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Erythrocyte and Brain Forms of Spectrin in Cerebellum: Distinct Membrane-Cytoskeletal Domains in Neurons

Abstract. *Chicken cerebellum expresses a polypeptide antigenically and biochemically related to the α subunit of spectrin, an erythrocyte membrane-cytoskeletal protein. Most of this polypeptide is associated with a brain specific spectrin subunit, γ-spectrin, and is localized in virtually all neuronal cell bodies and processes. Cerebellum also expresses polypeptides antigenically related to the β subunits of erythrocyte spectrin and these are also found in association with cerebellar α-spectrin but are confined to the plasmalemma of the neuronal cell bodies. This suggests that there is a mechanism for segregating different spectrin complexes into distinct membrane domains within a single cell.*

Red blood cells contain a protein network in close association with the cytoplasmic side of their plasma membrane. The principal component of this network is spectrin, a protein composed of two nonidentical subunits [α-spectrin, molecular weight, 240,000 (240K) and β-spectrin (220K)], which mediates linkage of actin oligomers to the plasma membrane (*1*). A number of investigators have shown that many nonerythroid cells express a polypeptide biochemically and antigenically related to erythrocyte α-spectrin (*2–5*). In avian and mammalian brain, α-spectrin specifically forms a complex with a 235K polypeptide (referred to here as γ-spectrin), which is antigenically distinct from both α- and β-spectrin and has a different peptide map (*6–8*). Brain spectrin (also termed fodrin) (*9*) has several biochemical properties similar to those of erythrocyte spectrin (*6–9*), giving rise to the suggestion that brain (αγ) spectrin is the nervous tissue analog of erythrocyte (αβ) spectrin. However, recent evidence has indicated that several nonerythroid tissues, in particular adult chicken skeletal and cardiac muscle, express polypeptides that are closely related antigenically and biochemically to the β subunit of erythrocyte spectrin and are distinct from brain γ-spectrin (*8*). In chickens, erythrocytes express two variants of β-spectrin, referred to as β and β′, in an approximate molar ratio of 5:1 (*8*). Chicken nonerythroid cells express variable amounts of polypeptides related to erythrocyte β- and β′-spectrins with the latter being the predominant polypeptide in certain cell types (*8*).

We now report that both the erythrocyte (αβ) and brain (αγ) forms of spectrin are expressed in nervous tissue. Analysis of whole extracts of chicken cerebellum by one-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis shows the presence of two high molecular weight polypeptides (Fig. 1). The upper component (brain α-spectrin) has the same electrophoretic mobility as chicken erythrocyte α-spectrin (Fig. 1, A and B) and has been shown previously to be antigenically related to chicken erythrocyte α-spectrin (*2–4, 6–8*). The lower component (235K, γ-spectrin) has a relative electrophoretic mobility slightly slower than the erythrocyte β- and β′-spectrins (Fig. 1, A and B) and is distinct from them by the criteria of antigenicity (*3, 7, 8*) (Fig. 1, C and D) and peptide mapping (*3, 7, 8*). Thus this polypeptide doublet appears to be identical to that previously identified as the component of axonal transport in retinal ganglion cells and termed fodrin (*9*). Immunoautoradiography of the same gel with antibodies to chicken erythrocyte β-spectrin reveals two cross-reacting polypeptides one of which comigrates with erythrocyte β′-spectrin (230K) and the other of which corresponds to erythrocyte β-spectrin; as previously shown for muscle β-spectrin (*8*), erythroid and cerebellar β-spectrins exhibit a slight difference in their relative electrophoretic mobility (Fig. 1, C and D).

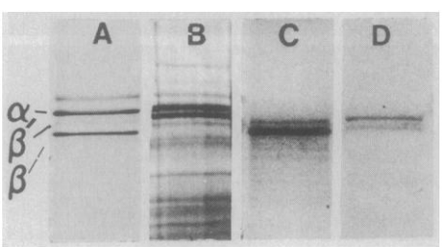

Fig. 1. Subunit composition of cerebellar spectrin. Coomassie blue stained 12.5 percent sodium dodecyl sulfate (SDS)-polyacrylamide gels (lanes A and B) and the corresponding autoradiograms after immunoautoradiography with antibodies to β-spectrin (lanes C and D) of chicken erythrocyte membranes (lanes A and C) and an SDS extract of whole chicken cerebellum (lanes B and D). Only the top portion of the gels are shown. The minor bands below β-spectrin in lanes C and D represent degradation products; SDS-polyacrylamide gel electrophoresis and immunoautoradiography were performed as described (*8, 11*). Autoradiograms were exposed without intensifying screens for 24 to 72 hours.

To examine further the presence of immunoreactive forms of β-spectrin in cerebellum, we "immunoprecipitated" spectrin from whole extracts of cerebellum with either α- or β-spectrin specific antibodies (Fig. 2). Under the conditions of immunoprecipitation used here, α-spectrin is coimmunoprecipitated with β-spectrin antiserum, and vice versa, even though each antibody is highly specific for its respective antigen. This is due to the fact that the spectrin subunits reassociate during the course of the immunoprecipitation reaction (*8, 10*). Antibodies to α-spectrin immunoprecipitate predominantly αγ-spectrin from whole cerebellar extracts (Fig. 2, lane C). Antibodies to β-spectrin do not immunoprecipitate any polypeptides detectable by Coomassie blue staining (Fig. 2, lane D). However, subsequent immunoautoradiography of the same β-spectrin immunoprecipitates with antibodies to β-spectrin establishes the presence of two polypeptides antigenically related to erythrocyte β- and β′-spectrins and that β′-spectrin is present in excess of β-spectrin in cerebellum (Fig. 2, lane I). Immunoautoradiography of the β-spectrin immunoprecipitates with α-spectrin antibodies shows also that a minor fraction of the total α-spectrin is coimmunoprecipitated as a complex with β-β′-spectrin (lane G).

To define the cell type (or types) in cerebellum that expresses the β-spectrin antigen or antigens, we used indirect immunofluorescence on frozen sections of cerebellum (*11*). Immunofluorescence with antibodies to α-spectrin reveals that, as expected (*2, 9*), the antigen is present in all three layers of the cerebellar cortex and in all discernible cell bodies and processes that populate the molecular, Purkinje cell, and granular layers (Fig. 3). In contrast, indirect immunofluorescence with antibodies to erythrocyte β-spectrin reveals that only a subpopula-

tion of these cell types react with this antiserum. Comparison of alternate sections stained with β-spectrin antibodies and Nissl stain reveals that the cell bodies of granule cells show a ring of fluorescence in association with their periphery similar to that observed with the α-spectrin antibodies (Fig. 3E). Occasionally the cell bodies of cells larger than granule cells, most likely Golgi type II cells, are also reactive. In Purkinje cells, the β-spectrin fluorescence is confined to the plasmalemma of the cell bodies and the processes that extend from the cell bodies into the molecular layer and which correspond to the initial dendritic trunks of these cells (Fig. 3, G and H). With the exception of a few cell bodies, which most likely correspond to Basket cells, the rest of the molecular layer does not show any substantial fluorescence above that of the preimmune β-spectrin serums, indicating that β-spectrin is not present in the numerous axonal and dendritic processes that populate this layer of the cerebellum. Furthermore, staining of alternate sections with antibodies to the glial fibrillary acidic protein or vimentin and β-spectrin to reveal the position of fibrous astrocytes (*12*), demonstrates that these cells do not contain β-spectrin (not shown). Finally, processes in the white matter are also uniformly negative with the antiserum to β-spectrin (Fig. 3E), suggesting that the axonal processes of mossy and climbing fibers and those of the Purkinje cells do not contain β-spectrin, but do contain α-spectrin (see above). A notable exception in the white matter are large cells, identified as the cell bodies of neurons in the cerebellar nuclei, which are reactive with the antiserum to β-spectrin antiserum. In this case, the fluorescence is also associated with the periphery of the cells and, where visible, is spread over a short distance into the main process emanating from the cell body (Fig. 3, F and I).

The fact that αγ-spectrin is found throughout the neuron whereas the distribution of the α-, β′-, and β-spectrins is restricted to the neuronal cell body suggests that during the process of axonal transport there is a mechanism for segregating certain cytoskeletal elements and confining them to the cell body. The mechanism by which the α-β′-β-spectrin complex is retained in the cell body is unknown. A simple hypothesis is that the membrane receptor for the α-β′-β complex is retained in the cell body and rapidly binds newly synthesized β′-β-spectrin while that for αγ-spectrin is axonally transported. The segregation of the two spectrin forms thus implies that there is an anisotropy in the molecular composition of the neuronal membrane-cytoskeleton which may result in differences in the biophysical properties of the membrane-cytoskeleton of cell bodies and axons.

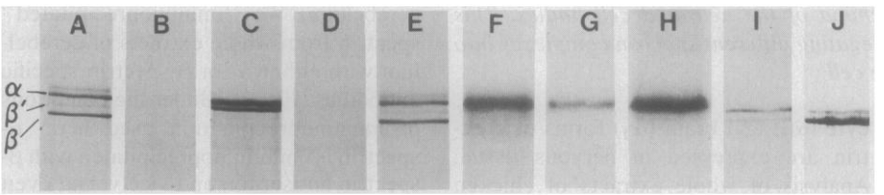

Fig. 2. Immunoprecipitation of chicken cerebellar spectrin with antibodies to erythrocyte α- and β-spectrin. Whole chicken cerebellum was solubilized in SDS and immunoprecipitated with antibodies to α- and β-spectrin (*8*, *10*). The resulting immunoprecipitates were separated on 12.5 percent SDS-polyacrylamide gels. (Lanes A and E) Chicken erythrocyte spectrin standard. (Lanes B to D) Coomassie blue–stained gels of immunoprecipitates of whole cerebellum with preimmune serum (lane B), antibodies to α-spectrin (lane C) and antibodies to β-spectrin (lane D). (Lanes F to J) Immunoautoradiography of the corresponding gels shown in lanes C to E with antibodies to α-spectrin (lanes F to H) and gels shown in lanes D and E with antibodies to β-spectrin (lanes I and J). The immunoautoradiogram shown in lane J was exposed for a shorter time than the rest. Autoradiograms were exposed without screens for 24 to 72 hours.

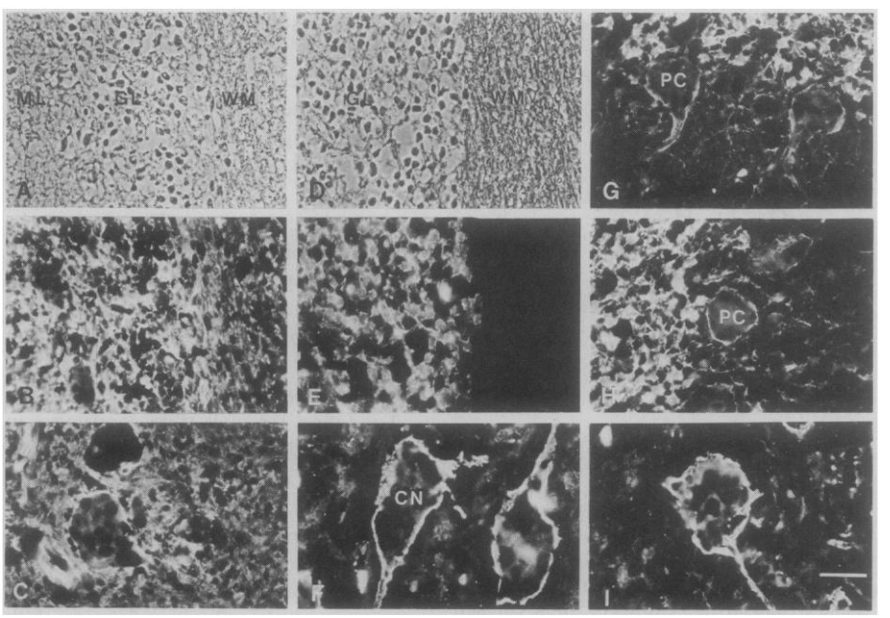

Fig. 3. Localization of α- and β-spectrin immunoreactive forms in chicken cerebellum by indirect immunofluorescence. Frozen sections of cerebellum from a 7-week-old chicken were treated with antibodies specific for chicken erythrocyte α-spectrin (B and C) or β-spectrin (E to I) as described (*8*). (A) and (D) are the phase contrast images of (B) and (E), respectively. The different layers of the cerebellum are denoted in A and D; *ML*, molecular layer; *GL*, granular layer; *WM*, white matter; *PC*, Purkinje cells; and *CN*, cell bodies of the large neurons in the deep cerebellar nuclei. The nuclei of granule cells in the granular layer are evident in (A) and (D) (bar = 24 μm).

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