The native molecular weight of the dystrophin-glycoprotein complex and its effect on actin depolymerization and polymerization were examined. First, we determined that the native molecular weight of purified dystrophin-glycoprotein complex is only large enough (M, 1,200,000) to contain one copy of each protein in the complex, including dystrophin. Using different approaches, we also demonstrated that dystrophin-glycoprotein complex significantly protected a fraction of actin filaments from disassembly, while individual recombinant actin binding fragments of dystrophin or calpain-digested dystrophin-glycoprotein complex had no effect on F-actin depolymerization. The protective effect of dystrophin-glycoprotein complex on F-actin depolymerization saturated at a dystrophin:actin molar ratio of 0.04, corresponding to 1 dystrophin/25 actin monomers, which is highly consistent with the 1:24 stoichiometry of dystrophin-glycoprotein complex binding to F-actin previously measured at equilibrium. However, dystrophin-glycoprotein complex did not bind G-actin or alter the kinetics or extent of actin polymerization. This excluded the possibility that dystrophin-glycoprotein complex inhibited actin depolymerization by capping the ends of actin filaments. It therefore appears that actin binding domains separated on the dystrophin molecule from each other by almost 1,200 amino acids act in concert to protect F-actin from depolymerization. Our data suggest that dystrophin stabilizes F-actin in vitro by binding alongside an actin filament and bridging actin monomers in a manner analogous to the actin side binding protein tropomyosin. It is noteworthy that we did not find any effect of skeletal muscle tropomyosin on dystrophin-glycoprotein complex binding to F-actin. This indicates that dystrophin-glycoprotein complex and tropomyosin may simultaneously bind the same actin filament and identifies another feature that distinguishes dystrophin from the other proteins in the actin-cross-linking superfamily.

Skeletal muscle dystrophin can be purified as part of a large oligomeric complex also containing integral and peripheral sarclemmal proteins, several of which are glycoproteins (1–3). The dystrophin-glycoprotein complex further interacts with the laminin family of extracellular matrix proteins by way of the 156-kDa dystrophin-associated glycoprotein (4, 5), now known as α-dystroglycan, as well as with F-actin via dystrophin (5–9). These studies suggest that the dystrophin-glycoprotein complex is an essential functional unit that links the actin-based membrane cytoskeleton with the extracellular matrix and may play a structural role in maintaining sarcolemmal membrane integrity during muscle contraction. In fact, numerous studies have demonstrated that the absence of, or abnormality in, the dystrophin-glycoprotein complex renders muscle more susceptible to necrosis (10, 11).

Striated muscle dystrophin is predominantly expressed as a 427-kDa, four-domain protein with the first three domains exhibiting significant sequence homology with the cytoskeletal proteins α-actinin and spectrin (12, 13): an amino-terminal, actin binding domain; a rod-like domain comprising 24 triple helical coiled-coil repeats; a cysteine-rich domain; and a carboxyl-terminal domain. Based on its sequence homology with spectrin and α-actinin (12, 13) and limited electron microscopy data (14), dystrophin was speculatively modeled as an antiparallel dimer that formed a hexagonal lattice by cross-linking different actin filaments through oppositely oriented amino-terminal actin binding domains. However, our recent characterization of dystrophin-glycoprotein complex binding to F-actin (15) indicated that this model for the interaction between intact dystrophin and F-actin may require substantial revision. First, dystrophin-glycoprotein complex bound to F-actin with a stoichiometry of 1 dystrophin/24 actin monomers and an average apparent Kd for dystrophin of 0.5 μM. Second, dystrophin-glycoprotein complex failed to induce cross-linking of actin filaments into supermolecular networks or bundles. Third, we identified a novel actin binding site located near the middle of the dystrophin rod domain. Recombinant dystrophin fragments corresponding to this novel actin binding site and the original amino-terminal actin binding domain of dystrophin both bound F-actin but with significantly lower affinity than was observed with intact dystrophin in the glycoprotein complex. Fourth, we found that dystrophin-glycoprotein complex significantly slowed depolymerization of F-actin. These experimental findings led us to propose a model in which dystrophin binds alongside an actin filament via two or more low affinity binding sites distributed throughout its amino-terminal and rod domains (15, 16).

The current study was designed to further test the actin side binding model for dystrophin by determining the oligomeric state of native dystrophin-glycoprotein complex and examining the mechanism by which dystrophin-glycoprotein complex protects actin filaments from depolymerization. Our results indicate that the native molecular weight of dystrophin-glycoprotein complex is only large enough to contain one copy of each protein in the complex, including dystrophin. Using different approaches, we have also confirmed that dystrophin-glycoprotein complex significantly protects a fraction of actin filaments from depolymerization. The protective effect of dystrophin-glycoprotein complex on F-actin depolymerization was dependent
on the dystrophin-glycoprotein complex concentration and saturated at a dystrophin:actin ratio of 0.04, corresponding to 1 dystrophin/25 actin monomers. This result is highly consistent with the 1:24 stoichiometry of dystrophin-actin complex binding to F-actin measured at equilibrium (15). However, recombinant proteins corresponding to the two identified actin binding domains of dystrophin failed to slow F-actin disassembly. We also studied actin polymerization in the absence and presence of dystrophin-glycoprotein complex and found that dystrophin-glycoprotein complex neither binds G-actin nor alters the kinetics or extent of actin assembly. This excluded the possibility that dystrophin-glycoprotein complex protects F-actin from depolymerization by nucleating assembly or capping filament ends. Our results suggest that dystrophin stabilizes F-actin by laterally bridging actin monomers over relatively long stretches of a filament. Interestingly, we did not find any effect of skeletal muscle tropomyosin on dystrophin-glycoprotein complex binding to F-actin, which suggests that these proteins can simultaneously bind to an actin filament. Taken together, these results suggest that dystrophin exists as a monomer in the glycoprotein complex and binds alongside an actin filament with 0.5 μM affinity via two or more discrete low affinity binding sites distributed throughout its amino-terminal and rod-like domains.

EXPERIMENTAL PROCEDURES

Proteins—Dystrophin-glycoprotein complex was purified from KCl-washed rabbit skeletal muscle membranes solubilized with either 1% digitonin or 1% Triton X-100 (15). Calpain-digested dystrophin-glycoprotein complex was prepared by incubation of 0.9 mg/ml dystrophin-glycoprotein complex with 0.06 mg/ml m-calpain (Sigma) at 25 °C for 60 min as described previously (15). Proteolysis was terminated with the addition of E-64 (Sigma) to a final concentration of 5 μg/ml. Recombinant dystrophin fragments DYS246 (amino acids 1–246) and DYS1416 (amino acids 1416–1880) were expressed and purified as described previously (15). Actin was purified from rabbit skeletal muscle using the method of Pardeck and Spudich (17) and further purified by gel filtration on a Sephacryl S-200 column (Pharmacia Biotech Inc.) according to MacLean-Fletcher and Pollard (18). Actin was labeled with pyrene iodide (Molecular Probes) following the procedures outlined by Cooper et al. (20). Actin concentration was determined by absorbance using E390 = 0.65 cm²/mg (18). The concentration of pyrene-G-actin and the pyrene:actin ratio were determined as described by Cooper et al. (20). Pyrene-G-actin was stored at −80 °C and thawed in a water bath immediately before use. Spectrin was purified from low ionic strength extracts of rabbit erythrocyte ghosts according to Bennett (21). Laminin-1 was the kind gift of Dr. Hynda Kleinman (National Institute of Health). Bovirin pseudouridine nucleoside 5′-phosphate was purchased from Sigma or Boehringer Mannheim. Rabbit skeletal muscle tropomyosin was generously provided by Dr. Marion Greaser (University of Wisconsin). Monoclonal antibody XIXC2 against dystrophin was the kind gift of Dr. Kevin Campbell (University of Iowa). Monoclonal antibody against α-sarcoglycan was purchased from Sigma.

Gel Filtration Chromatography—F-dystrophin was native molecular weight determined, 0.3–0.4 mg of dystrophin-glycoprotein complex was loaded onto a 1 × 50-cm Superose 6 HR prepacked column or a 2.5 × 48.5-cm Sephacryl-500 column (Pharmacia). The Superose 6 HR column was eluted at 0.15 ml/min in 50 mM Tris-HCl, pH 7.5, 0.18 M NaCl, 0.1% digitonin, while the Sephacryl-500 column was eluted at 0.5 ml/min in 50 mM Tris-HCl, pH 7.5, 0.18 M NaCl, 0.1% Triton X-100. The Stokes radius for dystrophin-glycoprotein complex was determined from a calibration curve of the protein standards catalase (52.2 Å), ferritin (61 Å), thyroglobulin (85 Å) (all from Pharmacia), spectrin dimer (123 Å; Ref. 34) and a previous measurement of its sedimentation coefficient (1,350,000) by sedimentation equilibrium analysis of dystrophin (15). The native molecular weight of the dystrophin-glycoprotein complex was calculated to be 1,200,000 with a frictional ratio of 2.3. Highly similar molecular weight values were obtained if the chromatography data were instead plotted as a function of the log molecular weight (not shown). These results were also highly consistent with the molecular weight obtained (1,350,000) by sedimentation equilibrium analysis of purified dystrophin-glycoprotein complex.1 Taken together, these data indicate that the native molecular weight of the purified dystrophin-glycoprotein complex is only large enough to contain one copy of dystrophin.

1 C. W. Dornin and T. M. Laue, personal communication.
with dystrophin-glycoprotein complex significantly slowed the depolymerization of actin filaments induced by dilution into low salt buffer conditions (15). For example, 80 min after dilution into buffer containing 2.3 mM NaCl and 0.05 mM MgCl₂ (final concentrations), 11.3% of actin alone was recovered in the high speed pellet, while 25.6% of actin pelleted when preincubated with dystrophin-glycoprotein complex at a 1:5 (dystrophin:actin) molar ratio (Fig. 2A). Virtually all actin (97.5%) was retained in the high speed pellet if F-actin was diluted into buffer containing 0.1 mM NaCl and 2 mM MgCl₂ (Fig. 2A). Dystrophin-glycoprotein complex also significantly protected pyrene labeled F-actin from depolymerization induced by rapid dilution into low salt buffer conditions (Fig. 3). Analysis over a wider time range (20 min to 20 h) indicated that dystrophin-glycoprotein complex significantly protected F-actin from disassembly for at least 4 h postdilution (15). The protective effect of dystrophin-glycoprotein complex on F-actin depolymerization was dependent on the dystrophin-glycoprotein complex concentration and saturated at a dystrophin:actin ratio of 0.04 corresponding to 1 dystrophin/25 actin monomers (Fig. 2B). This result is highly consistent with the 1:24 stoichiometry of dystrophin-glycoprotein complex binding to F-actin measured at equilibrium (15) and suggests that dystrophin binding to F-actin is responsible for the protective effect of dystrophin-glycoprotein complex on actin depolymerization.

Micromolar concentrations of deoxyribonuclease I can also induce depolymerization of actin filaments (Fig. 4A), probably through the combined effects of monomer sequestration and destabilization of actin monomer binding to the filament pointed end (33, 34). Although its effects on F-actin are complex, deoxyribonuclease I-driven depolymerization is advantageous (over the dilution method used above) in that it can be performed at constant actin and actin-binding protein concentrations under more physiological buffer conditions. Therefore, we were able to directly compare the effect of dystrophin-glycoprotein complex with that of two recombinant actin binding fragments of dystrophin (15) and skeletal muscle tropomyosin on F-actin depolymerization induced by deoxyribonuclease I (Fig. 4B). Both dystrophin-glycoprotein complex and tropomyosin significantly inhibited F-actin depolymerization induced by deoxyribonuclease I (Fig. 4B). 80 min after the addition of deoxyribonuclease I, 61.5 ± 3.9% of F-actin was recovered in the high speed pellet when preincubated with tropomyosin, while 40.9 ± 4.2% of F-actin pelleted in the presence of dystrophin-glycoprotein complex (Fig. 4B). In contrast, only 28 ± 3.3% of F-actin was recovered in the high speed pellet if depolymerized in the absence of tropomyosin or dystrophin-glycoprotein complex (Fig. 4B). The effects of tropomyosin and dystrophin-glycoprotein complex on actin depolymerization were not additive, since preincubation of F-actin with saturating concentrations of both tropomyosin and dystrophin-glycoprotein complex yielded a protective effect that was quantitatively indistinguishable from that observed in the presence of tropomyosin alone (Fig. 4C).

Neither dystrophin recombinant protein DYS246 nor DYS1416 had an effect on actin depolymerization by deoxyribonuclease I (Fig. 4B). 80 min after the addition of deoxyribonuclease I, only 28 ± 3.3% of F-actin was recovered in the high speed pellet when preincubated with tropomyosin, while 40.9 ± 4.2% of F-actin pelleted in the presence of dystrophin-glycoprotein complex (Fig. 4B). In contrast, only 28 ± 3.3% of F-actin was recovered in the high speed pellet if depolymerized in the absence of tropomyosin or dystrophin-glycoprotein complex (Fig. 4B). The effects of tropomyosin and dystrophin-glycoprotein complex on actin depolymerization were not additive, since preincubation of F-actin with saturating concentrations of both tropomyosin and dystrophin-glycoprotein complex yielded a protective effect that was quantitatively indistinguishable from that observed in the presence of tropomyosin alone (Fig. 4C).

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lymerization when compared with F-actin alone (Fig. 4B), even when both were present at molar ratios as high as 5:1 with respect to actin (not shown). These results suggested that dystrophin-glycoprotein complex stabilizes F-actin by the concerted effect of two or more actin binding sites distributed throughout its amino-terminal and rod-like domains. As a complementary approach, we examined the effect of dystrophin-glycoprotein complex on actin depolymerization after limited proteolysis. Limited calpain digestion of dystrophin-glycoprotein complex yields a stable series of actin binding dystrophin fragments, while other components of the dystrophin-glycoprotein complex remain intact but no longer cosediment with F-actin (15). The protective effect of dystrophin-glycoprotein complex on actin depolymerization was completely abolished upon digestion with calpain (Fig. 5). These results suggest that an intact dystrophin molecule is necessary to protect actin filaments from depolymerization.

An actin-binding protein that alters actin disassembly might also affect actin assembly. However, dystrophin-glycoprotein complex did not significantly affect the rate or extent of polymerization of pyrene-labeled actin at concentrations and molar ratios that inhibited depolymerization (Fig. 6). This result suggested that dystrophin-glycoprotein complex does not nucleate actin polymerization or alter the rate of filament elongation. As a further test of whether dystrophin-glycoprotein complex may nucleate actin polymerization, we incubated G-actin for 1 h in the absence or presence of dystrophin-glycoprotein complex (Fig. 7A). Neither actin nor dystrophin-glycoprotein complex was recovered in the high speed sedimentation analysis (Fig. 7A). The skeletal muscle tropomyosin used was functional, since it bound to F-actin cooperatively with an average apparent $K_d$ of 0.3 $\mu$M and a stoichiometry of 1 tropomyosin/6 actin monomers (not shown). Thus, in contrast to other members of the actin-cross-linking superfamily (35–37), dystrophin binding to F-actin does not appear to be inhibited by tropomyosin.

**DISCUSSION**

Based primarily on its sequence homology with spectrin and $\alpha$-actinin (12, 13), dystrophin has long been speculatively modeled as an antiparallel dimer, yet there is little direct experimental data addressing this issue. Rotary-shadowed images of dystrophin revealed predominantly dystrophin monomers with some dimers and higher ordered oligomers observed less frequently (14). Unfortunately, the dystrophin examined by electron microscopy was acid-eluted from an affinity column, which may have had some effect on the structures observed or their relative proportions. Dystrophin dimers would 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 $\alpha$-actinin as a positive control (15). While recombinant proteins corresponding to dystrophin repeats 2 and 3 of the rod domain have a conformation similar to that of spectrin repeats (38), it has been cautioned that conservation between dystrophin repeats is much lower than in the case of spectrin (39), particularly with regard to surface residues thought to be important in dimerization (40). In fact, recombinant dystrophin fragments consisting of eight triple helical repeats appear to assume a conformation analogous to tropomyosin. Moreover, previous studies have shown that tropomyosin inhibits actin binding by a number of dystrophin relatives (35–37). Therefore, it was necessary to examine whether tropomyosin also modulated the interaction between F-actin and the dystrophin-glycoprotein complex. However, tropomyosin had no effect on dystrophin-glycoprotein complex binding to F-actin, since the amount of dystrophin bound to F-actin did not change over a wide range of tropomyosin concentrations (Fig. 8). We observed no effect of tropomyosin on dystrophin-glycoprotein complex binding to F-actin even when F-actin was preincubated with tropomyosin at molar ratios as high as 1:1 prior to the addition of dystrophin-glycoprotein complex (not shown). Over a narrower range of concentrations, varying dystrophin-glycoprotein complex was similarly found to have no effect on the amount of tropomyosin that cosedimented with F-actin (Fig. 8B). The skeletal muscle tropomyosin used was functional, since it bound to F-actin cooperatively with an average apparent $K_d$ of 0.3 $\mu$M and a stoichiometry of 1 tropomyosin/6 actin monomers (not shown). Thus, in contrast to other members of the actin-cross-linking superfamily (35–37), dystrophin binding to F-actin does not appear to be inhibited by tropomyosin.

The data presented here (Figs. 2–5) and elsewhere (15) suggested that dystrophin stabilizes F-actin by laterally bridging filament subunits in a manner somewhat analogous to tropomyosin. Moreover, previous studies have demonstrated that tropomyosin inhibits actin binding by a number of dystrophin relatives (35–37). Therefore, it was necessary to examine whether tropomyosin also modulated the interaction between F-actin and the dystrophin-glycoprotein complex. However, tropomyosin had no effect on dystrophin-glycoprotein complex binding to F-actin, since the amount of dystrophin bound to F-actin did not change over a wide range of tropomyosin concentrations (Fig. 8). We observed no effect of tropomyosin on dystrophin-glycoprotein complex binding to F-actin even when F-actin was preincubated with tropomyosin at molar ratios as high as 1:1 prior to the addition of dystrophin-glycoprotein complex (not shown). Over a narrower range of concentrations, varying dystrophin-glycoprotein complex was similarly found to have no effect on the amount of tropomyosin that cosedimented with F-actin (Fig. 8B). The skeletal muscle tropomyosin used was functional, since it bound to F-actin cooperatively with an average apparent $K_d$ of 0.3 $\mu$M and a stoichiometry of 1 tropomyosin/6 actin monomers (not shown). Thus, in contrast to other members of the actin-cross-linking superfamily (35–37), dystrophin binding to F-actin does not appear to be inhibited by tropomyosin.

**FIG. 3. Effect of dystrophin-glycoprotein complex on depolymerization of pyrene-labeled F-actin.** 2 $\mu$M pyrene-labeled F-actin was preincubated in the absence (▲) or presence of dystrophin-glycoprotein complex (▲) and was induced to depolymerize by rapid dilution into low salt buffer conditions. The decrease in pyrene fluorescence intensity was followed over time. The data are plotted as the percentage of fluorescence relative to the amount of fluorescence at time zero.
coprotein complex bound to F-actin with a stoichiometry of 1 dystrophin/24 actin monomers and an apparent $K_d$ of 0.5 mM. As noted above, dystrophin in the glycoprotein complex did not cross-link F-actin. Furthermore, we obtained evidence for a novel actin binding site located near the middle of the dystrophin rod domain and observed that dystrophin-glycoprotein complex significantly protected F-actin from depolymerization (15). In the present study, we have confirmed that dystrophin-glycoprotein complex can protect F-actin from depolymerization (Figs. 2–5) using different protocols for inducing actin depolymerization (dilution into low salt buffers and below critical concentration, the addition of deoxyribonuclease I in constant buffer conditions) and methods of monitoring actin polymerization state (high speed sedimentation and pyrene-actin fluorescence). We have observed that the protective effect of dystrophin-glycoprotein complex on F-actin depolymerization saturated with a stoichiometry of 1 dystrophin/25 actin monomers, which is consistent with the stoichiometry of dystrophin-glycoprotein complex binding to F-actin determined at equilibrium (15).

Actin-binding proteins can influence the polymerization state of actin in a wide variety of ways (42). For example, the actin-cross-linking proteins $\alpha$-actinin and Dictyostelium discoideum 30-kDa actin bundling protein both inhibit the rate of actin depolymerization without effect on polymerization (43–46). However, inhibition of actin filament depolymerization by 30-kDa bundling protein does not strictly depend on its cross-linking activity (43). The actin bundling protein elongation or presence of tropomyosin (TM), dystrophin-glycoprotein complex (DGC), dystrophin fragments (DYS1416 and DYS246), or tropomyosin and dystrophin-glycoprotein complex together (TM + DGC). The proteins were present at a 1:5 molar ratio with respect to actin. 60 min after the addition of deoxyribonuclease I, the samples were centrifuged at 100,000 $\times$ g for 20 min. In A, B, and C, 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 data represent the average (± S.E.) of three or more independent determinations.

**FIG. 4. Effect of dystrophin-glycoprotein complex on F-actin depolymerization induced by deoxyribonuclease I.** Shown in A is the time course of F-actin depolymerization induced by deoxyribonuclease I. 2 $\mu$M F-actin was centrifuged at 100,000 $\times$ g for 20 min at various times after the addition of deoxyribonuclease I. The indicated time points include centrifugation time. In B, tropomyosin, dystrophin-glycoprotein complex, and two recombinant actin binding fragments of dystrophin were measured for effect on F-actin depolymerization. In C, tropomyosin and dystrophin-glycoprotein complex were measured for an additive effect on F-actin depolymerization. In B and C, 2 $\mu$M F-actin was incubated for 30 min at room temperature in the absence (control), presence of tropomyosin (TM), dystrophin-glycoprotein complex (DGC), dystrophin fragments (DYS1416 and DYS246), or tropomyosin and dystrophin-glycoprotein complex together (TM + DGC). The proteins were present at a 1:5 molar ratio with respect to actin. 60 min after the addition of deoxyribonuclease I, the samples were centrifuged at 100,000 $\times$ g for 20 min. In A, B, and C, 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 data represent the average (± S.E.) of three or more independent determinations.

**FIG. 5. Dystrophin-glycoprotein complex fails to protect F-actin from depolymerization after limited calpain digestion.** 6 $\mu$M F-actin in buffer containing 10 mM NaCl and 0.2 mM MgCl$_2$ was incubated for 30 min at room temperature in the absence (control) or presence of calpain-digested (Calpain DGC) or intact dystrophin-glycoprotein complex (DGC), each present at a 1:10 molar ratio with respect to actin. The samples were rapidly diluted 3-fold into buffer without NaCl and MgCl$_2$ and centrifuged at 100,000 $\times$ g for 20 min beginning 220 min after 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 data represent the average (± S.E.) of three independent experiments.
Stabilization of F-actin by Monomeric Dystrophin

Effect of dystrophin-glycoprotein complex on polymerization of pyrene-labeled actin. 2 μM pyrenyl G-actin was incubated at room temperature for 30 min in the absence (○) or presence of dystrophin-glycoprotein complex (□). Polymerization was initiated, and the increase in pyrene fluorescence intensity was followed over time. Symbols indicate the average (± S.E.) of selected time points performed in triplicate.

factor 1α alters both actin polymerization and depolymerization (47). In our previous study we demonstrated that dystrophin-glycoprotein complex does not have the capacity to cross-link F-actin (15). Therefore, the inhibitory effect of dystrophin-glycoprotein complex on F-actin depolymerization could not be explained as the result of actin filament cross-linking.

We have also observed that dystrophin-glycoprotein complex neither binds G-actin nor alters its rate of nucleation or elongation during polymerization (Figs. 6 and 7). Our results are in good agreement with previous studies of the recombinant amino-terminal domain of dystrophin (7) or utrophin (48) using similar methods but differ from the results of solid phase experiments (49, 50), which suggested that dystrophin binds monomeric and F-actin with similar avidity. These differences in results are almost certainly method-dependent, since α-actinin has also been observed to bind monomeric actin in solid phase assays (51) yet exhibits no affinity for G-actin or effect on actin polymerization in solution (45, 46). Nonetheless, our present results suggest that the dystrophin-glycoprotein complex exhibits no specific capping or nucleating activity that could provide an alternative explanation for its inhibitory effect on F-actin depolymerization.

Neither of the recombinant actin binding fragments of dystrophin (DYS246, DYS1416) affected F-actin depolymerization (Fig. 4B). DYS246 corresponds to the well characterized amino-terminal actin binding domain of dystrophin (6, 7, 9, 52) that is homologous to a conserved actin binding domain found in a number of proteins (13, 53, 54). DYS1416 corresponds to dystrophin amino acids 1416–1880 located near the middle of the dystrophin rod domain, which also exhibits actin binding activity (15). We also observed that limited calpain digestion of dystrophin-glycoprotein complex abolishes its capacity to protect actin filaments from depolymerization (Fig. 4C). It therefore appears that the binding sites represented by DYS246 and DYS1416, separated on the intact dystrophin molecule by almost 1,200 amino acids, act in concert to bind along an actin filament and protect it from depolymerization. In this regard, the in vitro behavior of dystrophin is more similar to that of tropomyosin (55) than it is to its sequence relatives α-actinin and spectrin.

Since the sum of our present and previous (15) data best support a side binding model for the interaction of dystrophin-glycoprotein complex with F-actin, it became relevant to determine whether dystrophin and tropomyosin can compete for binding to F-actin. For example, inhibition of dystrophin binding to tropomyosin could provide a basis for preventing dystrophin binding to sarcomeric thin filaments and support a default mechanism of targeting dystrophin to the sarcolemma membrane. Inhibition of dystrophin binding to tropomyosin could further explain why dystrophin overexpression is not detrimental to myofibrillar function (56). Previous studies have demonstrated that tropomyosin inhibits actin binding by filamin (35), spectrin (36), and α-actinin (37). Somewhat surprisingly, we did not observe any effect of skeletal muscle tropomyosin on dystrophin-glycoprotein complex binding to F-actin (Fig. 8), suggesting that dystrophin interacts with actin filaments in a novel manner. It is possible that the novel middle rod domain actin binding site corresponding to DYS1416 might support dystrophin-glycoprotein complex binding to F-actin, while the amino-terminal actin binding site is blocked by tropomyosin. However, a recombinant protein corresponding to the amino-terminal domain of dystrophin was previously found to have no effect on the binding of tropomyosin to F-actin (9). Thus, while the homologous actin binding domains of dystrophin, α-actinin, and β-spectrin seem likely to share a common core structure (54), it also appears that these domains exhibit distinct functional properties.
Dystrophin-glycoprotein complex and tropomyosin could both bind the same actin filament in vivo without steric hindrance or competition for actin binding sites. Alternatively, dystrophin-glycoprotein complex could substitute for tropomyosin by stabilizing actin filaments near the sarcolemmal membrane while simultaneously allowing or even facilitating the binding of other cortical actin-binding proteins. In support of this possibility, the apical region of myotendinous junctions contains bundles of actin filaments oriented in parallel with the dystrophin-enriched sarcolemmal membrane but lacking tropomyosin (57). Interestingly, these actin filament bundles become dissociated from the sarcolemmal membrane in dystrophin-deficient myotendinous junctions (58).

In summary, our present results provide support for a model (Fig. 9) in which monomeric dystrophin binds laterally along an actin filament through the concerted effect of at least two spatially separated low affinity binding sites. We have speculatively chosen to illustrate a minimal contact between dystrophin and F-actin with the amino-terminal half of a dystrophin molecule (containing both the amino-terminal and middle rod actin binding sites) contacting both strands of an actin filament composed of 24 actin molecules and spanning a length of 65 nm. In support of this model, recent *in situ* fluorescence resonance energy transfer microscopy analysis (59) of muscle sections labeled with an antibody (60) specific for dystrophin sequence overlapping with DYS1416 suggest that such a lateral association between dystrophin and F-actin occurs within cells. This model is also supported by experiments suggesting that the highly labile amino-terminal half of dystrophin is resistant to proteolysis *in situ* (61). Alternatively, the amino-terminal domain and each of the 24 spectrin-like repeats of the dystrophin rod domain may align with individual monomers in a single strand of an actin filament. Further refinement of the model will definitely benefit from more thorough mapping of the actin binding sites within the dystrophin rod domain as well as detailed ultrastructural analysis.

While our model (Fig. 9) can explain the relatively high affinity of intact dystrophin for F-actin in vitro, it may also hold important implications for the mechanical behavior of dystrophin in vivo. For example, Pollard and colleagues (62) have proposed that multiple, dynamically rearranging cross-links between α-actinin and actin filaments could explain the simultaneous rigid or deformable response of the cytomatrix in a deformation rate-dependent manner. Likewise, the presence of multiple, low affinity actin binding sites may enable the F-actin/dystrophin/sarcolemma linkage to re-

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**FIG. 8.** Effect of tropomyosin on dystrophin-glycoprotein complex binding to F-actin. Shown in A is a Coomassie Blue-stained SDS-polyacrylamide gel of 100,000 x g supernatants (S) and pellets (P) of dystrophin-glycoprotein complex and F-actin incubated in the absence (−TM) or in the presence (+TM) of tropomyosin. Actin (ACT) and tropomyosin (TM) were present at 2.4 and 0.6 μM, respectively. The concentration of dystrophin-glycoprotein complex was 0.09 mg/ml (0.2 mM dystrophin, DYS). In B, 24 μM F-actin was incubated with 0.2 μM dystrophin in the presence of increasing amounts of tropomyosin (O), or 2 μM F-actin was incubated with 0.3 μM tropomyosin in the presence of increasing amounts of dystrophin-glycoprotein complex (M). The samples were centrifuged at 100,000 x g for 20 min. The amount of dystrophin (O) or tropomyosin (M) bound to F-actin was determined densitometrically from Coomassie Blue-stained gels loaded with equal volumes of supernatants and pellets as shown in A. Average data (± S.E., n = 3) were expressed as the percentage of dystrophin or tropomyosin pelleted with F-actin, where 100% equals 0.019 ± 0.002 mol of dystrophin bound per mol of actin in the absence of tropomyosin and 0.096 ± 0.002 mol of tropomyosin bound per mol of actin in the absence of dystrophin-glycoprotein complex.

**FIG. 9.** Model for a side binding interaction between dystrophin and F-actin. As described in the “Discussion,” a minimal contact between the amino-terminal half of dystrophin and 24 actin monomers comprising both strands of an actin filament is depicted. However, a model in which the entire amino-terminal domain and 24 triple helical repeats of dystrophin are aligned along 24 or 25 monomers within a single strand of the filament cannot be ruled out.
spond in either an elastic or fluid manner, depending on the rate at which a stress is applied to the sarcolemmal membrane.

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