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ERM Proteins of the Lens

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Abstract Ezrin and radixin and protein 4.1 were detected in the lens of the eye. These proteins were mainly present in the young elongating cortical fiber cells and localized to the plasma membranes. Moesin was not detected. Ezrin, radixin, and protein 4.1 provide another means whereby actin is linked to the plasma membrane in addition to the known adherens junctions in the lens. J. Cell. Biochem. 92: 626–630, 2004. © 2004 Wiley-Liss, Inc.

Key words: ocular lens; ezrin; radixin; moesin; cytoskeleton

The ezrin-radixin-moesin (ERM) proteins link F-actin to the plasma membrane [Bretscher et al., 2000]. They are involved in the formation of microvilli, cell–cell adhesion, maintenance of cell shape, cell mobility, and membrane trafficking [Louvet-Vallee, 2000]. These proteins also appear to function in signal transduction and growth control [Bretscher et al., 2000, 2002; Louvet-Vallee, 2000]. Ezrin is concentrated at microvilli and membrane ruffles where it links actin to membrane proteins directly or via scaffolding proteins.

In a previous study, Dola et al. [1990] reported the specific extraction, by high-ionic-strength salt solution, of a 80-kDa protein from the bovine lens. In this study, we identified the protein as ezrin, determined its distribution, and analyzed the lens for other ERM proteins.

MATERIALS AND METHODS

Mammalian and chicken lenses were used for this study. Bovine eyes were obtained from Monarch Packing (Detroit, MI) and white Leghorn chicken eyes from Capitol Poultry (Detroit, MI). Lenses were removed from the eyes, decapsulated and homogenized as intact fiber masses, or dissected into separate fractions of epithelium, cortex, and nucleus. In some experiments, the superficial and deep cortex was dissected separately. The buffer consisted of 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitel (DTT), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 2 μg/ml pepstatin A, pH 7.5 (Buffer A). The fiber cell homogenates were centrifuged at 23,000 *g* for 15 min at 4°C. The water-soluble fraction (WSF) was collected and the water-insoluble fraction (WIF) was washed three times by homogenization in buffer A, followed by centrifugation. The final WIF was then extracted three times in 2 mM Tris-HCl, 1 mM EDTA, 0.5 mM PMSF, 2 μg/ml pepstatin A, and 1 mM DTT, pH 7.5 (buffer B). The supernatants were collected and the resultant WIF was extracted in buffer C consisting of 1 M KCl in buffer A. The resultant extracts and the final WIF were separately dialyzed against buffer A to remove the KCl. Aliquots of supernatants and the WIF were dissolved in 1% SDS. Cortical WIF was also prepared from human, cat, dog, and rat lenses. Human fetal lenses were obtained from the Body Bequest Program of the Department of Anatomy and Cell Biology (Atlanta, GA). Lenses of the other species were obtained from euthanised animals courtesy of the Division of Laboratory Animal Resources, Wayne State University (Detroit, MI).

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Sodium Dodecyl Sulphate (SDS)–Polyacrylamide Gel Electrophoresis (PAGE)

One-dimensional SDS–PAGE was performed according to Laemli [1970] with 10% (w/v) gels used for final resolution. Peptide analysis on proteins cut from the gel was done as previously described [Bagchi et al., 2003].

Immunoblotting

Protein was transferred from gels onto nitrocellulose paper according to Towbin et al. [1979]. Immunoblots were developed as previously described [Ireland and Maisel, 1984] with the secondary antibody conjugated to alkaline phosphatase. Polyclonal antibodies to ezrin were obtained from Dr. A. Bretscher, Cornell University, and polyclonal antibody to radixin [Woodward and Crouch, 2001] and to moesin [Li and Crouch, 2000] was a gift from Dr. D.H. Crouch, University of Dundee, UK. Monoclonal antibody to ezrin was a gift of Dr. O. Turunen, University of Helsinki, Finland.

Peptide Analysis

Gel fragments were cut from Coomassie stained gels and analyzed as previously described [Bagchi et al., 2003].

Immunofluorescence Analysis

Immunofluorescent studies on sections of 12-day chick embryo lens were performed according to Waggoner and Maisel [1978].

RESULTS

The SDS–PAGE protein pattern of bovine cortical lens fractions after extraction with low and high salt buffers is shown in Figure 1. Extraction with low salt (Buffer B) solubilized filensin and vimentin among other proteins. However, an 80 kDa protein remained with the WIF and was specifically, and nearly completely released by the high ionic strength buffer C (Fig. 1D). This protein reacted with a polyclonal antibody specific only for ezrin (Fig. 2). Peptide mapping of the protein cut from the gel revealed an N-terminal sequence of 13 amino acids—P I N V R V T T M D A E L—identical to that of bovine ezrin (Swiss-Prot: P31976). Based on the specificity of the antibody and the amino acid sequence, it was concluded that the 80 kDa bovine lens protein is ezrin. The high salt extract also contained protein 4.1, both the high (125 kDa) and low (80 kDa) molecular components as previously identified [Aster et al., 1984, 1986]. Other cytoskeletal proteins released by high salt included spectrin, ankyrin, and actin. The identity of these proteins was previously established [Dola et al., 1990]. Radixin was also detected in the high salt extract (not shown).

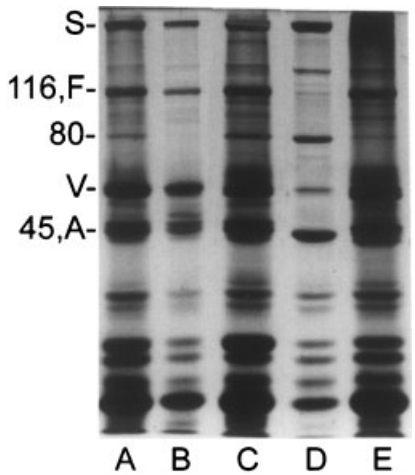

Fig. 1. One-dimensional SDS–PAGE gel of (**A**) WIF of bovine cortex; (**B**) low salt (buffer) extract of WIF; (**C**) resultant WIF. **D**: High salt extract of WIF shown in (C), (D) WIF after extraction with buffer.

Ezrin and radixin were present in all fractions of the bovine lens, but mainly in the cortical fiber cells (Figs. 3 and 4). Indeed only a faint reaction was noted for epithelial ezrin.

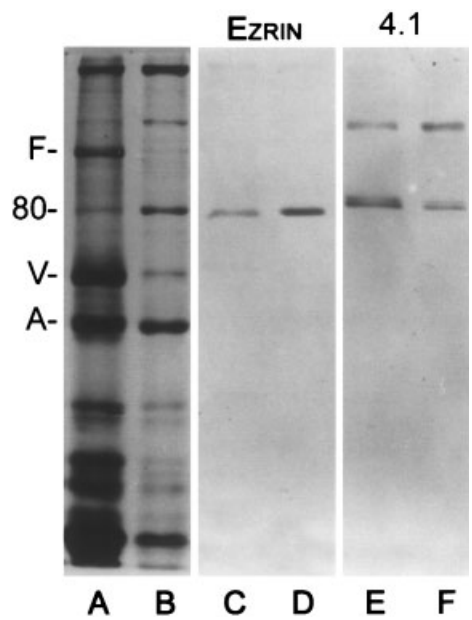

Fig. 2. Gel pattern of (**A**) bovine cortex WIF, and (**B**) high salt extract thereof. **Panel C–F** is the corresponding Western blot with antibody to Ezrin (C, D) and with antibody to protein 4.1 (E, F).

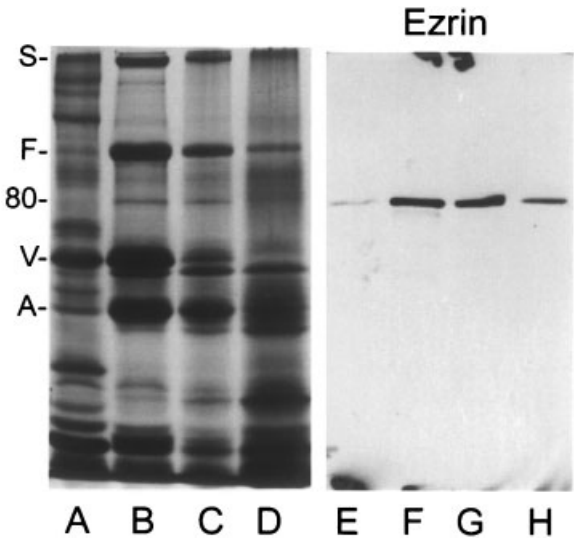

Fig. 3. SDS–PAGE of bovine lens cortex WIF. **A**: Epithelium. **B**: Superficial cortex. **C**: Deep cortex. **D**: Nucleus. The corresponding immunoblot is shown in (**E–H**).

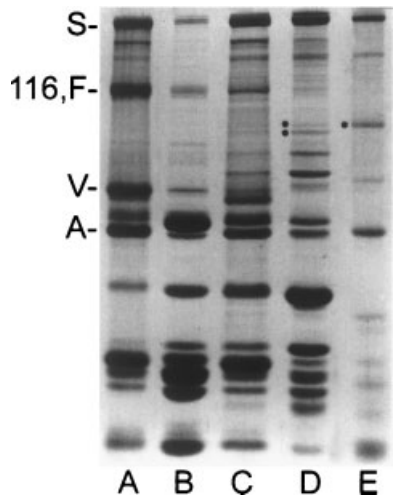

Fig. 5. SDS–PAGE of chicken lens. **A**: Cortex WIF. **B**: Low salt extract of (A). **C**: WIF after extraction with low salt. **D**: High salt extract. **E**: Bovine cortex high salt extract. The dots in (D) indicate two bands that reacted with anti-ezrin antibody (see Fig. 6). The dot in (E) is bovine ezrin.

Moesin was not detected in any fraction of the bovine lens.

High salt extraction of chicken lens WIF released two bands which reacted with the antibody to ezrin (Figs. 5 and 6). Ezrin and radixin were also detected in the lenses of several mammal species including the human fetal lens (Fig. 7).

An immunofluorescent study of 12-day-old embryonic chicken lenses clearly showed the localization of ezrin at the plasma membranes of annular pad and fiber cells (Fig. 8). Whereas immunofluorescence was uniform at the plasma membranes of the annular pad cells, the cortical fiber cell reactivity was concentrated at the angles where the cells interlock with each other.

DISCUSSION

This study shows that ezrin is present in the bovine lens as a ~80 kDa protein and that it co-extracted with actin and the spectrin–ankyrin complex. Ezrin was detected in the lens of all the species examined, including human, bovine, rat, dog, cat, and chicken. Radixin was also present in the lens, but moesin was not detected. These results are consistent with the findings of Ingraffea et al. [2002] that ezrin is found predominantly in polarized epithelial cells and moesin is enriched in endothelial cells and lymphocytes. As expected ezrin was localized to the plasma membrane of lens fiber cells. However Straub et al. [2003] found both ezrin and moesin in the bovine lens.

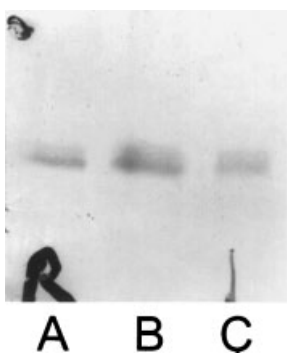

Fig. 4. Immunoblot of bovine epithelium, superficial cortex, and nucleus WIF (**A–C**) after reaction with the polyclonal antibody to radixin.

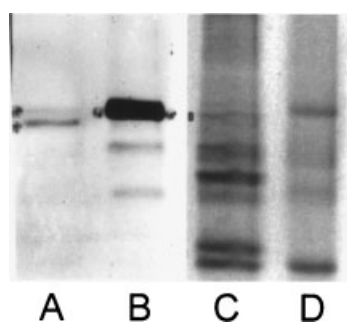

Fig. 6. Gel of high salt extracts of the WIF of chicken (**C**) and bovine (**D**) lenses and the corresponding immunoblot (**A**, **B**), with the polyclonal antibody to ezrin.

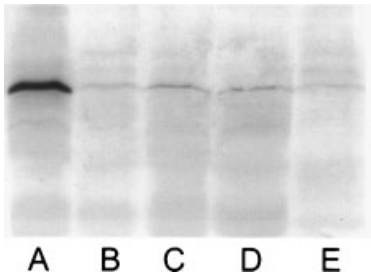

Fig. 7. Immunoblot with a monoclonal antibody to ezrin of the WIF of bovine, human, cat, dog, and rat lenses (**A–E**). Radixin was also detected in each lens.

The ezrin/radixin/moesin are highly homologous proteins which connect the cell membrane with the cytoskeleton, by cross-linking actin to plasma membrane proteins [Louvet-Vallee, 2000; Woodward and Crouch, 2001]. This linkage can be directed to integral membrane proteins or via scaffolding proteins such as EBP50 and E3KARP [Bretscher et al., 2002; Ingraffea et al., 2002].

The presence of ezrin and radixin provides an additional mechanism for linking actin to the plasma membrane of lens cells. The other modes of presumed linkages include the adherens junctions, and protein 4.1. In each case, the components of the system have been identified in the lens [Aster et al., 1986; Lo et al., 1997; Beebe et al., 2001; Straub et al., 2003]. However, the distribution of these proteins along the plasma membrane of fiber cells, have mainly been studied at the light microscopic level. Electron microscopic analyses are required to precisely define the location of these proteins with respect to the cytoskeleton and the plasma membrane in order to arrive at a structural interpretation of their role in lens differentiation and maturation. Moreover, the known participation of ERM proteins in the Rho-pathway [Bretscher et al., 2002] raises the possibility that these proteins play a functional role in lens development.

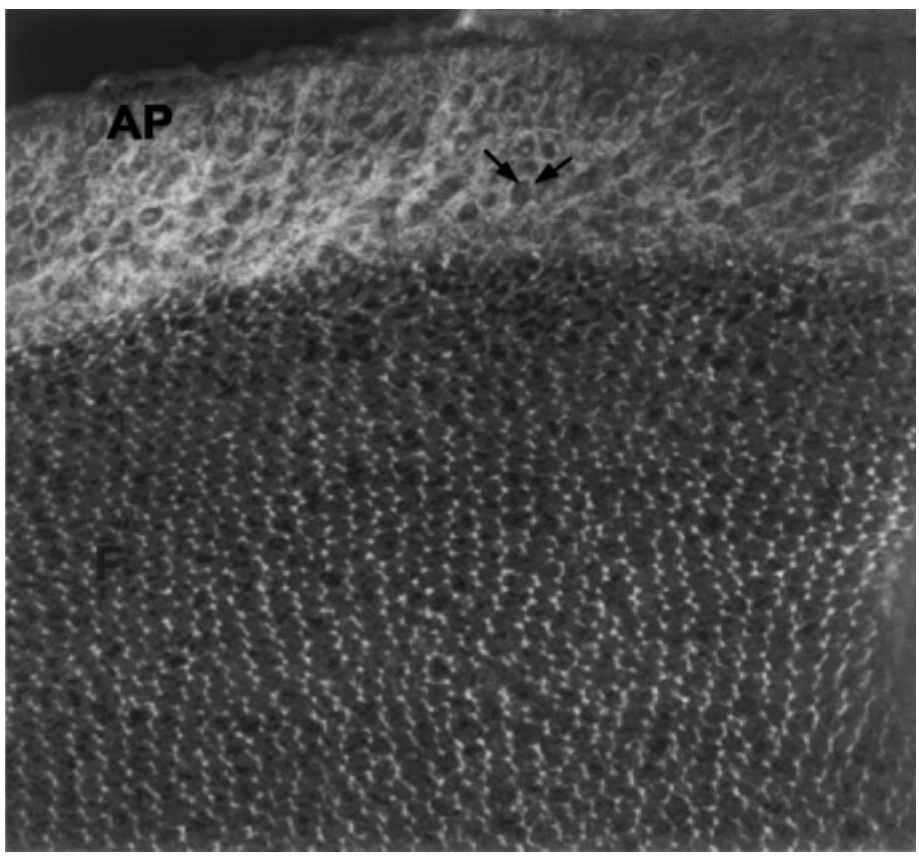

Fig. 8. Immunoflorescence pattern of ezrin in a 12-day chick embryonic lens. Note the concentration at the short sides of the fiber cells (F) and their angles, and along the plasma membranes (arrows) of the annular pad cells (A P, arrows).

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