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A monoclonal antibody against a synthetic peptide reveals common structures among spectrins and α-actinin

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A monoclonal antibody (Mab) against a synthetic peptide, SEDYGKDL, corresponding to one conserved sequence in the chicken α-fodrin repeats reacts in immunoblotting with avian α-spectrin and α-fodrin, both mammalian spectrins and with mammalian α-fodrin. This Mab also reacts with α-actinin in both chicken and human cells. Our results confirm the previously detected structural homology between spectrins and α-actinin and implicate their common evolutionary origin.

Cytoskeleton; Synthetic peptide; Spectrin; α-Actinin; Antibody

1. INTRODUCTION

Spectrin-like proteins have been identified in most non-erythroid cells [1–7]. Studies with antibodies raised to these proteins, fodrins, have suggested that in mammalian cells they are immunologically related to chicken erythroid α-spectrin but not to mammalian spectrins [1,3,5,6]. On the other hand, it has also been suggested that avian α-spectrin and mammalian α-fodrin are the most conserved members of the spectrin family [8]. Comparison of the partial cDNA sequences of chicken smooth muscle α-fodrin [9,10] and human α-fodrin [11] to the corresponding amino acid sequences of mammalian α-spectrin [12] has shown that these three proteins are closely similar: All contain 106-amino-acid repeats; this appears to be a structural hallmark of spectrins (cf. [6,12]). Comparison of the primary sequences of α-spectrin and α-fodrin also revealed distinctly well conserved regions (cf. [9,10]).

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Recently, it was shown by sequence comparison that chicken α-actinin and α-fodrin have considerable structural homology implicating their common evolutionary origin [14]. Here, we have continued to study this homology with a monoclonal antibody raised against a synthetic peptide, SEDYGKDL, which is a conserved sequence in avian α-fodrin. We show that this antibody reacts with distinct members of the spectrin family. Although any sequences identical to SEDYGKDL are not found in α-actinin, the monoclonal antibody clearly also recognizes this molecule.

2. MATERIALS AND METHODS

2.1. *Peptide synthesis*

The sequence of the synthetic peptide, SEDYGKDL (GC), was predicted from the partial cDNA sequence of the avian α-fodrin gene and corresponds to nucleotides 48–70 [9]. The peptide was synthesized by the solid-phase method using a Vega Coupler (250C) peptide synthesizer and t-Boc chemistry [15]. The protected *t*-butyloxycarbonyl (t-Boc) amino acids and Boc-Cys (MBzl)-O-resin

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(1.0 mM available amine/g resin) as solid-phase support were purchased from Peninsula Laboratories (Belmont, CA). Trifluoroacetic acid (TFA, Fluka, Buchs, Switzerland; 40% in CH_2Cl_2, v/v) was used to remove the t-Boc group from the resin-bound amino acid residues, and TFA-treated resin was neutralized by using triethanolamine (Merck, Darmstadt) (10% in CH_2Cl, v/v).

Protected amino acids were coupled to free amino groups using an equimolar amount of diisopropylcarbodiimide (Aldrich-Chemie, Steinheim). The efficiency of each coupling reaction was followed using the ninhydrin assay [16].

The additional Gly and Cys residues were added to the carboxy-terminus to facilitate coupling of the peptide to the carrier protein, ovalbumin (Sigma, St. Louis, MO). Coupling was carried out using *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester (Sigma) as described by Liu et al. [17].

2.2. *Monoclonal antibodies*

For in vitro monoclonal antibody production, spleen cells from Balb/c mice (1.7×10^7 cells/ml) were incubated in the presence of free peptide (2 μg/ml) and macrophages in the thymus-conditioned medium for 5 days [18]. The immunized spleen cells were fused with myeloma cells by standard techniques [19]. Positive hybridomas, producing IgM-type antibodies, were selected by a solid-phase enzyme immunoassay using the ovalbumin-coupled peptide and human erythrocyte spectrins, which were isolated from freshly made red blood cell ghosts [20] by the low ionic elution method [21]. One of the clones, IV12DG9, reacting strongly in both assays was further characterized. For comparison, we also raised monoclonal antibodies against purified chicken gizzard α-actinin [22] by standard techniques. Cloning of the antibodies was performed manually. The culture supernatant was used for experiments.

2.3. *Polyacrylamide gel electrophoresis and immunoblotting*

For SDS-PAGE analysis cultured human and chicken embryonal fibroblasts, chicken embryo red blood cells, human red blood cells and various chicken embryo tissues (brain, muscle, and intestine) were extracted in 0.5% Triton X-100 (50 mM Tris-HCl, pH 7.4) at 0°C. After boiling in sample buffer [22] the specimens were separated in 6.5% slab gels [23]. Transfer onto nitrocellulose sheets was carried out according to Towbin et al. [24]. For immunostaining the sheets were exposed to the Mabs or to rabbit antibodies to calf lens fodrin [3,9]. After washing the sheets were exposed to peroxidase-coupled rabbit anti-mouse immunoglobulins or swine anti-rabbit IgG (Dakopatts, Glostrup, Denmark). The peroxidase reaction was developed as in [24].

3. RESULTS

In immunofluorescence, the IV12DG9 Mab gave a bright membrane-associated reactivity in both human (fig.1a) and chicken red blood cells (fig.1b). Immunoblotting revealed that in human red blood cells this Mab reacts strongly with both α- and β-spectrins (fig.2, lane 2) as well as with chicken α-spectrin (fig.2, lane 4). In chicken fibroblasts, it not only detects the 240 kDa α-fodrin (fig.2, lane 7), similarly to the antibodies against calf lens fodrin (fig.2, lane 8), but reacts with a prominent 100 kDa polypeptide. The latter can also be detected with an Mab to α-actinin (fig.1, lanes 7,9). Similarly, in human fibroblasts (fig.2, lane 11) and other mammalian non-

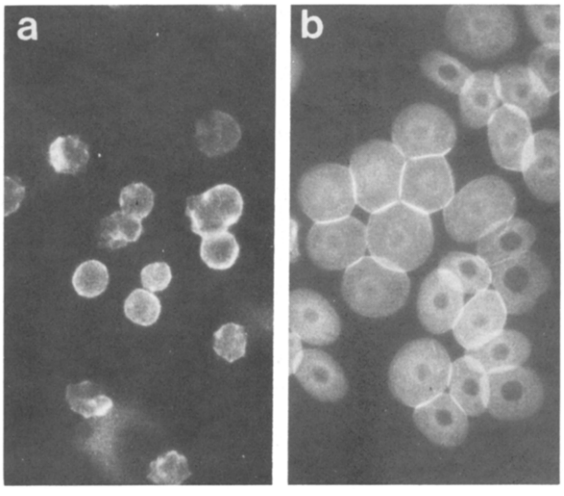

Fig.1. Indirect immunofluorescence staining of methanol-fixed cytocentrifuge preparations of human (a) and chicken erythrocytes (b) with the IV12DG9 antibody. Note the distinct membrane type of staining in both types of erythrocytes. × 800.

erythroid cells, the IV12DG9-Mab binds strongly to the 100 kDa α-actinin and also to α-fodrin. These are detected with the antibodies to α-actinin and calf lens fodrin, respectively (fig.2, lanes 12,13). In other chicken and mammalian tissues (brain, smooth muscle, cardiac muscle, intestine) immunoblotting revealed that our Mab only cross-reacts with the 240 kDa α-fodrin in addition to α-actinin (not shown).

The published sequences [9,12,14,25] show that although there are highly homologous regions in spectrin, fodrin and α-actinin that surround the SEDYG region in the repeat, a completely identical peptide is not present in the latter. Fig.3 compares some putative antigenic sites in α-actinin where our Mab may bind. The amino acids DYGK (fig.3, row 2) are homologous to the SEDYG region in α-fodrin (fig.3, row 1). Similarly, the amino acids EDYEK (fig.3, row 3) where glycine is changed to glutamic acid are homologous to SEDYG. Furthermore, there is a homologous tripeptide, LAS, present in both α-fodrin and α-actinin.

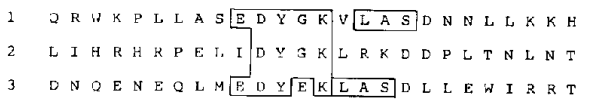

Fig.3. Two proposed antibody-binding sites on chicken α-actinin (amino acids 121–147, row 2 and 208–233, row 3) corresponding to chicken α-fodrin (amino acids 6–31, row 1). Putative antigenic determinants are boxed.

4. DISCUSSION

Early studies with polyclonal antibodies against mammalian spectrin failed to detect any spectrin-like proteins in nonerythroid cells (reviews [5–7]). This has been generally thought to be due to the complex nature of spectrin's immunologic reactivi-

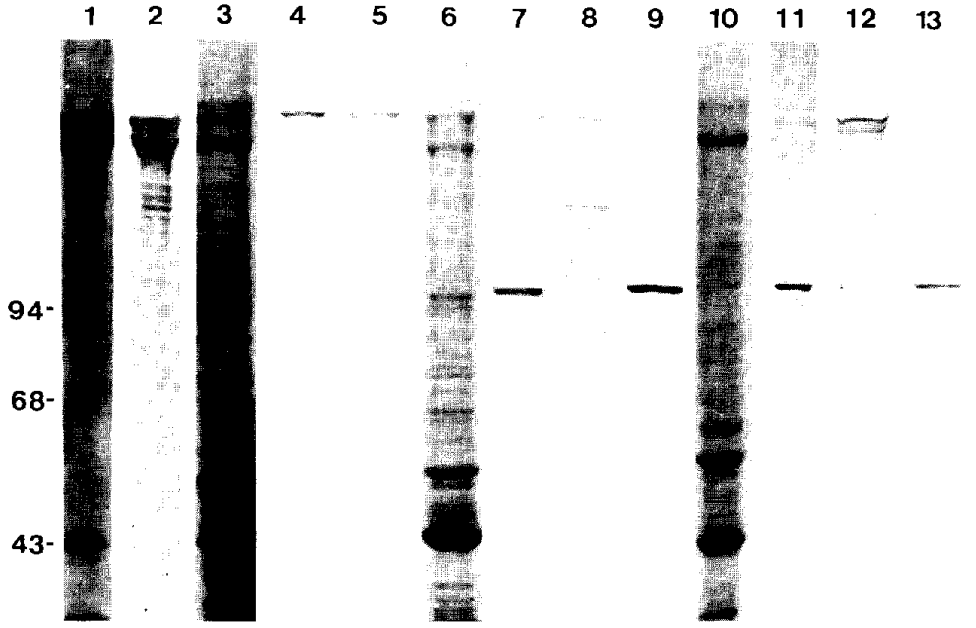

Fig.2. Immunoblotting analysis of SDS-PAGE separated chicken and human polypeptides recognized by the IV12DG9 Mab. Amido black was used for protein staining (lanes 1,3,6,10). Immunostaining with the IV12DG9-Mab revealed both α- and β-spectrins of human red blood cells (lane 2) but only α-spectrin in chicken erythroid cells (lane 4) as also anti-calf lens fodrin antibodies (lane 5). Among chicken fibroblast polypeptides the Mab identified α-fodrin (lane 7) as did the antibodies to calf lens fodrin (lane 8), and as well a prominent 100 kDa polypeptide also detected by the α-actinin Mab (lane 9). In human fibroblasts the monoclonal antibody also bound strongly to the prominent 100 kDa polypeptide and also to α-fodrin (lane 11) whereas antibodies to calf lens fodrin identified the α-fodrin as a distinct 240/235 kDa doublet (lane 12) and the α-actinin Mab revealed a similar 100 kDa polypeptide (lane 13).

ty [6] and to the narrow specificity of polyclonal antibodies [26]. Antibodies to synthetic peptides may in such a situation open up a new approach, because they are targeted to a defined sequence in the polypeptide [27]. Here, we have selected a conserved sequence of the chicken α-fodrin repeats for an antigen. The highly hydrophilic nature of this region and its probable folding to a β-turn suggested that it might be a good immunogen [27]. The sequence SEDYG is repeated three times in the cloned fragment of chicken α-fodrin [9] and once in the published fragment of human α-spectrin [12] where it occurs in a peptide identical to our antigen. Although in the α-actinin molecule there is only partial sequence homology with this sequence [14], our monoclonal antibody reacts with both α-actinin and some spectrins. This sequence homology must indicate that the shared domains in α-actinin and spectrin have very similar folds, otherwise the antibody would not detect these two proteins.

The identical immunoreactivities suggested to Lazarides and Nelson [28] that chicken α-fodrin might be identical to chicken α-spectrin. The present results support this possibility. However, it has also been suggested, mainly from analysis of peptide maps [8], that mammalian α-fodrin could be related closely to avian α-spectrin, whereas mammalian spectrins would be more distant from these. Interestingly, the present results would suggest that chicken α-spectrin and α-fodrin are closely related to mammalian erythroid α- and β-spectrins as well as to mammalian α-fodrin. The other members of the spectrin family may be more distinct proteins and therefore may not be revealed by our Mab.

Microfilaments, microtubules, intermediate filaments, nuclear lamina and spectrins together form a fibrillar complex in the cytoplasm of eukaryotic cells collectively called the cytoskeleton. Although all major components of the cytoskeleton appear to be distinct elements, recent studies have revealed homologies which may indicate a common evolutionary origin. Thus, nuclear lamins and intermediate filaments show considerable cross-reactivity in immunological studies as well as homology in sequence comparisons [29–31]. Similarly, some of the actin-associated proteins show similarities with actin itself which may suggest their common origin [32]. The sequence homology between spectrins and α-actinin [14], their common binding to actin [33,34] as well as a shared antigenic determinant found in this study, raise the question as to how closely these polypeptides are related structurally and functionally. Whether the Mab-binding sites might have any functional role in the cytoskeletal system remains to be established.

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REFERENCES

[1] Glenney, J.R. and Glenney, D. (1983) Cell Motil. 3, 671–678.
[2] Repasky, E.A., Granger, B.L. and Lazarides, E. (1982) Cell 29, 821–830.
[3] Lehto, V.-P. and Virtanen, I. (1983) J. Cell Biol. 96, 703–716.
[4] Virtanen, I., Badley, R.A., Paasivuo, R. and Lehto, V.-P. (1984) J. Cell Biol. 99, 1083–1091.
[5] Bennett, V. (1985) Annu. Rev. Biochem. 54, 273–304.
[6] Marchesi, V.T. (1985) Annu. Rev. Cell Biol. 1, 531–561.
[7] Virtanen, T., Thornell, L.-E., Damjanov, I., Damjanov, A., Hormia, M. and Lehto, V.-P. (1986) in: Membrane Skeletons and Cytoskeletal-Membrane Associations (Bennett, V. et al. eds) pp.635–647, Liss, New York.
[8] Glenney, J.R. and Glenney, P. (1984) Eur. J. Biochem. 144, 529–539.
[9] Wasenius, V.-M., Saraste, M., Knowles, J., Virtanen, I. and Lehto, V.-P. (1985) EMBO J. 4, 1425–1430.
[10] Birkenmeier, C.S., Bodine, D.M., Repasky, E.A., Helfman, D.M., Hughes, S.H. and Barker, J.E. (1985) Proc. Natl. Acad. Sci. USA 82, 5671–5675.
[11] MacMahon, A.P., Giebelhaus, D.H., Champion, J.E., Bailes, J.A., Lacey, S., Carritt, B., Henchman, S.K. and Moon, R.T. (1987) Differentiation 34, 68–78.
[12] Speicher, D.W. and Marchesi, V.T. (1984) Nature 311, 177–180.

[13] Speicher, D.W. (1986) J. Cell. Biochem. 30, 245–258.
[14] Wasenius, V.-M., Närvänen, O., Lehto, V.-P. and Saraste, M. (1987) FEBS Lett. 221, 73–76.
[15] Merrifield, R.B. (1964) Biochemistry 3, 1385–1390.
[16] Sarin, U.K., Kent, B.H., Tam, J.P. and Merrifield, R.B. (1981) Anal. Biochem. 117, 147–157.
[17] Liu, F., Zinnecker, M., Homaska, T. and Katz, D.H. (1979) Biochemistry 18, 690–697.
[18] Luben, R.A. and Möller, M.A. (1980) Mol. Immunol. 17, 635–639.
[19] Köhler, G. and Milstein, C. (1976) Eur. J. Immunol. 6, 511–519.
[20] Dodge, J., Mitchell, D. and Hanahan, D. (1963) Arch. Biochem. Biophys. 100, 119–130.
[21] Gratzer, W.B. (1981) Methods Enzymol. 85, 475–480.
[22] Feramisco, J.R. and Burridge, K. (1980) J. Biol. Chem. 255, 1194–1199.
[23] Laemmli, U.K. (1970) Nature 227, 680–682.
[24] Towbin, H., Staehelin, R. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[25] Baron, M.D., Davison, M.D., Jones, P., Patel, B. and Critchley, D.R. (1987) J. Biol. Chem. 262, 2558–2561.
[26] Harris, A.S., Green, L.A.D., Ainger, K.J. and Morrow, J.S. (1985) Biochim. Biophys. Acta 830, 147–158.
[27] Westhof, E., Altschuh, D., Moras, D., Bloomer, A.C., Mondragon, A., Klug, A. and Van Regenmortel, M.H.V. (1984) Nature 311, 123–128.
[28] Lazarides, E. and Nelson, W.G. (1982) Cell 31, 505–508.
[29] Franke, W.W. (1987) Cell 48, 3–4.
[30] Parry, D.A.D., Conway, J.F. and Steinert, P.M. (1986) Biochem. J. 238, 305–308.
[31] Aebi, U., Cohn, J., Buhle, L. and Gerace, L. (1986) Nature 323, 560–564.
[32] Maruta, H., Knoerzer, W., Hinssen, H. and Isenberg, G. (1984) Nature 312, 424–429.
[33] Weeds, A. (1982) Nature 296, 811–816.
[34] Stossel, T.P., Chaponnier, C., Ezzell, R.M., Hartwig, J.H., Janmey, P.A., Kwiatkowski, D.J., Lind, S.E., Smith, D.B., Southwick, F.S., Yin, H.L. and Zaner, K.S. (1985) Annu. Rev. Cell Biol. 1, 353–402.