunter reagent to brain membranes was measured as described [24]. Brain membranes were extracted with 0.8 M potassium iodide, 0.05 Tween 20, followed by 4 M urea to remove peripheral proteins.

The spectrin-binding site of erythrocyte ankyrin is localized in a M_r = 72,000 domain that is released by mild proteolysis [36]. Brain ankyrin also contains a spectrin-binding domain of M_r = 72,000 that has been purified from digests of brain membranes using a spectrin-affinity column [32]. The brain ankyrin M_r = 72,000 fragment binds to brain spectrin in the midregion of spectrin tetramers at sites equivalent to those for the intact ankyrin molecule [32]. Peptide maps of spectrin-binding domains of brain and erythrocyte ankyrin are distinct [32], indicating that these proteins have a different sequence. However, the similarity in domain size suggests a substantial sequence homology will be found when such data become available.

Brain ankyrin has a binding site for the cytoplasmic domain of the erythrocyte anion transporter [24]. Brain ankyrin binds to inside-out erythrocyte vesicles, and this binding is blocked by either erythrocyte ankyrin or purified cytoplasmic fragment of the transporter. The domain of erythrocyte ankyrin involved in binding to the transporter fragment has been identified as a protease-resistant region of M_r = 90,000, while the transporter-binding domain of brain ankyrin is of M_r = 95,000 [24]. The finding that brain ankyrin has a binding site for the erythrocyte transporter suggests that a protein related to the ankyrin-binding region of the transporter should be present in brain. Evidence for such a protein comes from measurements of association of brain ankyrin with brain membranes at a site(s) that is blocked by the cytoplasmic fragment of the erythrocyte transporter (Fig. 4). Current research is directed toward identification of this brain ankyrin-binding protein. Antibodies raised against erythrocyte transporter fragment have not been useful, since they react with many membrane proteins, including those that are not integral proteins. An anion transporter in amounts comparable to ankyrin has not been noticed previously in brain by physiologists, and it is possible that the ankyrin-binding region is not linked to a transporter channel. In this case, erythrocytes, but not other tissues, may have evolved a unique

composite protein formed by gene fusion events that has roles both as a structural protein and an ion channel.

Data presented so far supports, but does not prove, the hypothesis that ankyrin is a major membrane attachment site for spectrin. Additional, although still circumstantial, evidence for such a role of ankyrin is that ankyrin is present, as estimated by radioimmunoassay, in sufficient numbers of copies, and, in fact, is in a threefold excess over spectrin tetramers [24]. Further evidence for linkage of spectrin and ankyrin is localization of ankyrin to the plasma membrane of neuronal cells as has already been observed with spectrin [Detlev Drenckhahn, personal communication]. Ankyrin also colocalizes with spectrin by immunofluorescence in pheochromocytoma cells, which have been grown in the presence of nerve growth factor (Fig. 5). Such cells have extended processes with endings where staining with antibody against ankyrin and spectrin is intense. Both ankyrin and spectrin also are present over the cell body, and, by light microscopy, have an identical pattern. It will be important in future work to extend the morphology to the level of the electron microscope.

Brain ankyrin is present at 100 pmol/mg of membrane protein or about threefold in excess of spectrin tetramers [24]. Ankyrin thus may link proteins other than spectrin to the membrane. Tubulin is a likely candidate for such a protein. Erythrocyte ankyrin was demonstrated previously to bind to microtubules polymerized from pure brain tubulin in a saturable manner with a K_D of about 2 μM [37]. Brain ankyrin also has a tubulin-binding activity, based on cosedimentation with microtubules and association with tubulin on protein blots [24]. The domains of brain and erythrocyte ankyrin involved in tubulin binding have been identified in both proteins as the same domain that binds the cytoplasmic fragment of the anion transporter. A feature of the association of erythrocyte and brain ankyrin with tubulin is that neither protein alters the extent of tubulin polymerization at equilibrium and, in this respect, differs from currently recognized microtubule-associated proteins. It will be important in the

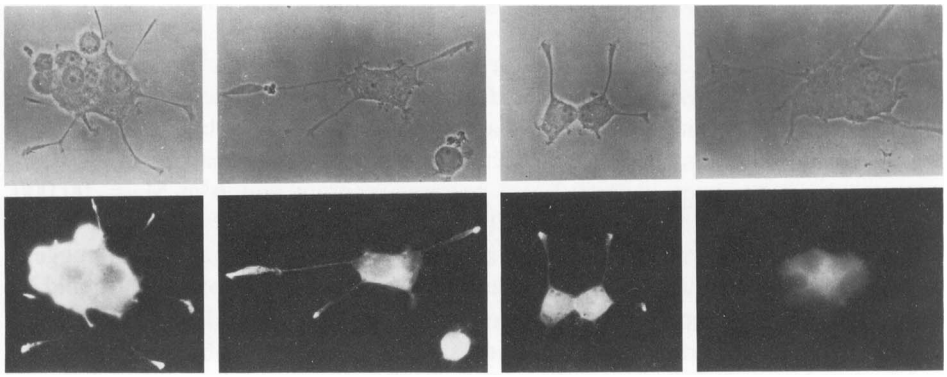

Fig. 5. Immunofluorescent distribution of brain spectrin (left panel) and brain ankyrin (middle two panels) in pheochromocytoma cells grown with nerve growth factor. Pheochromocytoma cells were grown on glass coverslips in the presence of nerve growth factor, and they were fixed in 3% formaldehyde and permeabilized with Triton X-100 as described [37]. Coverslips were incubated with affinity-purified rabbit IgG against brain spectrin [21], brain ankyrin [24], or nonimmune IgG (right panel), followed by rhodamine-labeled goat anti-rabbit IgG as described [37]. Phase contrast micrographs are shown (top panels) of corresponding fluorescent images (bottom panel).

future to examine the effect of ankyrin on the rate of tubulin polymerization and on possible nucleation and severing of microtubules.

A substantial fraction of tubulin in the brain is associated with membranes [38]. The amount of membrane-bound tubulin is in considerable excess over the number of ankyrin molecules, suggesting membrane attachment sites in addition to a direct ankyrin linkage. It is not presently known whether ankyrin can simultaneously bind tubulin, spectrin, and the membrane site. Ankyrin, most likely, can associate with both spectrin and tubulin or both spectrin and the membrane site, since the spectrin-binding activity resides in a different domain of the ankyrin molecule from the other sites. Thus, more data is required to determine if ankyrin can function as a direct membrane attachment site for tubulin.

BRAIN 4.1 (SYNAPSIN I)

Erythrocyte protein 4.1 is a membrane-associated spectrin-binding protein that promotes association between spectrin and actin, and it consists of two polypeptides of $M_r \sim 78{,}000$ and 80,000 that are closely related in sequence. Erythrocyte 4.1 also binds to the cytoplasmic domain of the anion transporter, although 4.1 cannot bind simultaneously to spectrin and the transporter [39]. Erythrocyte 4.1 may have another membrane-binding site in addition to the transporter, which has been suggested to involve sialoglycoproteins [40,41]. Erythrocyte 4.1 does not participate in the high-affinity spectrin-binding to membranes that has been measured in assays of reassociation of spectrin with inside-out vesicles, since complete removal of 4.1 does not reduce spectrin binding [42,43] and since 4.1 does not displace binding of spectrin [44]. Protein 4.1 thus could be viewed as a modulator that regulates associations between spectrin, actin, and possibly membrane sites, but may not perform a continuous structural linkage as is the case with ankyrin.

Immunoreactive forms of erythrocyte 4.1 were first detected in fibroblasts [45], and subsequently in brain [46], as well as other tissues [47–49]. Brain 4.1 has been purified, and consists of two basic polypeptides of $M_r = 75{,}000$ and 77,000 with nearly identical peptide maps [50]. Brain 4.1 binds to spectrin from erythrocyte and brain at sites present in both alpha and beta subunits as determined by protein blots [50]. Binding of erythrocyte 4.1 to spectrin also occurs at both subunits [51]. Brain and erythrocyte 4.1 both bind at the same site on spectrin, since erythrocyte 4.1 can displace binding of brain 4.1 to erythrocyte spectrin in blot assays [50]. Another feature in common between erythrocyte 4.1 and the immunoreactive protein from brain is that both proteins are substrates for endogenous cyclic-AMP-dependent protein kinases in their respective tissues (Fig. 6).

The properties of brain 4.1 are identical to those of the protein synapsin I. Both are basic proteins that are substrates for cAMP-dependent protein kinase and appear on SDS gels as doublets of $M_r \sim 75$–80,000. Synapsin I is a protein discovered in brain about 15 years ago by Greengard and his colleagues, and it is a substrate for the cyclic-AMP-regulated protein kinase [52] as well as two calmodulin/calcium-regulated protein kinases [53] and protein kinase C [54]. Synapsin I is concentrated at nerve endings, where it is associated with secretory vesicles [55,56] and is phosphorylated in intact nerves following excitation [57]. For these reasons, synapsin has been thought to play some important role in neural secretion, although its functions have not been determined.

Fig. 6. Brain 4.1 is a substrate for cAMP-dependent kinase. Myelin-depleted brain membranes (2 mg/ml protein) were incubated at 37°C with 1 μM [^{32}P]-ATP in the presence or absence of 10 μM cAMP as described [52]. After 1 min, the incubation was terminated by the addition of SDS to a final concentration of 1%. The samples were heated to 60°C for 10 min, and samples were withdrawn for electrophoresis or immunoprecipitation. For immunoprecipitation, 0.1 ml of the mixture was diluted to 2.3 ml with a buffer containing 0.15 M NaCl, 10 mM Na phosphate, 1 mM EDTA, 1% Triton X-100, 2 mg/ml bovine serum albumin (BSA), 0.5 mg/ml cytochrome c, and 5 μg/ml pancreatic trypsin inhibitor. This was divided into 0.4-ml aliquots to which were added 2 μg anti-brain 4.1 or 2 μg nonimmune IgG and in some cases 15 μg brain 4.1. Protein A-bearing Staphylococci (0.1 μl) were added to each tube, and the mixtures were incubated for 3 hr at 4°C with agitation. To terminate the incubation, 3.5 ml of 2 M urea, 1% Triton X-100, 0.1 M glycine was added to each tube, and bacteria with adsorbed immune complexes were recovered by centrifugation at 4000g for 15 min. The precipitates were solubilized in SDS for electrophoresis on a 5–15% gel, an autoradiograph of which is shown. Lanes 1–3) Immunoprecipitates from membranes incubated without cAMP. Lanes 4–6) Immunoprecipitates from membranes incubated with cAMP. Lanes 1 and 4) Anti-brian 4.1 immunoprecipitate. Lanes 2 and 5) Immunoprecipitate from nonimmune IgG. Lanes 3 and 6) Immunoprecipitate from anti-brain 4.1 with excess unlabeled brain 4.1. Lane 7) Brain membranes phosphorylated in the presence of cAMP. Lane 8) Brain membranes phosphorylated in the absence of cAMP.

Several lines of evidence [50] suggest that synapsin I purified by the method of Ueda and Greengard [58] is identical to the immunoreactive form of erythrocyte 4.1 in brain: (1) these proteins comigrate exactly on SDS-gels; (2) both proteins cross-react with antibody raised against erythrocyte 4.1 and against the brain 4.1; (3) antibody against brain or erythrocyte 4.1 recognizes multiple polypeptides in digests of synapsin I and brain 4.1, indicating that cross-reactivity is not restricted to a single region of these proteins; (4) brain 4.1 and synapsin I bind spectrin equivalently in blot assays; and (5) two-dimensional peptide maps are identical of synapsin I, brain

4.1, and mixtures of these proteins. Further circumstantial evidence of similarity between erythrocyte 4.1 and synapsin is that in both cases a 10,000-dalton peptide is labeled by cyclic-AMP-dependent protein kinase [53,59]. Furthermore, the amount of synapsin in brain (0.4% of the total protein [60]) is about the same relative to spectrin as that of 4.1 in erythrocytes, where in both systems these proteins are present in 2:1 ratios with respect to spectrin tetramers.

The identity of synapsin I and brain 4.1 was unexpected, since synapsin was thought to be uniquely present in the nervous system based on lack of cross-reactivity with antibody [55,56,60]. However, these negative results with antibody should be viewed in the context of the general experience with erythrocyte proteins that frequently cross-react poorly with analogs in other tissues. Synapsin and erythrocyte 4.1, like spectrin, differ in peptide maps and sequence while retaining functional homology. Another aspect of synapsin that is surprising is the localization in secretory vesicles lacking spectrin. It should be kept in mind that such localization reflects where the protein is most concentrated, but it does not exclude lower levels of synapsin in other areas of neurons. For example, synapsin in axons would be distributed over a much larger membrane surface than in secretory vesicles and may be difficult to visualize. Moreover, vesicle-bound synapsin in principle could associate with spectrin that is present on the plasma membrane. Such a vesicle-synapsin-spectrin-membrane linkage may be transitory and subject to regulation, and it would not require the colocalization of these proteins on vesicles.

Identification of synapsin as an immunoreactive analog of an erythrocyte membrane skeletal protein suggests that synapsin could be approached experimentally as either a structural protein or a protein that modulates associations between structural proteins. It is pertinent in this regard that recent evidence suggests that synapsin and erythrocyte 4.1 bind to microtubules [61]. Synapsin could therefore be involved in coupling of vesicles to microtubules during axonal transport, linkage of microtubules to spectrin on the plasma membrane, or interaction with coated vesicles, which are known to contain tubulin [62]. A role of synapsin in promoting binding of spectrin to actin may be less important in brain than erythrocytes, since brain spectrin binds well to actin in the absence of accessory proteins. Clearly, elucidation of the functions of synapsin will be a challenging task, especially in view of the fact that even in erythrocytes, 4.1 is only partially understood.

CONCLUSION

Proteins closely related to erythrocyte ankyrin and protein 4.1 have been isolated from brain, where one aspect of their function involves association with spectrin. Ankyrin in brain and other tissues is a likely candidate for a high-affinity attachment of spectrin to the plasma membrane. The role of membrane-associated spectrin is not clear, but important clues may be available once the membrane protein(s) has been identified that links ankyrin to the membrane. Possible roles of these membrane proteins in cell-cell interactions and associations with receptors or ion channels can then be evaluated. Protein 4.1 in brain is equivalent to synapsin, a vesicle-associated protein, and it may be involved in linking secretory vesicles to plasma membrane-bound spectrin and to microtubules. The erythrocyte membrane has been useful in that experience in this simple system led to the finding of ankyrin, a newly discovered protein in brain, and to the discovery that synapsin, a previously

characterized protein of unknown function, is closely related to erythrocyte 4.1. The activities of these proteins in brain are clearly much more complex (and interesting) than in erythrocytes, and they involve intracellular organelles, regulatory systems, and additional structural proteins such as microtubules. It is likely that erythrocytes will continue to provide new proteins that will be found in brain, and also that elucidation of functions of these proteins will require extensive studies of the brain proteins themselves.

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