Properties of brain spectrin (fodrin)

N.R. Burns, V. Ohanian and W.B. Gratzer

Medical Research Council Cell Biophysics Unit, King's College, Drury Lane, London WC 2B 5RL, England

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Fodrin, a protein from bovine brain, immunologically related to spectrin, is shown, unlike some other proteins of generally similar appearance in the electron microscope, to resemble spectrin closely in its most distinctive structural characteristic, the very high α-helix content. Like spectrin, it is also insoluble below pH 5. One of the subunits only is phosphorylated by the cAMP-independent red cell membrane kinase, that phosphorylates the smaller subunit of spectrin. Fodrin also forms a ternary complex with F-actin and the third constituent of the red cell membranes cytoskeleton, protein 4.1. In the presence of 4.1 the interaction between fodrin and F-actin is enhanced. It is surmised that fodrin plays an analogous functional role in neuronal cells to that of spectrin in the red cell.

Spectrin *Fodrin* *Cytoskeleton* *Actin-binding protein* *Membrane*

1. INTRODUCTION

It has recently emerged that spectrin, the high-M_r protein of the membrane-associated cytoskeleton of the mammalian red blood cell, has counterparts in other cell types, by the criterion of immunological cross-reactivity [1,2]. Proteins, immunologically related to spectrin and sharing certain of its physical characteristics, have been isolated from brain [3–5] and from intestinal microvilli [6]. They contain two different polypeptide chains, resemble spectrin in the electron microscope, though the contour length of brain spectrin (termed fodrin [4] or calspectin [3]) appears to be appreciably greater than that of red cell spectrin [5], and they bind calmodulin and F-actin [3–5], as well as, in the case of fodrin, attaching to the spectrin binding sites (ankyrin) on red cell membrane vesicles [4,7]. Fodrin also has in common with spectrin a high ionic-strength dependence of Stokes' radius [7]. Nevertheless, the extent of the structural and functional similarity of spectrin to fodrin is not established. Thus for example, fodrin exists as a tetramer, difficult to dissociate into its constituent dimers under non-denaturing conditions [4,5], whereas spectrin tetramers dissociate readily into their heterodimer parts; fodrin appears to bind strongly to F-actin, whereas human spectrin at least binds only weakly in the absence of a third cytoskeletal protein, 4.1 [8]. Moreover, attention has in the past been drawn to the similarity of gross shape of spectrin to a number of other actin-binding proteins [9], that in other respects appear to be unrelated. One unusual structural feature of spectrin, the very high α-helix content (70–80%), is not shared by these proteins, and indeed a high-M_r species that cross-reacts immunologically with spectrin, and was thought to belong to the same class, has been reported to have a vanishingly low α-helicity [10].

Our purpose here is to examine fodrin with respect to the most characteristic properties of red cell spectrin, so as to establish the likely extent of functional analogy between them.

2. MATERIALS AND METHODS

Fodrin was prepared from fresh bovine brain, obtained from the slaughterhouse, by a procedure based on that in [4]. A myelin-depleted crude membrane fraction was prepared and washed as described in [4], and the membranes were extracted for 30 min at 37°C with 0.2 mM sodium EGTA, 0.5 mM dithiothreitol and 0.3 mM phenylmethylsulphonyl fluoride (pH 8.0). The membrane debris were removed by centrifugation and the superna-

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tant was made 26% (w/v) in ammonium sulphate. The precipitated protein was collected and dialysed against 1 M urea, 20 mM glycine, 1 mM sodium azide, 0.2 mM dithiothreitol (pH 7.0) and then fractionated on a Sepharose 4B column, equilibrated with the same buffer. Fractions were screened for purity by gel electrophoresis in the presence of sodium dodecyl sulphate.

Actin from chicken breast muscle was prepared as in [11]. Protein 4.1 from red cells was prepared by a simplified procedure, based on the dissociation of Triton-insoluble cytoskeletons [12] with 1 M Tris (pH 7.6) followed by gel filtration chromatography (details to be published). Binding studies were performed by incubating F-actin at 0.1 mg/ml with fodrin at 0.1 mg/ml and protein 4.1 at 0.07 mg/ml at 25°C in 0.1 M sodium chloride, 30 mM Tris, 0.1 mM magnesium chloride, 0.1 mM ATP, 0.5 mM dithiothreitol (pH 7.8) for 45 min. The mixtures were then centrifuged at 100000 × g in a Beckman Airfuge for 25 min. This brings down the F-actin. The supernatants and pellets were made 1% in sodium dodecyl sulphate, heated for 5 min in a boiling water bath and examined by gel electrophoresis [13]. Gels were stained with Coomassie brilliant blue and evaluated by microdensitometry.

To examine the phosphorylation of fodrin, a preparation of cAMP-independent red cell membrane kinase [14], containing 2 mg protein/ml, was added at 80 μl/ml of fodrin (0.35 mg/ml), together with 0.5 μCi [γ-^{32}P]ATP (825 μCi/mmol). After incubation at 37°C for 30 min, the protein was subjected to gel electrophoresis in a high-resolution system [15] in the presence of SDS. The gel was stained and then examined by autoradiography.

Concentrations of fodrin were determined by micro-Kjeldahl colorimetric nitrogen analysis [16] or by spectrophotometry at 205 and 210 nm [17,18]. Circular dichroism was measured in a Cary 61 instrument.

3. RESULTS AND DISCUSSION

As judged by gel electrophoresis, the fodrin prepared from bovine brain resembled that of porcine, and consisted of two components present in equal proportion. Circular dichroism showed the conformation to be very similar to that of spectrin, with a molar residue ellipticity at 222 nm of 25600 deg.cm^2.dmol^{-1}, indicative of some 70% α-helix (fig.1). By contrast with the immunologically related microtubule-associated protein [19], examined in [10], therefore, there is a strong presumption that fodrin is in gross structural terms very similar to red cell spectrin.

A further characteristic of spectrin is its insolubility below pH 5 [20]. Fodrin solutions, adjusted to pH 5 or below, exhibit similar isoelectric precipitation.

An important property of spectrin is that it is a substrate for the cAMP-independent kinase of the red cell membrane [21], only the smaller subunit being phosphorylated. Fig.2 shows that the same kinase also phosphorylates one subunit only of fodrin, which therefore presumably contains an analogous tract of polypeptide sequence.

It is already known [4,6] that tetrameric fodrin will bind to and cross-link F-actin. In the case of human red cell spectrin [8] (though not necessarily that from other species, such as the sheep [22]), binding to F-actin is very weak, unless the

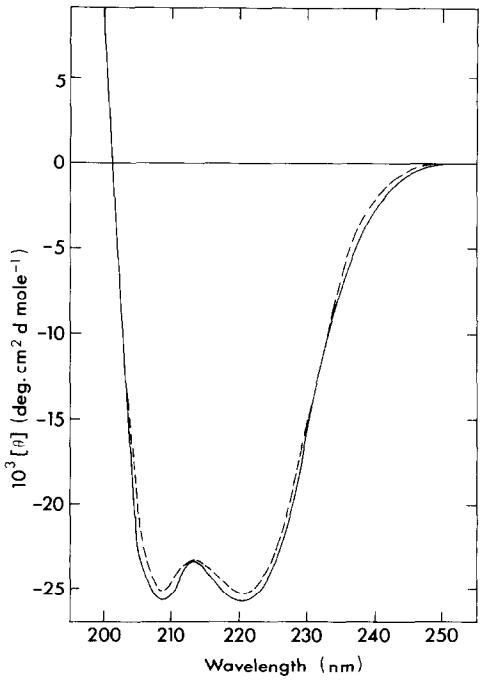

Fig.1. Ultraviolet circular dichroism of bovine brain fodrin (——) and human red cell spectrin dimer (– – –), showing similarity of conformation.

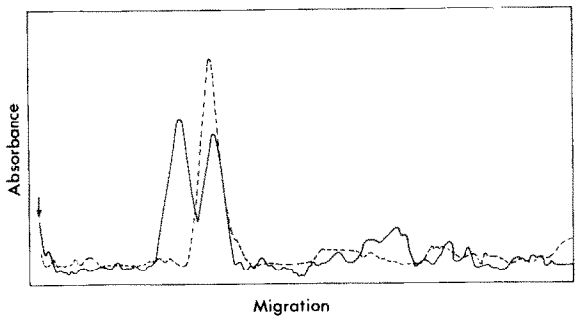

Fig.2. Phosphorylation of fodrin by red cell membrane cAMP-independent kinase. Polyacrylamide gel electrophoresis of protein after treatment with kinase and [γ-^{32}P]ATP. Electrophoresis was done in a 6% gel in the discontinuous buffer system in [14]. Microdensitometer trace: (——) of the stained gel; (– – –) the corresponding autoradiograph; (→) origin.

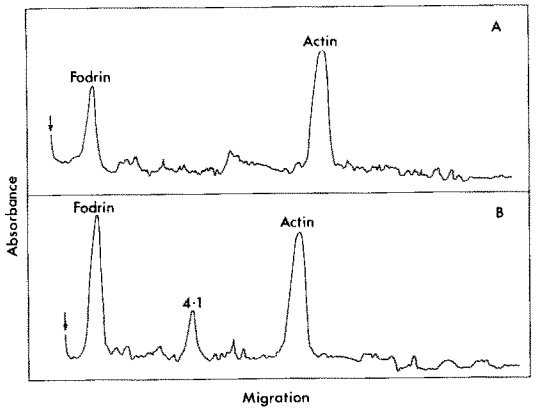

Fig.3. Formation of ternary complex between fodrin, F-actin and protein 4.1 from human red cell membrane. Gel electrophoresis of pellets recovered from incubated mixture of F-actin and fodrin by centrifugation (A), and from mixture of F-actin, fodrin and 4.1 (B). Note the presence of 4.1 in the pellet in B, and also the increased amount of fodrin carried down. In this electrophoretic system [13] the two components of the fodrin are not resolved; (→) Origin.

cytoskeletal constituent, protein 4.1 is present. The formation of a tight ternary complex between F-actin, spectrin and 4.1 is thus one of the most distinctive functional attributes of spectrin. Fig.3 shows that when a mixture of F-actin, fodrin and red cell 4.1 are incubated together, a ternary complex is pelleted. Moreover, the amount of fodrin brought down is markedly increased, indicating that the binding is significantly stronger in the presence of 4.1.

The function of fodrin in neuronal cells is not known. However, it may be conjectured that it is required for the maintenance of mechanical rigidity of the cell and stability of the plasma membrane, by analogy to the purpose served by spectrin in the red cell, in which the mechanical demands are undoubtedly much more stringent. In this light, it is reassuring that the structural characteristics of the two proteins, on which the mechanical properties conferred by the cytoskeletal network on the membrane must depend, are similar, and also that the pattern of functionally important interactions is conserved. It should be borne in mind that fodrin also retains the property of binding to ankyrin, the membrane-attachment protein for spectrin [4,7], as well as to calmodulin [4,5].

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REFERENCES

[1] Goodman, S.R., Zagon, I.S. and Kulikowski, R.R. (1981) Proc. Natl. Acad. Sci. USA 78, 7570–7574.
[2] Repasky, E.A., Granger, B.L. and Lazarides, E. (1982) Cell 29, 821–823.
[3] Kakiuchi, S., Sobue, K., Kanda, K., Morimoto, K., Tsukita, S., Tsukita, S., Ishikawa, H. and Kurokawa, M. (1982) Biomed. Res. 3, 400–410.
[4] Bennett, V., Davis, J. and Fowler, W.E. (1982) Nature 299, 126–131.
[5] Glenney, J.R., Glenney, P. and Weber, K. (1982) J. Biol. Chem. 257, 9781–9787.
[6] Glenney, J.R., Glenney, P., Osborn, M. and Weber, K. (1982) Cell 28, 843–854.
[7] Burridge, K., Kelly, T. and Mangeat, P. (1982) J. Cell Biol. 95, 478–486.
[8] Ungewickell, E., Bennett, P.M., Calvert, R., Ohanian, V. and Gratzer, W.B. (1979) Nature 280, 811–814.
[9] Tyler, J.M., Anderson, J.M. and Branton, D. (1980) J. Cell Biol. 85, 489–495.
[10] Voter, W.A. and Erickson, H.P. (1982) J. Ultrastruct. Res. 80, 374–382.

[11] Spudich, J.A. and Watt, S.J. (1971) J. Biol. Chem. 246, 4866–4871.
[12] Yu, J., Fischman, D.A. and Steck, T.L. (1975) J. Supramol. Struct. 1, 233–248.
[13] Kendrick-Jones, J., Szentkiralyi, E.M. and Szent-Györgyi, A.G. (1976) J. Mol. Biol. 104, 747–775.
[14] Laemmli, U.K. (1970) Nature 227, 680–685.
[15] Tao, M., Conway, R. and Cheta, S. (1980) J. Biol. Chem. 255, 2563–2568.
[16] Jaenicke, L. (1974) Anal. Biochem. 61, 623–627.
[17] Scopes, R.K. (1974) Anal. Biochem. 59, 277–282.
[18] Tombs, M.P., Soutar, F. and Maclagan, N.F. (1959) Biochem. J. 73, 167–171.
[19] Davis, J. and Bennett, V. (1982) J. Biol. Chem. 257, 5816–5820.
[20] Gratzer, W.B. and Beaven, G.H. (1975) Eur. J. Biochem. 58, 403–409.
[21] Hosey, M.M. and Tao, M. (1976) Biochemistry 15, 1561–1568.
[22] Brenner, S.L. and Korn, E.D. (1979) J. Biol. Chem. 254, 8620–8627.