A New Model for the Interaction of Dystrophin with F-actin

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Abstract. The F-actin binding and cross-linking properties of skeletal muscle dystrophin-glycoprotein complex were examined using high and low speed cosedimentation assays, microcapillary falling ball viscometry, and electron microscopy. Dystrophin–glycoprotein complex binding to F-actin saturated near 0.042 ± 0.005 mol/mol, which corresponds to one dystrophin per 24 actin monomers. Dystrophin–glycoprotein complex bound to F-actin with an average apparent K_d for dystrophin of 0.5 μM. These results demonstrate that native, full-length dystrophin in the glycoprotein complex binds F-actin with some properties similar to those measured for several members of the actin cross-linking superfamily of proteins. However, we failed to observe dystrophin–glycoprotein complex-induced cross-linking of F-actin by three different methods, each positively controlled with α-actinin. Furthermore, high speed cosedimentation analysis of dystrophin–glycoprotein complex digested with calpain revealed a novel F-actin binding site located near the middle of the dystrophin rod domain. Recombinant dystrophin fragments corresponding to the novel actin binding site and the first 246 amino acids of dystrophin both bound F-actin but with significantly lower affinity and higher capacity than was observed with purified dystrophin–glycoprotein complex. Finally, dystrophin–glycoprotein complex was observed to significantly slow the depolymerization of F-actin, suggesting that dystrophin may lie along side an actin filament through interaction with multiple actin monomers. These data suggest that although dystrophin is most closely related to the actin cross-linking superfamily based on sequence homology, dystrophin binds F-actin in a manner more analogous to actin side-binding proteins.

THE gene defective in Duchenne muscular dystrophy encodes a number of alternatively promoted and spliced transcripts which give rise to several structurally distinct proteins collectively referred to as dystrophins (Ahn and Kunkel, 1993). Striated muscle dystrophin is predominantly expressed as a 427 kD, four domain protein with the first three domains exhibiting significant sequence homology with the cytoskeletal proteins α-actinin and spectrin (Koenig et al., 1988; Matsudaira, 1991): an amino-terminal, putative actin-binding domain; a rod-like domain comprised of 24 triple helical coiled coil repeats; a cysteine-rich domain and a carboxy-terminal domain. Skeletal muscle dystrophin has been isolated as part of a large, tightly associated oligomeric complex of sarcolemmal proteins commonly referred to as the dystrophin–glycoprotein complex (Ervasti et al., 1990; Yoshida and Ozawa, 1990; Ervasti and Campbell, 1991). Integral and peripheral membrane components of the glycoprotein complex bind to the cysteine-rich and/or carboxy-terminal domains of dystrophin (Suzuki et al., 1992; Kramarcy et al., 1994; Suzuki et al., 1994), ultimately linking dystrophin to a highly glycosylated, extracellular component named α-dystroglycan (Ibraghimov-Beskrovnaya et al., 1992). α-Dystroglycan in turn binds to the laminin family of proteins (Ibraghimov-Beskrovnaya et al., 1992; Ervasti and Campbell, 1993*a*; Sunada et al., 1994; Pall et al., 1996). Many studies have demonstrated that both intact dystrophin (Ervasti and Campbell, 1993*a*; Fabbrizio et al., 1993; Senter et al., 1993; Lebart et al., 1995) and recombinant proteins corresponding to its amino-terminal domain (Hemmings et al., 1992; Way et al., 1992; Fabbrizio et al., 1993; Corrado et al., 1994; Jarrett and Foster, 1995) are capable of binding F-actin. These data support a model for the dystrophin-glycoprotein complex in linking the actin-based, cortical cytoskeleton with the extracellular matrix (Ervasti and Campbell, 1993*b*). The importance of the dystrophin–glycoprotein complex is evident from numerous studies demonstrating that the loss of or deletions within dystrophin, components of the glycoprotein complex or laminin-2, can all lead to forms of muscular dystrophy (Campbell, 1995; Worton, 1995). However, the exact cellular function(s) of the dystrophin–glycoprotein complex remains to be elucidated.

The transmembrane linkage formed by the dystrophin–glycoprotein complex may play a structural role in main-

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taining sarcolemmal membrane integrity during contraction (Menke and Jockusch, 1991; Stedman et al., 1991; Petrof et al., 1993; Pasternak et al., 1995). As an alternative, or perhaps additional function, the dystrophin–glycoprotein complex may further serve to organize or modulate other proteins involved in signal transduction (Sealock and Froehner, 1994; Yang et al., 1995; Brenman et al., 1996). Regarding a structural function, it is not clear whether or how the linear association of proteins comprising the dystrophin–glycoprotein complex may be further organized into higher ordered networks. Since full-length dystrophin is most closely related to the actin cross-linking superfamily of proteins based on sequence homology (Koenig et al., 1988; Matsudaira, 1991), it has been speculated that dystrophin may form antiparallel dimers that cross-link different actin filaments, ultimately forming a hexagonal lattice (Koenig and Kunkel, 1990; Ahn and Kunkel, 1993). Alternatively, there may exist additional actin binding sites within dystrophin yielding a bivalent monomer that cross-links actin filaments in a manner similar to fimbrin (Matsudaira, 1991). However, neither of these possibilities has been examined experimentally.

To address these issues, we have quantitatively characterized the F-actin binding properties of purified dystrophin–glycoprotein complex and show that purified skeletal muscle dystrophin–glycoprotein complex binds F-actin with some biochemical properties similar to several other native members of the actin cross-linking superfamily. However, in contrast with the other members of the F-actin cross-linking superfamily of proteins, dystrophin in the glycoprotein complex does not appear to cross-link F-actin. Furthermore, we present evidence for a novel actin binding site located near the middle of the dystrophin rod domain. Finally, we observed that dystrophin–glycoprotein complex significantly slows depolymerization of F-actin. Taken together, our results suggest that dystrophin may bind along side an actin filament with 0.5 μM affinity via multiple lower affinity binding sites distributed throughout its amino-terminal and rod domains.

Materials and Methods

Purification of Dystrophin–Glycoprotein Complex

Dystrophin–glycoprotein complex was prepared from KCl-washed rabbit skeletal muscle membranes as previously described (Ervasti et al., 1991) with slight modifications. In brief, the digitonin solubilate obtained from 1 g of KCl-washed membranes was circulated overnight on a 60-ml succinylated WGA-agarose column (Vector Laboratories, Burlingame, CA) at 4°C. The succinylated WGA-agarose column was washed with 224 ml of buffer A (0.1% digitonin, 50 mM Tris-HCl, pH 7.4, 0.75 mM benzamidine, 0.1 mM PMSF) containing 0.5 M NaCl, followed by 224 ml of buffer A, pH 7.8, and finally eluted with 224 ml of buffer A, pH 8.0, containing 0.3 M *N*-acetylglucosamine. The *N*-acetylglucosamine eluate was adjusted to pH 7.4 with HCl and applied to a 3-ml DEAE-cellulose (Whatman DE52) column. The column was step-eluted with buffer A containing the following NaCl gradient profile: 0 M (20 ml), 0.08 M (20 ml), 0.08–0.11 M (4 ml), 0.11 M (20 ml), 0.11–0.18 M (4 ml), 0.18 M (20 ml), 0.18–1 M (4 ml), 1 M (28 ml). The dystrophin–glycoprotein complex eluted with 0.18 M NaCl was concentrated to 2.5 ml in a Centriplus 100 (Amicon, Beverly, MA), loaded onto a PD-10 column (Pharmacia Biotech, Piscataway, NJ), and eluted with 3.5 ml of actin binding buffer (10 mM Tris-HCl, pH 8.0, 1 mM NaN_3, 0.1 mM ATP, 0.1 M NaCl, 2 mM $MgCl_2$, 0.2 mM dithiothreitol 0.1% digitonin, 1 mM EGTA). Dystrophin–glycoprotein complex was also prepared from KCl-washed rabbit skeletal muscle membranes solubilized with 1% Triton X-100 in the presence of 0.15 M NaCl. Chromatographic procedures were performed as described above except that all buffers contained 0.1% Triton X-100 instead of 0.1% digitonin and the succinylated WGA-agarose column was washed with 224 ml of buffer A containing 0.15 M NaCl instead of 0.5 M NaCl. Purified dystrophin–glycoprotein complex was concentrated to 0.2–0.3 ml in a Centricon 100 (Amicon) and assayed for protein with the Bio-Rad DC Protein Assay kit using BSA as standard. The dystrophin concentrations referred to in the text and figure legends were based on the measured dystrophin–glycoprotein complex protein concentration, the observation that dystrophin comprises 50% of the Coomassie Blue–stained protein in electrophoretically separated dystrophin-glycoprotein complex (Yoshida and Ozawa, 1990; Ervasti and Campbell, 1991) and the predicted molecular weight of dystrophin as 427,000 (Koenig et al., 1988).

Purification of Actin

Actin was purified from rabbit skeletal muscle using the method of Pardee and Spudich (1982) and further purified by gel filtration on a Sephacryl S-200 column (Pharmacia Biotech) according to MacLean-Fletcher and Pollard (1980*a*). Gel filtered G-actin was either concentrated from 15 ml to ~0.5 ml in a Centricon 10 (Amicon) or polymerized to F-actin and concentrated by sedimentation at 100,000 *g* and resuspended in 0.5–1.0 ml of 2 mM Tris-HCl, pH 8.0, 1 mM NaN_3, 0.1 mM ATP, 0.2 mM $CaCl_2$, 50 mM KCl, 2 mM $MgCl_2$. Actin concentration was determined by absorbance using $E_{290} = 0.65$ cm²/mg (MacLean-Fletcher and Pollard, 1980*a*).

High Speed Sedimentation Assay

Various amounts of purified dystrophin–glycoprotein complex in actin binding buffer were mixed with concentrated F-actin in a total volume of 60 μl and incubated for 30 min at room temperature. The samples were subjected to centrifugation at 100,000 *g* for 20 min, supernatants carefully decanted and the pellets redissolved in an equivalent volume of actin binding buffer. To quantify the amount of dystrophin-glycoprotein complex bound to F-actin, equal volumes of the supernatants and resuspended pellets were separated electrophoretically on 3–12% SDS–polyacrylamide gels (Ervasti and Campbell, 1993*a*). Coomassie blue–stained gels were analyzed densitometrically using a Bio-Rad Model GS-670 Imaging Densitometer. The intensities of the scanned bands were quantitated by volume integration after background subtraction. Binding data were analyzed by nonlinear regression analysis with the computer program Kinetics (Brooks, 1992). Dystrophin–glycoprotein complex binding to F-actin was also measured in the presence of 5 μM bovine brain calmodulin (Calbiochem Novabiochem, San Diego, CA), or wheat germ calmodulin (the kind gift of Dr. Gale Strasburg) in actin binding buffer or in actin binding buffer containing 0.2 mM $CaCl_2$ instead of EGTA.

Low Speed Sedimentation Assay

Chicken gizzard α-actinin (Sigma) or dystrophin-glycoprotein complex in 10 mM Tris-HCl, pH 8.0, 1 mM NaN_3, 0.1 mM ATP, 0.2 mM dithiothreitol and 0.1% digitonin, was mixed at the desired molar ratio with G-actin (final concentration 6.5 μM) in a total volume of 60 μl. Actin polymerization was initiated by the addition of KCl to 100 mM and $MgCl_2$ to 2 mM. After incubation for 1 h at room temperature, the samples were subjected to centrifugation at 20,000 *g* for 20 min (Meyer and Aebi, 1990) followed by analysis of the supernatants and resuspended pellets on Coomassie blue-stained SDS–polyacrylamide gels.

Measurement of Viscosity

Viscosity measurements were made by microcapillary falling ball viscometry as described by MacLean-Fletcher and Pollard (1980*b*). Concentrated G-actin was mixed at the desired molar ratio with α-actinin or dystrophin–glycoprotein complex (final actin concentration 6 μM) in 10 mM Tris-HCl, pH 8.0, 1 mM NaN_3, 0.1 mM ATP, 0.2 mM dithiothreitol, 0.1% digitonin, 100 mM KCl, and 2 mM $MgCl_2$, and in the absence or presence of 9 μM PIP_2 (the kind gift of Dr. Richard Anderson, University of Wisconsin, Madison, WI). Immediately after adding actin, the samples were drawn into 100-μl capillary tubes (VWR Scientific, Inc., Philadelphia, PA) and held horizontally. After incubation for 1 h, the velocity of a 0.64-mm diameter stainless steel ball (kindly provided by Dr. Tom Pollard, Salk Institute, La Jolla, CA) was measured at 25°C by recording the time required for the ball to travel 2-cm through the capillary tube inclined at 80°C. The viscosity values were obtained after calibration of the viscometer against various concentrations of glycerol (MacLean-Fletcher and Pollard, 1980*b*).

Electron Microscopy

3 μM G-actin was polymerized for 30 min at room temperature in actin binding buffer in the absence or presence of 0.42 mg/ml dystrophin–glycoprotein complex (0.5 μM dystrophin) or 0.110 mg/ml (0.5 μM) α-actinin. Samples were applied to carbon-coated copper grids and negatively stained with 2% uranyl acetate before examination in a Hitachi 600 electron microscope.

Calpain Digestion

0.342 mg/ml dystrophin–glycoprotein complex was incubated with 0.083 mg/ml m-calpain (Sigma) for 60 min at 25°C in actin binding buffer containing 2 mM $CaCl_2$ instead of EGTA. Proteolysis was terminated with the addition of E-64 (Sigma) to a final concentration of 5 μg/ml and the digested dystrophin–glycoprotein complex was incubated for an additional 30 min at 25°C in the absence or presence of 6.5 μM F-actin. After centrifugation at 100,000 *g* for 20 min, equal volumes of supernatants and pellets were electrophoretically separated on 3–12% SDS polyacrylamide gels, transferred to nitrocellulose, and stained with dystrophin-specific antibodies as previously described (Ervasti and Campbell, 1993*a*).

Antibodies

Rabbit 47 polyclonal antisera and monoclonal antibody XIXC2 were the kind gift of Dr. Kevin Campbell (University of Iowa, Iowa City, IA). Rabbit 47 antisera were raised against a synthetic peptide representing the amino-terminal 15 amino acids of human dystrophin (Ervasti et al., 1991) while monoclonal antibody XIXC2 (Ervasti et al., 1990) recognizes an epitope located within dystrophin amino acids 1416-1494 (K. Campbell, personal communication). Monoclonal antibody DYS1, raised against a recombinant protein corresponding to dystrophin amino acids 1181-1388, monoclonal antibody 50DAG against adhalin/α-sarcoglycan amino acids 217-289 and monoclonal antibody 43DAG against the COOH-terminal 15 amino acids of β-dystroglycan were all purchased from Novocastra Laboratories (Newcastle, UK) through Vector Laboratories. A monoclonal antibody specific for PIP_2 (Fukami et al., 1992) was generously provided by Dr. Tadaomi Takenawa (University of Tokyo, Tokyo, Japan). The PIP_2-specific monoclonal antibody was tested for reactivity with dystrophin–glycoprotein complex preincubated in the absence or presence of 9 μM PIP_2 using skeletal muscle α-actinin as a positive control (Fukami et al., 1992).

Recombinant Dystrophin Fragment Expression and Purification

The plasmid pRSVDy, encoding full-length dystrophin (Acsadi et al., 1991), was the kind gift of Dr. Jon Wolff. A DNA fragment encoding amino acids 1-246 of dystrophin was PCR-amplified from pRSVDy using the following pairs of oligonucleotide primers (Integrated DNA Technologies, Coralville, IA): 5′-ATCACATGTTGTGGTGGGAAGAAGTAGAGGACTG3′, and 5′-ATCCATGGGTTCAATGCTCACTTGTTGAGGC-3′. The resulting PCR product was subcloned into the EcoRV site of pCR3, cut out with AflIII and NcoI, and inserted into the NcoI site of pET16b (Novagen, Inc., Madison, WI) to produce pDYS246.

A DNA fragment encoding amino acids 1416-1880 of dystrophin was PCR-amplified from pRSVDy and the following pair of oligonucleotide primers (GIBCO BRL, Gaithersburg, MD): 5′-ATCCATATGGATTTGACAAGTCATGAGATC-3′, and 5′-ATCGGATCCATACCACTGATGAGAAATTTC-3′. The PCR product was subcloned into the TA site of PCRII using the Original TA Cloning Kit (Invitrogen Corp., San Diego, CA). The insert was then cut out with NdeI and BamHI and ligated into pET16b previously digested with NdeI and BamHI, producing the plasmid pDYS1416.

The recombinant proteins DYS246 and DYS1416 were purified from cell lysates obtained after IPTG induction of *E. coli* transformed with pDYS246 or pDYS1416 using the His-Bind system (Novagen) per manufacturer's instructions. DYS246 or DYS1416 in elution buffer (250 mM imidazole, 500 mM NaCl, 20 mM Tris-HCl, pH 7.9) were dialyzed into 50 mM Tris-HCl, pH 8.0, containing either 100 mM or 500 mM NaCl, and concentrated using a Centricon-3 (Amicon). Recombinant protein concentrations were determined with the Bio-Rad DC Protein Assay using BSA as a standard. DYS246 was confirmed by Western blot analysis with rabbit 47 polyclonal antisera (Ervasti et al., 1991) while DYS1416 was confirmed by Western blot analysis with monoclonal antibody XIXC2 (Ervasti et al., 1990; Ervasti and Campbell, 1991).

Results

Characterization of Dystrophin–Glycoprotein Complex Binding to F-Actin

As previously demonstrated (Ervasti and Campbell, 1993*a*), incubation of submicromolar concentrations of purified dystrophin–glycoprotein complex with F-actin followed by high-speed sedimentation (100,000 *g* for 20 min) resulted in the cosedimentation of a significant amount of dystrophin–glycoprotein complex with the F-actin pellet (Fig. 1). Even at eightfold higher concentrations, virtually no (<2%) dystrophin–glycoprotein complex sedimented in the absence of F-actin (Fig. 1 *A*). Neither dystrophin–glycoprotein complex nor actin sedimented at 100,000 *g* if the assay was instead performed by incubation of dystrophin–glycoprotein complex with 6 μM G-actin in the absence of KCl and $MgCl_2$ (not shown). Incubation of increasing amounts of dystrophin–glycoprotein complex with a fixed amount of F-actin followed by high speed sedimentation demonstrated that dystrophin–glycoprotein complex bound F-actin in a saturable manner (Fig. 1 *B*). Nonlinear regression analysis of the binding data from three independent experiments with different dystrophin–glycoprotein complex and actin preparations (Fig. 1 *B*) yielded an average dissociation constant (K_d) of 0.5 μM and saturation (B_{max}) at 0.042 ± 0.005 mol/mol, which corresponds to one dystrophin per 24 actin monomers. The shape of the curve as well as the calculated Hill coefficient (1.40 ± 0.33) suggested that dystrophin in the glycoprotein complex bound F-actin with little or no cooperativity. The NaCl concentration and pH dependence of dystrophin–glycoprotein complex binding to F-actin was measured at a dystrophin concentration of 0.5 μM in order to detect whether these changes in buffer conditions altered the affinity and/or maximal binding of dystrophin–glycoprotein complex to F-actin. Dystrophin–glycoprotein complex cosedimentation with F-actin was independent of different pH conditions in the range of pH 6.5 to 8.0 but was found to be sensitive to increasing NaCl concentrations ranging from 0.02 to 0.5 M (Fig. 2). The results presented in Figs. 1 and 2 demonstrate that purified skeletal muscle dystrophin–glycoprotein complex binds specifically to F-actin with biochemical properties similar to other native members of the actin cross-linking superfamily.

Because the effect of calmodulin on the binding of recombinant dystrophin amino-terminal domain to F-actin has been examined by several groups yielding conflicting results (Bonet-Kerrache et al., 1994; Jarrett and Foster, 1995; Winder and Kendrick-Jones, 1995), we further tested calmodulin for effect on dystrophin–glycoprotein complex binding to F-actin. We detected no effect of 5 μM bovine brain calmodulin on dystrophin–glycoprotein complex cosedimentation with F-actin when tested in the presence of either 1 mM EGTA or 0.2 mM $CaCl_2$ (Fig. 2). The bovine brain calmodulin was determined to be functional by its calcium-dependent binding to purified calcium/calmodulin kinase II (Ervasti and Campbell, 1993*a*) as well as its ability to stimulate myosin light chain kinase activity with corresponding effects on myofibrillar contraction (J.R. Patel and R.L. Moss, personal communication). 5 μM calmodulin purified from wheat germ also had no effect on dys-

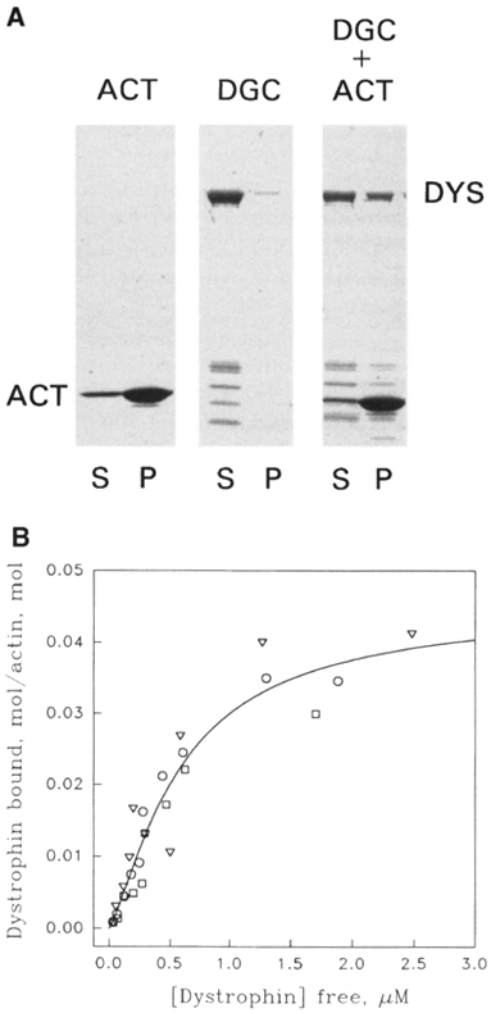

Figure 1. Cosedimentation of the dystrophin–glycoprotein complex with F-actin. Shown in *A* are Coomassie blue–stained SDS–polyacrylamide gels of 100,000 *g* supernatants (*S*) and pellets (*P*) of dystrophin–glycoprotein complex incubated in the presence (DGC + ACT) or absence (*DGC*) of F-actin, or F-actin alone (*ACT*). The concentration of dystrophin–glycoprotein complex was 0.21 mg/ml (0.25 μM dystrophin) in (DGC + ACT) and 1.83 mg/ml (2.14 μM dystrophin) in DGC; F-actin was present at 6 μM in all panels. The molecular weight standards ($\times 10^{-3}$) are indicated on the left. (*B*) Increasing amounts of dystrophin–glycoprotein complex were incubated with 6.5 μM F-actin with subsequent centrifugation at 100,000 *g*. The amount of free and bound dystrophin was determined densitometrically from Coomassie blue–stained gels of 100,000 *g* supernatant and pellet fractions as shown in *A*. The binding data were fitted using nonlinear regression analysis. Different symbols denote the data of three independent experiments performed with different dystrophin–glycoprotein complex and F-actin preparations.

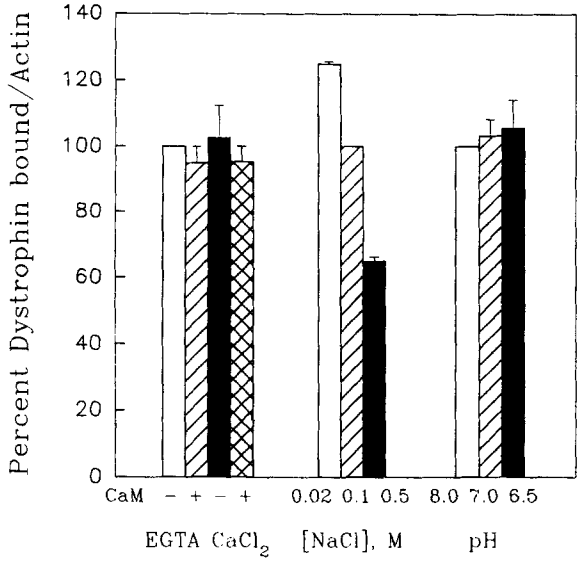

Figure 2. Effect of NaCl, pH , calcium and calmodulin on dystrophin–glycoprotein complex cosedimentation with F-actin. High-speed cosedimentation assays were performed in the presence of 6.5 μM F-actin and 0.43 mg/ml dystrophin–glycoprotein complex (0.5 μM dystrophin). The binding data were normalized against the amount of actin pelleted and expressed as percent of the dystrophin–glycoprotein complex pelleted with F-actin under control conditions (0.1 M NaCl, 1 mM EGTA, pH 8.0) where 100% equals 0.019 ± 0.002 mol dystrophin bound per mol actin. The effect of 0.2 mM $CaCl_2$ was examined in the absence of EGTA. The concentration of calmodulin (*CaM*) was 0.08 mg/ml (5 μM).

trophin–glycoprotein complex binding to F-actin (not shown).

Failure of Dystrophin–Glycoprotein Complex to Cross-link F-Actin

Dystrophin is commonly modeled as an antiparallel dimer based primarily on its sequence homology with spectrin (Koenig et al., 1988). If dystrophin exists as a dimer in the glycoprotein complex it would be expected to cross-link actin filaments into supermolecular networks or bundles. To test this hypothesis, G-actin was polymerized to F-actin in the absence or presence of dystrophin–glycoprotein complex or α-actinin, then negatively stained with uranyl acetate and examined by electron microscopy (Fig. 3). F-actin bundles were readily apparent when actin was polymerized in the presence of α-actinin at a ratio of one α-actinin per six actin monomers. However, no F-actin bundles were observed when actin was polymerized alone or in the presence of dystrophin–glycoprotein complex at ratios as high as one dystrophin per six actin monomers.

The failure of dystrophin–glycoprotein complex to bundle F-actin (Fig. 3) did not rule out the possibility that dystrophin may cross-link actin filaments into loose networks that were not discernible by electron microscopy. Therefore, low speed sedimentation analysis of dystrophin–glycoprotein complex/F-actin mixtures was performed. It has been previously shown (Meyer and Aebi, 1990) that F-actin does not sediment under low speed (20,000 *g*) centrifugation conditions unless complexed into supermolecular aggregates by a cross-linking protein such as α-actinin (Fig. 4 *A*). The presence of both F-actin and dystrophin–glycoprotein complex in the supernatant fraction after centrifugation at 20,000 *g* (Fig. 4 *B*) indicated that dystrophin–glycoprotein complex did not cross-link F-actin. Dystrophin–glycoprotein complex was unable to effect the low speed sedimentation of F-actin even at a dystrophin/actin molar ratio as high as 1:4 (not shown). High speed sedimentation analysis performed in parallel with the low speed experiment

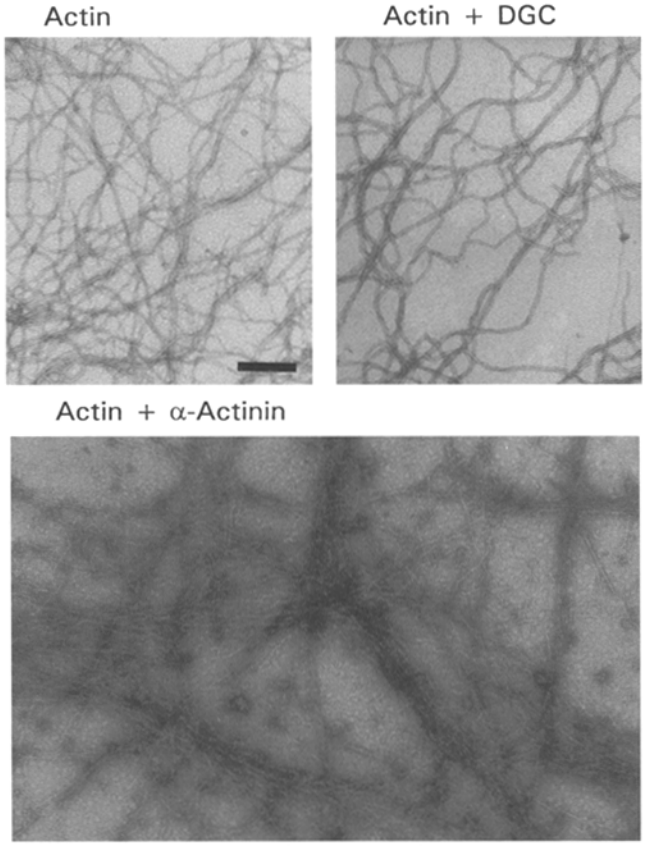

Figure 3. Electron micrographs of negatively stained F-actin polymerized in the absence or presence of dystrophin–glycoprotein complex or α-actinin. 3 μM G-actin was polymerized alone or in the presence of dystrophin–glycoprotein complex or α-actinin, the samples stained with uranyl acetate and visualized by electron microscopy. The dystrophin/F-actin and α-actinin/F-actin molar ratio was 1:6. Bar, 200 nm.

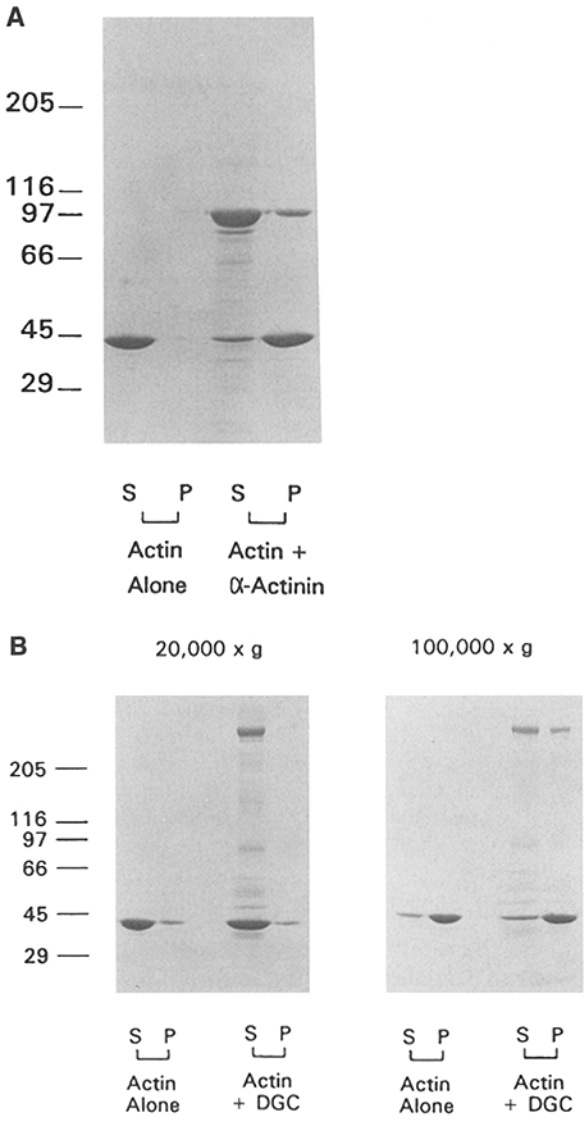

Figure 4. Low speed sedimentation analysis of F-actin cross-linking by α-actinin and dystrophin-glycoprotein complex. Shown in *A* is the Coomassie blue–stained SDS–polyacrylamide gel of 20,000 *g* supernatants (*S*) and pellets (*P*) of F-actin incubated in the absence or presence of α-actinin. The concentration of α-actinin was 0.2 mg/ml (1 μM); F-actin was present at 5 μM. Shown in *B* are the Coomassie blue–stained SDS–polyacrylamide gels of 20,000 *g* and 100,000 *g* supernatants (*S*) and pellets (*P*) of F-actin incubated in the presence or absence of dystrophin–glycoprotein complex. The concentration of dystrophin–glycoprotein complex was 0.42 mg/ml (0.5 μM dystrophin); F-actin was present at 6.5 μM. The molecular weight standards ($\times 10^{-3}$) are indicated on the left.

(Fig. 4 *B*) confirmed that G-actin had polymerized to F-actin and that dystrophin–glycoprotein complex bound F-actin. The inability of dystrophin–glycoprotein complex to cosediment with F-actin at low speed was not the result of proteolytic cleavage of the amino-terminal domain of dystrophin, as an antibody specific for the first 15 amino acids of dystrophin (Ervasti et al., 1991) strongly reacted on Western blots containing the 20,000 *g* supernatant (not shown). Furthermore, neither 0.2 mM $CaCl_2$ nor 5 μM calmodulin induced dystrophin–glycoprotein complex to cross-link F-actin as assessed by the low speed sedimentation assay (not shown).

Since F-actin cross-linked by dystrophin–glycoprotein complex may be so rigid that it is not sedimented at low speed (Pollard and Cooper, 1982), the effect of dystrophin–glycoprotein complex on gelation of actin-filament solutions was examined by falling-ball viscometry. α-Actinin increased the viscosity of F-actin more than 2.5-fold when present at a 1:20 molar ratio with F-actin and formed a gel when α-actinin and actin were mixed at a 1:5 molar ratio (Fig. 5). In contrast with α-actinin, dystrophin–glycoprotein complex had no effect on the gelation of F-actin as the viscosity of F-actin did not change in the presence of dystrophin–glycoprotein complex (Fig. 5) at molar ratios as high as 1:5. Whereas the dystrophin-glycoprotein complex remains intact during membrane solubilization in 1% digitonin and 0.5 M NaCl (Ervasti et al., 1990; Yoshida and Ozawa, 1990; Ervasti and Campbell, 1991; Ervasti et al., 1991), it is possible that these solubilization conditions may specifically dissociate dystrophin dimers or alter the complex in some other manner such that it is incapable of crosslinking F-actin. To address this possibility, dystrophin–glycoprotein complex was solubilized from membranes with 1% Triton X-100 and 0.15 M NaCl and chro-

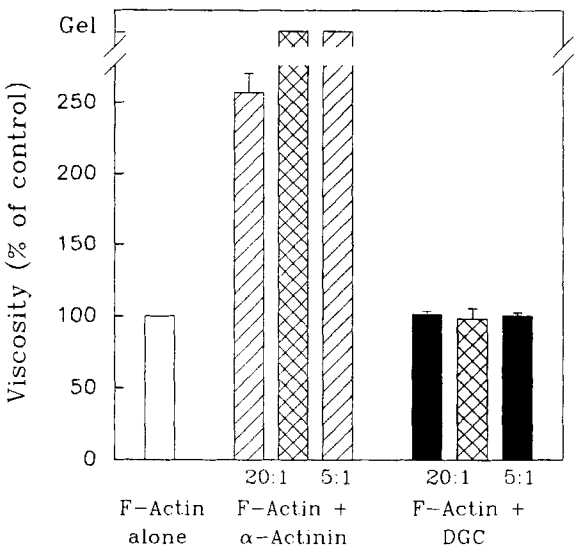

Figure 5. Effect of α-actinin and dystrophin–glycoprotein complex on the viscosity of F-actin. Average viscosity measurements (± SEM, $n \geq 3$) obtained by falling ball viscometry at 25°C are expressed as the percent of that obtained for 6 μM F-actin alone (60.37 ± 2.01 cP). Gel indicates a viscosity at which the ball does not fall. The concentrations of α-actinin were 0.06 mg/ml (0.3 μM) and 0.24 mg/ml (1.2 μM). Dystrophin–glycoprotein complex was present at concentrations 0.26 mg/ml and 1 mg/ml (0.3 μM and 1.2 μM dystrophin, respectively). F-actin was present at 6 μM in all assays. The cross-hatched bars illustrate data obtained in the presence of 9 μM PIP_2.

matographically purified in the presence of 0.1% Triton X-100 instead of digitonin. Whereas the complex purified from low salt Triton X-100 solubilates cosedimented specifically with F-actin in the high speed assay, it also failed to cross-link F-actin as assessed in the low speed sedimentation assay and by falling-ball viscometry (not shown). It is also possible that association with the glycoprotein complex prevents dystrophin from cross-linking F-actin. However, dystrophin dissociated from the glycoprotein complex by treatment with β-octylglucoside (Yoshida et al., 1994) also exhibited no measureable F-actin cross-linking activity (not shown). These results suggest that the failure of dystrophin–glycoprotein complex to cross-link F-actin was not due to an adverse affect of its purification or interaction with associated proteins.

As PIP_2 has been shown to inhibit the actin binding activity of dystrophin purified from *Torpedo* electric organ (Mejean et al., 1995), it remained possible that the failure of dystrophin–glycoprotein complex to cross-link F-actin might be due to presence of copurifying polyphosphoinositides. However, purified dystrophin–glycoprotein complex failed to react with PIP_2-specific antibodies (Fukami et al., 1992) while the presence of 9 μM PIP_2 or pretreatment with phosphatidylinositol-specific phospholipase C had no effect on dystrophin–glycoprotein complex binding to F-actin as assessed in the high-speed and low-speed sedimentation assays (not shown). Furthermore, 9 μM PIP_2 had no effect on the gelation of F-actin in the presence of dystrophin–glycoprotein complex even though the same concentration of PIP_2 dramatically stimulated the F-actin gelating activity of smooth muscle α-actinin (Fig. 5). Taken together, the results presented in Figs. 3–5 demonstrate that purified dystrophin–glycoprotein complex is unable to cross-link actin filaments into supermolecular networks or bundles.

Evidence for a Novel F-Actin Binding Site in Dystrophin

In the initial study that identified the region of dystrophin responsible for interaction with the glycoprotein complex (Suzuki et al., 1992), it was shown that limited calpain digestion of purified dystrophin–glycoprotein complex yielded a stable series of dystrophin fragments while integral membrane components of the glycoprotein complex were not digested. Identification of calpain-derived dystrophin fragments with sequence-specific antibodies revealed a stable 31 kD fragment that corresponded to the amino-terminal, putative actin-binding domain of dystrophin (Suzuki et al., 1992). Using a similar strategy, we sought to identify dystrophin fragments that cosedimented with F-actin after limited calpain digestion of dystrophin–glycoprotein complex. Surprisingly, the 31 kD dystrophin fragment recognized by antibodies specific for the amino-terminal 15 amino acids of dystrophin did not cosediment with F-actin when 0.4 μM of digested dystrophin–glycoprotein complex was analyzed in the high speed sedimentation assay (Fig. 6). However, dystrophin monoclonal antibody XIXC2 (Ervasti et al., 1990), which maps to an epitope contained in dystrophin amino acids 1416-1494 (Campbell, K., personal communication) did identify a 50 kD fragment of the dystrophin rod domain that retained F-actin binding activity (Fig. 6). The 50 kD, actin binding fragment of dystrophin was further localized within the rod domain by the absence of reactivity with

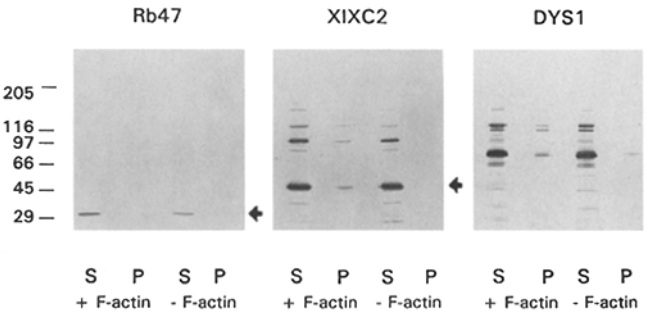

Figure 6. Cosedimentation of dystrophin fragments with F-actin after limited digestion of dystrophin–glycoprotein complex with calpain. Shown are identical nitrocellulose transfers stained with rabbit polyclonal antibodies raised against a synthetic peptide corresponding to the first 15 amino acids of dystrophin (Rb 47), or monoclonal antibodies XIXC2 and DYS1 that are specific for adjacent (but not overlapping) epitopes located in the rod domain of dystrophin (refer to Fig. 7). The nitrocellulose transfers contained electrophoretically separated supernatants (*S*) and pellets (*P*) of calpain-digested dystrophin–glycoprotein complex incubated in the presence (+ F-actin) or absence (− F-actin) of F-actin and sedimented at 100,000 *g*. The arrows identify the 31-kD and 50-kD dystrophin fragments detected by rabbit 47 and XIXC2 antibodies, respectively. The concentration of calpain-digested dystrophin–glycoprotein complex was 0.34 mg/ml (0.4 μM dystrophin) and F-actin was present at 6.5 μM. The molecular weight standards ($\times 10^{-3}$) are indicated on the left.

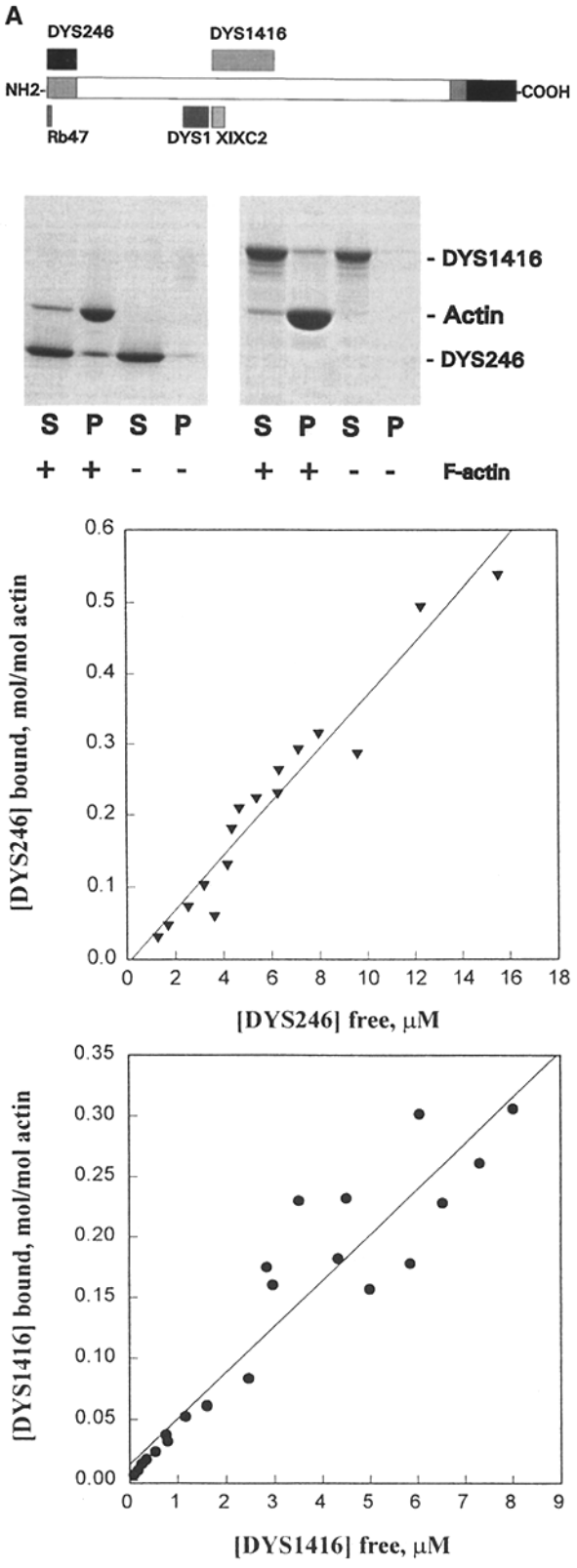

Figure 7. Cosedimentation of recombinant dystrophin fragments with F-actin. Shown in *A* is a diagram illustrating the location of epitopes for Rb 47 (amino acids 1-15), XIXC2 (amino acids 1416-1494) and DYS1 (amino acids 1181-1388) in the dystrophin primary sequence as well as the relative location of the dystrophin sequences encoded by recombinant proteins DYS246 (amino acids 1-246) and DYS1416 (amino acids 1416-1880). Also shown in *A* are Coomassie blue–stained SDS–polyacrylamide gels of supernatants (*S*) and pellets (*P*) of recombinant DYS246 and DYS1416 sedimented at 100,000 *g* in the presence or absence of F-actin. (*B*) Increasing amounts of DYS246 or DYS1416 were incubated with 6.5 μM F-actin with subsequent centrifugation at 100,000 *g*. The amount of free and bound protein was determined densitometrically from Coomassie blue-stained gels of 100,000 *g* supernatant and pellet fractions as shown in *A*.

monoclonal antibody DYS1 (Fig. 6), which recognizes an epitope located within dystrophin amino acids 1030-1388 (Nicholson et al., 1989). Nitrocellulose transfers stained with monoclonal antibodies specific for adhalin/α-sarcoglycan and β-dystroglycan indicated that neither of these integral membrane components of the dystrophin–glycoprotein complex was digested by m-calpain or cosedimented with F-actin after calpain digestion of the dystrophin–glycoprotein complex (not shown). These results suggest that a portion of the dystrophin rod domain may also play a role in the actin binding activity of dystrophin.

In order to confirm the actin binding activity of the 50-kD fragment of the dystrophin rod domain (Fig. 6), recombinant proteins corresponding to dystrophin amino acids 1-246 (DYS246) and 1416-1880 (DYS1416) were evaluated for interaction with F-actin using the high speed sedimentation assay. Both recombinant proteins were observed to specifically cosediment with F-actin (Fig. 7 *A*). Neither 5 μM calmodulin or 9 μM PIP_2 had any effect on the cosedimentation of DYS246 with F-actin (not shown). It was not possible to accurately measure the affinity or stoichiometry of either protein for F-actin because higher but subsaturating protein concentrations sedimented in the absence of F-actin. However, it was apparent from the binding data obtained over the measurable concentration range that DYS246 and DYS1416 both bound F-actin with substantially lower affinity and higher capacity (Fig. 7 *B*) than was observed with purified dystrophin–glycoprotein complex (Fig. 1). These results could be explained by a model whereby intact dystrophin binds with 0.5 μM affinity to F-actin through the combined effect of multiple lower affinity contact sites.

Effect of Dystrophin–Glycoprotein Complex on F-Actin Depolymerization

The stoichiometry of dystrophin–glycoprotein complex binding to F-actin (1 dystrophin per 24 actin monomers, Fig. 1), the lack of cross-linking activity (Figs. 3–5) and the evidence for an additional actin binding site located in the rod domain (Figs. 6 and 7) raise the possibility that full-length dystrophin may bind along an actin filament through interaction with multiple actin monomers in a manner analogous to side-binding proteins like tropomyosin (Broschat, 1990). If dystrophin indeed binds along an actin filament, it might further be expected to prevent or retard the dissociation of actin monomers from the ends of filaments to which it is bound (Broschat, 1990; Schafer and Cooper, 1995). To test this hypothesis, F-actin alone, or F-actin pre-equilibrated with dystrophin–glycoprotein complex was rapidly diluted into buffer conditions that favored actin depolymerization. At various times post-dilution, the amount of F-actin remaining was determined by

high-speed sedimentation analysis (Fig. 8). As hypothesized, preequilibration of dystrophin–glycoprotein complex with F-actin significantly slowed the depolymerization of F-actin when compared to F-actin alone (Fig. 8). For example, 4 h after dilution, 4.6 ± 1.9% of F-actin alone pelleted while 14.9 ± 0.2% pelleted when dystrophin–glycoprotein complex was present at a 1:5 molar ratio with F-actin. We further determined the molar ratio of dystrophin that cosedimented with actin using the densitometrically determined fraction of each protein that pelleted and the total protein concentrations. Over the time range of 50–80 min post-dilution, the average molar ratio of dystrophin that cosedimented with the actin pellet was 0.13 ± 0.02. Preliminary fluorescence experiments using pyrene-labeled actin (Cooper et al., 1983) confirmed that dystrophin–glycoprotein complex significantly slowed F-actin depolymerization but had no effect on F-actin polymerization (not shown). While it is possible that the protective effect of dystrophin–glycoprotein complex on actin depolymerization is a general feature of proteins that bind F-actin (Schafer and Cooper, 1995), α-actinin only slowed actin depolymerization at the earliest measurable time point (20 min) while the protective effect of dystrophin–glycoprotein complex persisted for at least 4 h post-dilution (Fig. 8). These data, together with the stoichiometry of dystrophin–glycoprotein complex binding to F-actin (Fig. 1), suggest that an intact dystrophin molecule has the capacity to interact directly with multiple actin monomers within a filament.

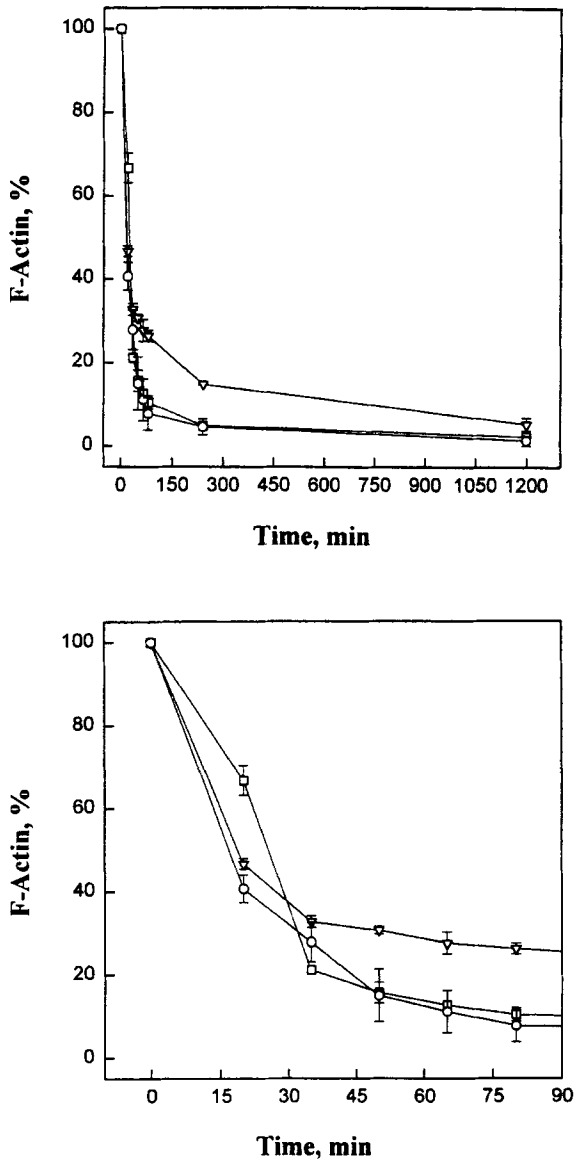

Figure 8. Effect of dystrophin–glycoprotein complex and α-actinin on the depolymerization of F-actin. 7.5 μM F-actin in G-buffer containing 10 mM NaCl and 0.2 mM $MgCl_2$ was incubated 30 min at room temperature alone (*circles*) or in the presence of dystrophin–glycoprotein complex (*inverted triangles*) or α-actinin (*squares*), both present at a 1:5 molar ratio with respect to actin. The samples were rapidly diluted threefold into G-buffer and centrifuged at 100,000 *g* for 20 min after various incubation times post-dilution. The fraction of actin (% F-actin) remaining in the pellet was determined densitometrically from Coomassie blue–stained gels loaded with equal volumes of supernatants and pellets. The upper panel shows the entire time range evaluated while time points between 0 and 80 min are shown in expanded form in the lower panel. Time points include centrifugation time and represent the average (± SEM) of three or more independent determinations.

Discussion

Although dystrophin is clearly an essential muscle protein, little is known about its physiological role(s) in maintaining cellular viability. The functional importance of the cysteine-rich and carboxy-terminal domains of dystrophin is evident from several studies that correlate specific mutations or deletions in these domains with presentation of the most severe forms of muscular dystrophy (Hoffman et al., 1991; Helliwell et al., 1992; Matsumura et al., 1993). Localization of the binding sites for dystrophin-associated proteins to the cysteine-rich and carboxy-terminal domains (Suzuki et al., 1992; Kramarcy et al., 1994; Suzuki et al., 1994) demonstrated that these two domains are essential for normal dystrophin function in skeletal muscle. On the other hand, transgenic expression of a nonmuscle isoform of dystrophin (DP71) lacking the amino-terminal and rod domains in dystrophin-deficient muscle leads to the restoration of dystrophin-associated proteins but fails to correct the dystrophic phenotype (Cox et al., 1994; Greenberg et al., 1994). These data indicate that the cysteine-rich and carboxy-terminal domains of dystrophin alone are insufficient to confer normal muscle function and leave unresolved the functional role of the remaining 80% of dystrophin sequence comprising the amino-terminal and rod-like domains.

Certainly, the strong sequence homology of the amino-terminal 246 amino acids of dystrophin with the actin binding domains of several well-characterized F-actin cross-linking proteins (Koenig et al., 1988; Matsudaira, 1991) and accumulating data (Hemmings et al., 1992; Way et al., 1992; Fabbrizio et al., 1993; Corrado et al., 1994; Jarrett and Foster, 1995) support its hypothesized role in binding F-actin. In addition, several studies have noted a correlation between mutations or deletions in the amino-terminal domain with the expression of intermediate to severe forms of muscular dystrophy (Beggs et al., 1991; Prior et al., 1993; Winnard et al., 1993; Comi et al., 1994; Mun-

toni et al., 1994). However, it is not clear whether the more severe phenotypes presented by patients with defects or deletions in the amino-terminal domain are due to the absence of an important functional domain or simply to the low abundance of an unstable truncated protein. In fact, transgenic *mdx* mice expressing normal levels of a dystrophin construct lacking amino acids 45-273 present with a benign phenotype, indicating that the amino-terminal domain is not essential for normal dystrophin function (Corrado et al., 1996). In this study, we present evidence suggesting that both the amino-terminal and rod domains of dystrophin are involved in binding to actin filaments.

Dystrophin in the glycoprotein complex bound F-actin in a specific and saturable manner with an average K_d of 0.5 μM (Fig. 1). Other studies have estimated 0.1–1.0 μM F-actin binding affinities for purified *Torpedo* dystrophin (Lebart et al., 1995) as well as maltose binding protein/dystrophin chimeras comprising the first 90 to 385 amino acids of dystrophin (Corrado et al., 1994; Jarrett and Foster, 1995). These results could be taken to suggest that the amino-terminal domain of dystrophin is sufficient to account for the actin binding activity of dystrophin. However, binding constants were estimated using a direct ELISA assay which, for a variety of reasons (Goldberg and Djavadi-Ohaniance, 1993), is generally considered inappropriate for measuring ligand binding affinity. Furthermore, it is unclear what conformational effect the >40 kD maltose binding protein portion of the chimeras may have exerted on the normally unconstrained amino terminus of native dystrophin. We expressed a recombinant protein initiated at the start methionine of dystrophin (DYS246). DYS246 comprises the amino-terminal 246 amino acids of dystrophin with a small (~5 kD), carboxy-terminal polyhistidine tag which facilitated rapid purification to >95% purity. Although saturation was not measurable, DYS246 clearly bound F-actin in the high-speed sedimentation assay with at least an order of magnitude lower affinity and substantially higher capacity (Fig. 7) than was observed for purified dystrophin–glycoprotein complex (K_d = 0.5 μM, B_{max} = 1:24, Fig. 1). Consistent with our results, it was previously demonstrated (Way et al., 1992) that a non-fusion recombinant protein corresponding to dystrophin amino acids 1-246 bound F-actin with 1:1 stoichiometry and an apparent K_d of 44 μM when measured in the high-speed sedimentation assay. Taken together, these data suggest that the amino-terminal, putative actin binding domain of dystrophin alone is insufficient to explain the F-actin binding properties of full-length dystrophin.

Regarding regulation of dystrophin binding to F-actin, we observed no effect of either calmodulin or PIP_2 as reported by others (Jarrett and Foster, 1995; Mejean et al., 1995). The reason for these different results is not presently clear. In the case of PIP_2, our conflicting results may reflect a difference in sequence between mammalian skeletal muscle dystrophin and *Torpedo* electric organ dystrophin that confers sensitivity to PIP_2. While the complete amino acid sequence for *Torpedo* dystrophin has not been determined, the PIP_2 binding site in the actin binding domain of α-actinin (Fukami et al., 1996) is poorly conserved with the analogous sequence in skeletal muscle dystrophin. With regard to calmodulin, we and others (Bonet-Kerrache et al., 1994; Winder and Kendrick-Jones, 1995) have not been able to confirm an effect of calmodulin on recombinant dystrophin amino-terminal domain binding to F-actin using a solution phase assay. In the study reporting an effect of calmodulin (Jarrett and Foster, 1995), a direct ELISA assay was used to show that fusion protein binding to immobilized actin was fourfold higher in the presence of calcium than in the presence of EGTA and that addition of 5 μM calmodulin reduced apparent fusion protein binding in the presence of calcium towards the values obtained in EGTA. In the absence of additional control experiments, it is possible that the observed calmodulin inhibition of fusion protein binding to actin was due to calcium buffering by calmodulin that may have affected the solubility of fusion protein or the colorimetric reaction used for detection. Resolution of this controversy awaits demonstration of a calmodulin effect on intact dystrophin binding to F-actin.

Three additional experimental findings suggest that dystrophin interacts with F-actin in a manner that dramatically distinguishes it from the other proteins that comprise the actin cross-linking superfamily (Matsudaira, 1991). First, dystrophin is commonly modeled as an antiparallel dimer based primarily by analogy to α-actinin and spectrin (Koenig et al., 1988; Matsudaira, 1991) and would therefore be expected to cross-link actin filaments into supermolecular networks or bundles. However, dystrophin in the glycoprotein complex was unable to cross-link F-actin when examined by three different methods, all carried out in parallel with α-actinin as a positive control (Figs. 3–5). While recombinant proteins corresponding to dystrophin repeats two and three of the rod domain have a conformation similar to that of spectrin repeats (Kahana et al., 1994), it has been cautioned that conservation between dystrophin repeats is much lower than in the case of spectrin (Tinsley et al., 1992), particularly with regard to surface residues thought to be important in dimerization (Winder et al., 1995). Images obtained from rotary-shadowed dystrophin preparations (Pons et al., 1990) revealed the presence of dystrophin monomers and dimers as well as higher ordered oligomers. As noted by the authors of this study (Pons et al., 1990), however, the purification of dystrophin involved an acid elution step from an affinity column which may have had some effect on the structures observed. While gel permeation chromatography (Yoshida and Ozawa, 1990) of purified dystrophin–glycoprotein complex yielded a molecular weight estimate (>1,000,000) suggestive of dimeric complex, the method relies on the assumption that the protein under investigation is globular, which is probably not valid in the case of dystrophin.

The second novel finding of this study was the identification of an additional F-actin binding site located near the middle of the dystrophin rod domain and separated from the amino-terminal, putative actin binding domain of dystrophin by almost 1,200 amino acids (Figs. 6 and 7). While remarkable, this finding is not unprecedented. For example, the first α-helical repeat of β-spectrin was recently shown to participate in the binding of its amino-terminal domain to F-actin (Li and Bennett, 1996). Tensin exhibits homology to the consensus site found in the F-actin cross-linking superfamily of proteins (Matsudaira, 1991), yet nonhomologous sequences were shown to be responsible for its actin binding activity (Lo et al., 1994). Further-

more, the domain of c-Abl tyrosine kinase responsible for its actin binding activity shows weak homology with the triple helical repeats common to dystrophin, α-actinin and spectrin (Van Etten et al., 1994). Interestingly, the portion of the dystrophin rod domain that exhibits actin binding activity (amino acids 1416-1880, Fig. 7) also contains repeats 10 and 14, which are noted for conforming poorly with the stereotypical repeat pattern (Koenig and Kunkel, 1990; Winder et al., 1995). As with the amino-terminal, putative actin binding domain of dystrophin, a recombinant protein corresponding to dystrophin amino acids 1416-1880 appeared to bind F-actin with substantially lower affinity and higher capacity (Fig. 7) than was observed for intact dystrophin in the glycoprotein complex (Fig. 1). These data support the possibility that multiple lower affinity sites distributed throughout the dystrophin amino-terminal and rod domains may act in concert to bind dystrophin to F-actin with 0.5 μM affinity. It may be relevant that one study of different dystrophin fusion proteins noted actin binding activity for a construct encoding the most distal repeats of the dystrophin rod domain (Jarrett and Foster, 1995).

As a third distinguishing feature of dystrophin binding to F-actin, we observed that dystrophin–glycoprotein complex significantly protected a fraction of actin filaments from depolymerization (Fig. 8). It is noteworthy that the actin side-binding protein tropomyosin binds to F-actin with a stoichiometry of one tropomyosin per seven actin monomers and also protects F-actin from depolymerization (Broschat, 1990). On the other hand, it has been argued that bivalent actin binding proteins should also retard F-actin depolymerization as a consequence of cross-linking activity (Schafer and Cooper, 1995). Consistent with a previous study (Cano et al., 1992), we observed that α-actinin significantly stabilized F-actin for brief periods (<30 min) after initiation of depolymerization (Fig. 8). However, α-actinin failed to protect F-actin for longer time periods while dystrophin–glycoprotein complex inhibited F-actin depolymerization for as long as 4 h (Fig. 8). We hypothesize that the early protection of F-actin from depolymerization by α-actinin is due to the presence of bundles which rapidly dissociate upon dilution (Meyer and Aebi, 1990). Since dystrophin–glycoprotein complex does not have the capacity to cross-link actin filaments (Figs. 3–5), the more prolonged protective effect of dystrophin–glycoprotein complex on F-actin depolymerization (Fig. 8) is best explained by a side binding interaction between dystrophin and F-actin. Interestingly, although dystrophin is highly susceptible to proteolysis in vitro (Koenig and Kunkel, 1990; Suzuki et al., 1992), the amino-terminal half of the molecule appears to be protected from proteolysis in situ (Hori et al., 1995), perhaps through a lateral association with F-actin.

In light of these results, we propose a model in which the amino-terminal and rod domains both participate in dystrophin binding along an actin filament through the concerted effect of multiple low affinity binding sites. An actin side binding function for dystrophin may be particularly important for stabilizing lateral associations between thin filaments and the sarcolemma at myotendinous junctions (Tidball and Law, 1991). In addition, dystrophin could play a structural role at costameres (Porter et al., 1992) through a side binding interaction with peripheral actin filaments emanating from the Z- and M-lines of myofibers (Bard and Franzini-Armstrong, 1991). Both of these specialized sites of force transmission are disrupted in dystrophin-deficient *mdx* mouse muscle (Tidball and Law, 1991; Ridge et al., 1994). The redundancy provided by multiple actin binding sites may also help to explain why restoration of a truncated dystrophin comprising only the cysteine-rich and carboxy-terminal domains is insufficient to correct the pathologies observed in dystrophic muscle (Cox et al., 1994; Greenberg et al., 1994), yet no specific sequences within the amino-terminal (Corrado et al., 1996) and rod domains (England et al., 1990) also appear to be essential for normal dystrophin function. Perhaps some mild forms of muscular dystrophy are due to gene deletions that allow the expression of sufficient portions of the amino-terminal and/or rod domains (in any combination with cysteine-rich and carboxy-terminal domains) to conserve at least partial actin binding activity. The presence of actin binding sites within the rod domain of dystrophin would also provide a basis by which dystrophin isoforms lacking the amino-terminal domain (D'Souza et al., 1995) may still function in a manner analogous to full-length dystrophin.

Based on the stoichiometry of dystrophin–glycoprotein complex binding to F-actin (Fig. 1), a dystrophin molecule has the capacity to interact with 24 actin monomers which should span a length of 130 nm if arranged in a single strand of a filament or one-half of this length when comprising both strands of the filament. Either possibility would be sufficient to allow linear contact with amino acids 1-246 and 1416-1880 of an extended dystrophin molecule with a length of 120-140 nm (Cullen et al., 1990; Pons et al., 1990). Further refinement of the model will require unambiguous resolution of the oligomeric state of native dystrophin. Toward this end, experiments are currently underway to accurately determine the native molecular weight of dystrophin–glycoprotein complex and purified dystrophin.

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