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A CYTOSKELETAL PROTEIN TALIN FROM GIZZARD SMOOTH MUSCLE

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Please refer to Folio 36A.

INTRODUCTION

Talin is a 230-kDa cytoskeletal protein localized at the membrane/cytoskeleton attachment sites in association with the extracellular matrix receptor integrins (Burridge and Connel, 1983a; 1983b; Horwitz *et al.*, 1986; Burridge *et al.*, 1988; Isenberg and Goldmann, 1992). In muscle tissues, talin is localized at the myotendinous junctions (Tidball *et al.*, 1986) and intercalated discs (Belkin *et al.*, 1986) and therefore it is assumed to play a key role in the organization of the tissue architecture. Recently, talin has been shown to bind directly to both G- and F-actin and to increase the velocity and extent of polymerization of actin greatly (Muguruma *et al.*, 1990; Goldmann and Isenberg, 1991; Kaufmann *et al.*, 1991). Furthermore, talin has been shown to possess a low level of activity to cross-link actin filaments (Muguruma *et al.*, 1992). These results appear to reinforce the crucial role of talin in organizing the supramolecular architecture at the membrane/cytoskeleton attachment sites. It is also known that it undergoes rapid proteolysis by Ca^{2+}-dependent protease (CDP) in vitro and even in living cells under some conditions (Fox *et al.*, 1985: Beckerle *et al.*, 1987). In order to study on the molecular level the roles of talin in the organization of the membrane/ cytoskeleton attachment sites and the effects of its proteolytic degradation on these tissue architectures, the interactions of talin and its proteolytic fragments with other cytoskeletal proteins were studied in this report.

MATERIALS AND METHODS

Talin was purified from chicken gizzard as described by Molony *et al.* (1987) with the following modifications: Talin was extracted from washed myofibrils by incubating over night at 4°C in a buffer containing 10mM Tris-HCl (pH9.1), 1mM EDTA, 1mM NaN_3, 5mM 2-mercaptoethanol and 0.5mM phenylmethylsulfonyl fluoride (PMSF) instead of incubating at 37°C for 30 minutes and the subsequent step of actin precipitation by 10mM $MgCl_2$ was omitted. Actin was purified from rabbit skeletal muscle by the method of Spudich and Watt (1971) and purified further by gel filtration on a Sephacryl S-200 column equilibrated with a G-actin buffer. α-Actinin was prepared from chicken gizzard as described by Feramisco and Burridge (1980). CDP was purified from chicken gizzard as described by Szpacenko *et al.* (1981). Sodium dodesyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed as described by Laemmli (1970) using 7.5-17.5% gradient slab gels unless otherwise noted. High shear viscosity of actin solution was measured at 25°C by using an Ostwald viscometer with a buffer flow time of about 31 seconds and the low shear viscosity was measured by using a falling ball viscometer (Maclean-Fletcher and Pollard, 1980). Anti-chicken gizzard talin antibodies were prepared by injecting purified talin into rabbit back subcutaneously. For immunoblots of talin and its proteolytic fragments, various freshly dissected chicken tissues were homogenized extensively in Waring blender with 2.5 volumes of 50mM Tris-acetate buffer (pH7.5) containing 10mM EDTA, 5mM 2-mercaptoethanol, 0.5mM PMSF and 100mg/l trypsin inhibitor, boiled immediately in an SDS-PAGE sample buffer and then subjected to SDS-PAGE and immunoblots.

RESULTS AND DISCUSSION

Talin purified from chicken gizzard as described above consists of a 230-kDa polypeptide (Figure 1a). Upon limited digestion of talin with CDP, the 230kDa polypeptide was cleaved into two polypeptides of about 190 and 47kDa. We have shown previously that the 190kDa fragment but not the 47kDa fragment possesses the ability to bind to F-actin (Muguruma *et al.*, 1990). When the 190 and 47kDa fragments were mixed with actin in the presence of α-actinin and centrifuged, large fractions of both α-actinin and 190kDa fragments were sedimented with actin, while the 47kDa fragment remained in the supernatant (Figure 1b). The proportion of the α-actinin that cosedimented with F-actin in the presence of the 190kDa fragment did not differ to a significant extent from that cosedimented with F-actin in the absence of the fragment (data not shown). Similarly, the proportion of the 190kDa fragment that cosedimented with F-actin in the presence of α-actinin was also similar to that obtained in the absence of α-actinin. These results appeared to indicate that α-actinin and the 190kDa fragment did not interact with each other and that these proteins interacted with actin in different manners.

Effects of talin and the 190kDa fragment on the polymerization of actin are shown in Figure 2. The 190-kDa fragment stimulated both the initial velocity and final extent of polymerization of actin. The magnitudes of these effects of the fragment were higher than those of intact talin, whereas the reason for this is not known.

Effects of talin and the 190kDa fragments on gelation of actin are shown in Figure 3. Whereas talin alone or the 190kDa fragment alone (not shown) did not exhibit a significant level of actin-gelating activity, they augmented the gelation of actin in the presence of low concentrations of α-actinin. The effect of the 190kDa fragment was slightly stronger than that of intact talin.

The actin-gelation activity of talin and the 190kDa fragment shown in Figure 3 is likely to be ascribed to the multi-valent actin-binding capacity of these proteins, as known for several other actin-cross-linking proteins such as spectrin and filamin (Korn,1982; Pollard and Cooper, 1986; Burridge *et al.*, 1990). On SDS-PAGE of the 190kDa fragment that had been incubated with a cross-linking reagent 1-ethyl-3[3-(dimethylamino)propyl] carbodiimide (EDC), the 190kDa band disappeared from the gel and a few higher molecular aggregates were detected (Figure 4) indicating that the fragment is present as dimeric or oligomeric molecules in the medium. These oligomeric molecules may serve to cross-link actin filaments, even if the fragment contains only one actin-binding region on each monomeric molecule.

Immunoblots with anti-chicken gizzard talin antibodies revealed the presence of immuno-reactive 230kDa polypeptide in freshly prepared crude homogenates from various chicken tissues including skeletal and cardiac muscles, magnum, isthmus, shell gland, brain, eye and gizzard (Figure 5). It was noted, however, that almost every tissue extract contained immuno-reactive, approximately 190kDa polypeptide and minor smaller polypeptides in addition the intact 230kDa talin polypeptide. The levels of the immuno-reactive 190kDa polypeptide in most of the tissue extracts were very high and were comparative to or even higher than those of the 230kDa bands. These results appear to indicate that talin was very susceptible to proteolytic fragmentation, whereas it is not known what proportion of the 190kDa polypeptide was present within the living cells (Fox *et al.*, 1985; O'Halloran and Burridge, 1986).

The 190kDa fragment of talin retained full or higher levels of actin-nucleation and actin-gelation activities that the intact talin possessed. It is apparent that both of these activities associated with talin are due to the function of the C-terminal 190kDa fragments. It has been reported that the 190kDa fragment bind to integrin (Horwitz *et al.*, 1986). The vinculin-binding site has been localized on two regions located between residues 483 and 1652 and between residues 1653 to 1848 of talin (Lee *et al.*, 1992). The role of the 47kDa region of talin is not known. The amino acid sequence of the 47kDa region has been reported to be homologous to those of several other cytoskeletal proteins which are localized underneath the plasma membranes such as spectrin, ezrin and band 4.1 protein (Rees *et al.*, 1990). Recently Goldmann *et al.* (1992) have shown that talin interact with lipid vesicles in vitro. The 47kDa domain is thus likely to have some role in interacting with membranes.

CONCLUSION

The 190kDa domain of talin possessed comparable or higher level capacities to interact with actin to those of the intact talin possessed in stimulating nucleation of actin and crosslinking of actin filaments. Considerable levels of the 190kDa fragment of talin were present in freshly prepared crude extracts from various chicken tissues including smooth muscle tissues. Talin may play multiple roles in organizing membrane/cytoskeleton attachment sites through the C-terminal, 190kDa domain, and there is a possibility that the proteolytically generated 190kDa fragment may be functioning even in living cells to a certain extent.

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