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Tubulin–Myosin Interaction. Some Properties of Binding between Tubulin and Myosin†

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ABSTRACT: This report presents evidence suggesting the direct binding between tubulin and myosin: (1) coprecipitation of tubulin with myosin occurred at a low ionic strength at which no precipitation of tubulin by itself occurred; (2) the amount of tubulin coprecipitated was unchanged when the coprecipitate was washed thoroughly; (3) about 2 mol of tubulin dimer could bind per mol of myosin at the maximum under our experimental conditions. The binding of about 1 mol of tubulin dimer was influenced by the presence of F-actin, but that of the other 1 mol of tubulin dimer was uninfluenced. In the former binding, tubulin or actin which bound first to myosin was suggested to have a priority. With regard to the priority of the binding, a similar result was obtained from the experiments of tubulin interference in actin activation of myosin Mg^{2+}-ATPase. The tubulin–myosin binding occurred moderately even at 0 °C and was not affected by Ca^{2+} (2 mM), colchicine (200 μM), or Mg-ATP (4 mM), reflecting that the ability of tubulin to bind to myosin was different from the ability of tubulin to form microtubules and that the nature of tubulin–myosin binding was different from that of F-actin–myosin binding. Besides tubulin–myosin interaction, a possible interaction between microtubule-associated proteins (MAPs) and actomyosin was suggested from the data that MAPs activated actomyosin Mg^{2+}-ATPase activity while purified tubulin inhibited the activity.

Attention has been increasingly directed to the roles of the actomyosin system and microtubule (tubulin–dynein) system in cell motility [for review, see Goldman et al. (1976)]. Furthermore, the idea that the two systems may function coincidentally in a certain cellular motile phenomenon has been widely entertained; for example, axoplasmic transport (Hoffmann & Lasek, 1975), release of neurotransmitters at synaptic endings (Thoa et al., 1972), movement of pigment granules in pigment cells (Malawista, 1975), chromosome movement in mitosis (Sanger, 1975), and modulation of cell surface proteins (Nicolson, 1976). It is possible to infer that both systems function not only independently but also cooperatively through the interactions between the components contained in both systems.

As one of such interactions, a tubulin–myosin interaction has so far been studied. Mohri & Shimomura (1973) observed a superprecipitation-like phenomenon in a tubulin–myosin mixture. Alicea & Renaud (1975) showed tubulin–myosin interaction by measuring myosin ATPase activity which was markedly stimulated by tubulin. However, this result was not reproducible as shown by Castle et al. (1976). On the other hand, Gozes et al. (1975) reported that tubulin and actin synthesized in vitro from brain mRNA failed to separate from each other even if they were treated by the purification procedures specific for the respective proteins. The result strongly suggested the occurrence of actin–tubulin or tubulin–myosin interaction. This urged us to investigate the tubulin–myosin interaction in detail. The present paper describes the evidence suggesting that about 2 mol of tubulin dimer can bind per mol of myosin and that the binding attributes of the 2 mol are likely to be different from each other.

Materials and Methods

Preparation of Proteins. Microtubule protein was prepared from porcine brain by the procedure of Shelanski et al. (1973) except that the reassembly buffer of Weisenberg (1972) was replaced by that of Kuriyama (1975). As "microtubule protein", the fraction obtained after two cycles of polymerization and depolymerization was used. For the preparation of purified tubulin and microtubule-associated proteins (MAPs), the microtubule protein was applied to a phosphocellulose column preequilibrated with 5 mM imidazole hydrochloride buffer (pH 6.6) containing 50 mM KCl (buffer A) and eluted with buffer A. The protein fraction eluted in the void volume was used as purified tubulin (PC-tubulin). The MAPs fraction was then eluted with 5 mM imidazole hydrochloride buffer (pH 6.6) containing 0.8 M KCl and desalted by passage through a column of Sephadex G-25 preequilibrated with buffer A.

Myosin was extracted from rabbit skeletal muscle as described by Perry (1955) and purified by ammonium sulfate precipitation. Purified actin was prepared from rabbit skeletal muscle by the method of Hirabayashi & Hayashi (1970).

Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. This was performed by using 7.5% polyacrylamide gel by the method of Weber & Osborn (1969) unless otherwise specified. Staining of gels was done with 0.25% Coomassie brilliant blue solution containing 45.4% methanol and 9.2% acetic acid for more than 6 h, and free dye was rinsed out with 20% ethanol containing 10% acetic acid for 2–3 days.

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Table I: Effect of Temperature on Tubulin–Myosin Binding[a]

temp (°C)	bound tubulin dimer/myosin (mol/mol)
0	0.6
10	0.8
20	1.1
30	1.4

[a] Aliquots of a mixture of myosin (0.25 mg/mL) and microtubule protein (0.29 mg/mL) in buffer A were incubated for 15 min at various temperatures and centrifuged at 5200*g* for 10 min. The resulting pellets were analyzed by electrophoresis.

ATPase Assay. A reaction mixture (2 mL) was composed of proteins, 50 mM KCl, 4 mM $MgCl_2$, 2 mM ATP, and 5 mM imidazole hydrochloride buffer (pH 6.6). The mixture was incubated at 30 °C for 20 min, and the reaction was stopped by the addition of cold trichloroacetic acid solution. After centrifugation, the amount of inorganic phosphate liberated in the supernatant was measured by the method of Fiske & Sabbarow (1925).

Assay for Tubulin Contained in a Tubulin–Myosin Coprecipitate. A mixture of microtubule protein (or PC-tubulin) and myosin at low ionic strength (0.05 M with respect to KCl concentration) was centrifuged at 5200*g* for 10 min, and the pellet was dissolved in 0.01 M sodium phosphate buffer (pH 7.2) containing 25% glycerol and 1% sodium dodecyl sulfate. The sample was then subjected to electrophoresis. Protein bands in the gel were stained and scanned in a Fujiox densitometer (Type FD-A) at 600 nm. Densitometric areas of myosin heavy chain and tubulin were traced on a paper, cut out, and weighed. Standard curves showed that myosin heavy chain and tubulin were stained quantitatively up to 11 and 5.5 μg, respectively. In the quantitative region, tubulin was stained with 70% efficiency as compared with myosin heavy chain. The equation to estimate the molar ratio of tubulin dimer per myosin in a tubulin–myosin coprecipitate is

$$\frac{T}{M_{hc} \times 0.7} \times \frac{400\,000}{110\,000}$$

where T is the weight of the densitometric area of tubulin coprecipitated by myosin, M_{hc} is that of myosin heavy chain, and 0.7 is a factor showing a difference in staining efficiency between the two proteins. Molecular weights used in the equation were as follows: tubulin dimer, 110000; myosin heavy chain, 200000 (myosin, 485000).

Results

Gozes et al. (1975) showed that both actin and tubulin newly synthesized in vitro from brain mRNA were precipitated by myosin. From this hint, we first carried out a similar experiment using actin and myosin from skeletal muscle and microtubule protein from brain. When a mixture of actin and microtubule protein was mixed with myosin and brought to low ionic strength, both actin and tubulin were precipitated by myosin (Figure 1). When actin alone and microtubule protein alone were mixed with myosin, actin and tubulin were also precipitated by myosin, respectively (lanes b and c of Figure 1). Under the conditions, microtubule protein alone without myosin was not precipitated at all (Figure 1d). The tubulin coprecipitated by myosin was composed of approximately equal amounts of α and β subunits (Figure 1f). The tubulin–myosin coprecipitation occurred to nearly the same extent in the pH range between 5.9 and 7.6 at 30 °C, and more than 5 min of incubation was found to be sufficient for the coprecipitation (data not shown). For the incubation temperature, 30 °C was selected (Table I).

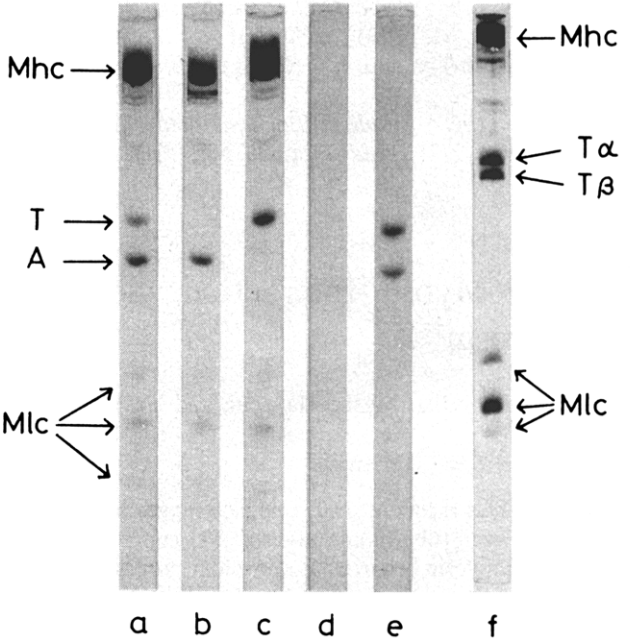

FIGURE 1: Tubulin–myosin coprecipitation revealed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. (a) A mixture of 0.2 mL of actin solution (5 mg/mL in 0.1 mM ATP and 4 mM Tris-HCl buffer, pH 7.6) and 0.2 mL of microtubule protein solution (5 mg/mL in 0.4 M KCl and 20 mM Tris-HCl buffer, pH 7.6) was incubated for 20 min at 30 °C, added to 0.2 mL of myosin solution (15 mg/mL in 0.4 M KCl and 20 mM Tris-HCl buffer, pH 7.6), and then diluted with 3.4 mL of cold distilled water. The mixture was centrifuged at 5200*g* for 5 min, and the resulting pellet was washed once with 2 mL of 0.04 M KCl and 2 mM Tris-HCl buffer (pH 7.6) and subjected to electrophoresis. (b) The same as (a) except that microtubule protein was omitted. (c) The same as (a) except that actin was omitted. (d) The same as (a) except that actin and myosin were omitted. (e) A mixture of actin and microtubule protein samples used in this experiment. (f) The same as (c) except that electrophoresis was carried out by the method of Mabuchi & Shimizu (1974) instead of Weber & Osborn (1969). Protein bands in each gel are shown by arrows with symbols: Mhc, myosin heavy chain; T, tubulin; Tα and Tβ, α and β subunits of tubulin; A, actin; Mlc, myosin light chain.

To ascertain whether or not tubulin was precipitated by nonspecific entanglement of myosin precipitation, we thoroughly washed the coprecipitate with buffer A. Even if the precipitate was washed 4 times, the amount of tubulin contained in the tubulin–myosin precipitate was not changed at all. Next, an experiment was carried out in which a fixed amount of myosin was added to an increasing amount of microtubule protein. As shown in Figure 2a, the amount of bound tubulin increased in proportion to the amount of added microtubule protein and reached a saturation point where about 1.5 mol of tubulin dimer bound per mol of myosin. The amount of bound tubulin was not influenced whether microtubule protein was added to myosin at high ionic strength and diluted to low ionic strength or added to myosin at low ionic strength.

However, the possibility still remained that tubulin might indirectly be linked to myosin through MAPs, neurofilament, or a certain protein included in the microtubule protein. PC-tubulin was therefore used to prove direct binding between tubulin and myosin. As shown in Figure 2b, PC-tubulin could bind to myosin and about 2 mol of tubulin dimer bound per mol of myosin at the saturation point. This shows that direct binding between tubulin and myosin occurs whether PC-tubulin or microtubule protein is used.

For the characterization of the tubulin–myosin binding, effects of colchicine, Ca^{2+}, and Mg-ATP on the binding were investigated. Preincubation of 0, 50, 100, and 200 μM col-

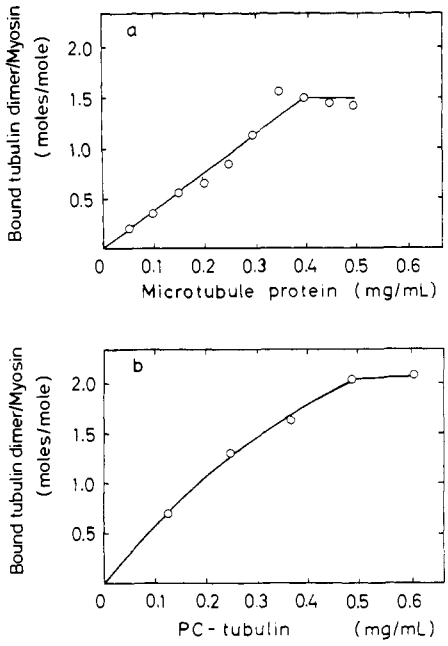

FIGURE 2: Stoichiometry of tubulin–myosin binding. (a) 0.2 mL of myosin solution (6.2 mg/mL in 0.5 M KCl and 5 mM imidazole hydrochloride buffer, pH 6.6) was added to 0.2 mL of variously concentrated microtubule protein solution (0.5 M KCl and 5 mM imidazole hydrochloride buffer, pH 6.6). The mixture was diluted with 3.6 mL of 5 mM imidiazole hydrochloride buffer (pH 6.6), incubated for 15 min at 30 °C, and centrifuged at 5200*g* for 10 min at 30 °C. The precipitate was analyzed by electrophoresis. Amounts of tubulin in tubulin–myosin precipitates are expressed as molar ratios of tubulin to myosin. (b) A mixture (2.4 mL) of myosin (0.54 mg) and PC-tubulinn (an increasing amount) in buffer A was incubated for 5 min at 30 °C and centrifuged at 5200*g* for 10 min. The precipitate was analyzed as in (a).

chicine and of 1 and 2 mM $CaCl_2$ with microtubule protein resulted in 1.2, 1.2, 1.2, 1.3, 1.3, and 1.1 mol of bound tubulin per mol of myosin, respectively, showing that colchicine and Ca^{2+} of the above-mentioned concentrations, which completely prevent microtubule assembly, do not exert any influence upon the tubulin–myosin binding. On the other hand, when aliquots of a tubulin–myosin complex having a molar ratio (bound tubulin dimer/myosin) of 1.6 were treated with 1, 2, 3, and 4 mM Mg-ATP for 5 min at 30 °C, the molar ratios of complexes after centrifugation showed the values of 1.7, 1.7, 1.6, and 1.5, respectively. This indicates that the tubulin–myosin binding is not affected at all by the indicated concentrations of Mg-ATP.

For elucidation of the nature of tubulin binding to myosin, binding competition between tubulin and actin against myosin was examined. Effects of increasing amounts of F-actin on the tubulin–myosin binding were investigated by experiments of two types: (i) microtubule protein or PC-tubulin was added to myosin before F-actin addition and (ii) F-actin was added to myosin before the addition of microtubule protein or PC-tubulin. In these experiments, there exists a dilemma: ATP needed for preventing actin denaturation might elicit superprecipitation in the tubulin–actin–myosin mixture, whereas use of actin solution with no ATP does not bring about superprecipitation but might result in actin denaturation to some extent. Accordingly, we carried out the experiments i and ii by using microtubule protein under the conditions with ATP (Figure 3a) and without ATP (Figure 3b). As shown in parts a and b of Figure 3, these different experimental conditions did not make any difference in the results obtained. Therefore, we then carried out the experiments i and ii by using PC-tubulin under the ATP-containing conditions (Figure 3c). The

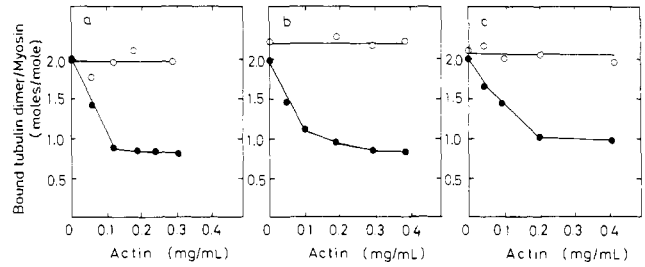

FIGURE 3: Effects of increasing amounts of F-actin on tubulin–myosin binding. Experiments of type i (○). 1 mL of myosin solution (a fixed amount in buffer A) was mixed with 0.2 mL of microtubule protein or PC-tubulin solution (a fixed amount in buffer A), incubated for 15 min at 30 °C, and added to 0.3 mL of F-actin solution (an increasing amount in buffer A with or without 0.05 mM ATP). The mixture was incubated for 15 min at 30 °C and centrifuged at 5200*g* for 10 min. Experiments of type ii (●). 1 mL of myosin solution (a fixed amount in buffer A) was mixed with 0.3 mL of F-actin solution (an increasing amount in buffer A with or without 0.05 mM ATP), incubated for 15 min at 30 °C, and added to 0.2 mL of microtubule protein or PC-tubulin solution (a fixed amount in buffer A). The mixture was incubated for 15 min at 30 °C and centrifuged at 5200*g* for 10 min. The precipitates were analyzed by electrophoresis. Amounts of bound tubulin are expressed as molar ratios of tubulin to myosin. (a) F-Actin suspended in buffer A containing 0.05 mM ATP was used. Protein concentrations in the final mixtures: myosin, 0.34 mg/mL; microtubule protein, 0.40 mg/mL; F-actin, amounts indicated on the abscissa. (b) F-Actin suspended in buffer A containing no ATP was used. Protein concentrations in the final mixtures: myosin, 0.23 mg/mL; microtubule protein, 0.29 mg/mL; F-actin, amounts indicated on the abscissa. (c) The same as (a) except that PC-tubulin was used instead of microtubule protein. Protein concentrations in the final mixtures: myosin, 0.23 mg/mL; PC-tubulin, 0.27 mg/mL; F-actin, amounts indicated on the abscissa.

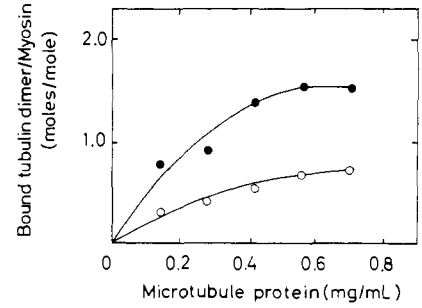

FIGURE 4: Effect of preexisting F-actin on tubulin–myosin binding. A mixture of 0.8 mL of F-actin solution (1.7 mg/mL in buffer A) and 2.0 mL of myosin solution (0.33 mg/mL in buffer A) was incubated for 15 min at 30 °C and added to 0.2 mL of microtubule protein (an increasing amount in buffer A). After centrifugation the precipitates were analyzed by electrophoresis. (○) Amounts of bound tubulin. (●) Amounts of bound tubulin in a control experiment where F-actin was omitted.

results shown in Figure 3 indicate that in all experiments of the type i the molar ratio of the tubulin–myosin binding (tubulin dimer/myosin) was about 2 without added F-actin and the value was uninfluenced by increasing amounts of F-actin, and in all experiments of the type ii the molar ratio of the tubulin–myosin binding gradually decreased from 2 to about 1 as the amounts of added F-actin in the reaction mixture increased and the ratio (about 1) remained constant even in the presence of larger amounts of added F-actin. Hence, it is possible to consider that about 1 mol of tubulin dimer binds per mol of myosin independently of F-actin competition and the other 1 mol of tubulin dimer is capable of competing with F-actin for binding to myosin. However, the latter does not compete with F-actin when tubulin is added to myosin before F-actin addition, suggesting that it acquires a priority for the binding to myosin.

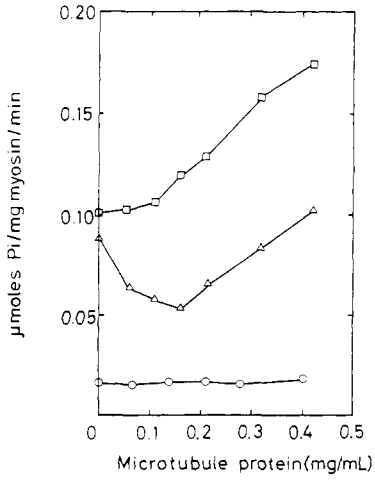

FIGURE 5: Effects of microtubule protein on myosin or actin activation of myosin Mg^{2+}-ATPase. (○) A mixture (1.6 mL) of myosin (0.30 mg) and microtubule protein (an increasing amount) in buffer A was added to 0.2 mL of Mg solution (50 mM KCl, 40 mM $MgCl_2$, and 5 mM imidazole hydrochloride buffer, pH 6.6), incubated for 15 min at 30 °C, and added to 0.2 mL of 20 mM ATP solution. ATPase assay was performed as described under Materials and Methods. (△) A mixture (1.4 mL) of myosin (0.32 mg) and microtubule protein (an increasing amount) in buffer A was added to 0.2 mL of 40 mM Mg solution, incubated for 15 min at 30 °C, and mixed with 0.2 mL of F-actin solution (1.5 mg/mL in buffer A containing 0.05 mM ATP). The mixture was incubated for 15 min at 30 °C and added to 0.2 mL of 20 mM ATP solution. After that, ATPase activity was measured. The amount of inorganic phosphates liberated during the preincubation period was very small (<2% of the total inorganic phosphate was liberated during the ATPase assay) and did not obscure the results. (□) A 0.7-mL myosin solution (0.47 mg/mL in buffer A) was mixed with 0.2 mL of F-actin solution (2.0 mg/mL in buffer A containing 0.05 mM ATP), added to 0.2 mL of 40 mM Mg solution, and incubated for 15 min at 30 °C. The mixture was added to 0.7 mL of microtubule protein solution (an increasing amount in buffer A), incubated for 15 min at 30 °C, and added to 0.2 mL of 20 mM ATP solution. ATPase activity was then measured.

We then attempted an experiment in which aliquots of an actomyosin mixture were mixed with increasing amounts of microtubule protein. As shown in Figure 4, by the preexistence of F-actin, amounts of bound tubulin fell to about half in each microtubule protein concentration as compared with values of the control experiment in which F-actin was omitted in the reaction mixture. The result implies that F-actin which bound first to myosin also acquires a priority for 1 of 2 mol of tubulin to bind to myosin.

In order to support the results of tubulin–myosin interaction as obtained by the coprecipitation method, we investigated the effects of microtubule protein or PC-tubulin on myosin Mg^{2+}-ATPase activity and actin activation of myosin Mg^{2+}-ATPase. The addition of an increasing amount of microtubule protein to myosin had no effect on myosin Mg^{2+}-ATPase activity (Figure 5, ○) but exerted some influence on actin-activated myosin Mg^{2+}-ATPase (Figure 5, △ and □). In one experiment (Figure 5, △), a fixed amount of myosin was mixed with an increasing amount of microtubule protein and then mixed with a fixed amount of F-actin. The result showed that in the range of low microtubule protein concentrations increasing amounts of microtubule protein brought about a gradual activity drop of actin-activated Mg^{2+}-ATPase and the inhibition reached a maximum at which the ATPase activity became nearly half the initial activity (with no microtubule protein). On the contrary, with further increase of microtubule protein concentration, acceleration of actomyosin Mg^{2+}-ATPase activity gradually occurred. In the other experiment (Figure 5, □), a fixed amount of actomyosin

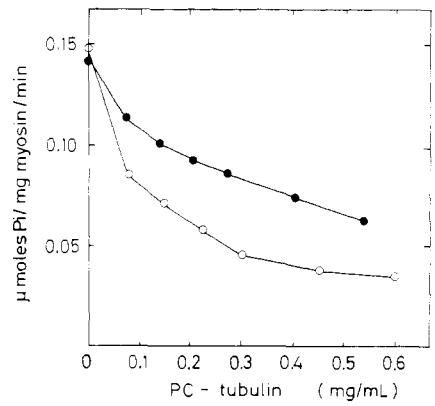

FIGURE 6: Effect of PC-tubulin on actin activation of myosin Mg^{2+}-ATPase. (○ and ●) Experimental procedures were the same as those in (△) and (□) of Figure 5, respectively, except that PC-tubulin was used instead of microtubule protein. Protein concentrations: myosin, 0.13 mg/mL; actin, 0.20 mg/mL; PC-tubulin, increasing amounts (0–0.6 mg/mL, see abscissa).

was mixed with an increasing amount of microtubule protein. In this case, the inhibition of actomyosin Mg^{2+}-ATPase activity was not recognized but a gradual acceleration was exclusively observed.

As a trial to separate respective factors that inhibit and accelerate actomyosin Mg^{2+}-ATPase from the microtubule protein fraction, effects of PC-tubulin and MAPs on actomyosin Mg^{2+}-ATPase were examined. As for PC-tubulin, the following two experiments were done. In the first experiment, myosin was mixed with PC-tubulin and then F-actin was added (Figure 6, ○), and in the second experiment, actomyosin was mixed with PC-tubulin (Figure 6, ●). PC-tubulin showed only an inhibitory effect, and the degree of the inhibition was larger in the first experiment than that in the second one, which suggests that the priority phenomenon appears in the experiments using PC-tubulin, as is the case with microtubule protein.

On the other hand, increasing amounts of MAPs exerted only an accelerative effect on actomyosin Mg^{2+}-ATPase. Under the conditions used, MAPs alone showed very little ATPase activity. Furthermore, the acceleration of ATPase activity by MAPs did not occur when MAPs were added to actin or myosin, but occurred only when MAPs were added to actomyosin (Figure 7).

Discussion

In the present paper, we applied a tubulin–myosin coprecipitation method to the detection of direct binding between tubulin and myosin (Figure 1) and found that about 2 mol of tubulin dimer bound per mol of myosin at the maximum whether microtubule protein or PC-tubulin was used (Figure 2). Under our experimental conditions, the binding attributes of the 2 mol of tubulin are likely to be different from each other in terms of the competition with F-actin (Figures 3 and 4). The binding of about 1 mol of tubulin was not influenced by the presence of F-actin, but the other 1 mol was influenced. In the latter binding, tubulin or actin which bound first to myosin showed a priority for binding to myosin. The priority phenomenon was supported, from a different angle, by investigating the effects of tubulin on actin activation of myosin Mg^{2+}-ATPase (Figures 5 and 6).

We have considered that the tubulin–myosin binding found under our experimental conditions is sure to occur, but its quantitative analysis has not completely been made, since we have investigated it at a low ionic strength at which myosin and actomyosin are aggregated. For the actual quantitation,

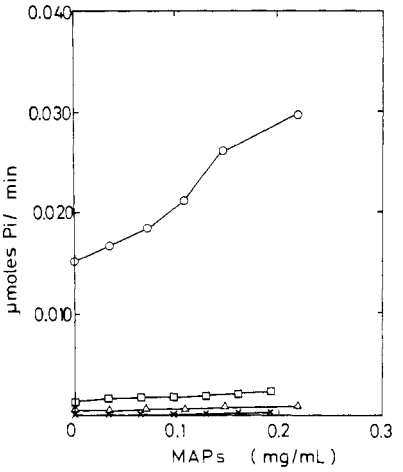

FIGURE 7: Effect of MAPs on actin activation of myosin Mg^{2+}-ATPase. (○) A 0.7-mL myosin solution (0.32 mg/mL in buffer A) was mixed with 0.2 mL of F-actin solution (2.0 mg/mL in buffer A containing 0.05 mM ATP), added to 0.2 mL of 40 mM Mg solution, and incubated for 15 min at 30 °C. The mixture was added to 0.7 mL of MAPs solution (an increasing amount in buffer A), incubated for 15 min at 30 °C, and added to 0.2 mL of 20 mM ATP solution. After the addition of final ATP, ATPase assay was performed. (△) The same as (○) except that myosin was omitted. (□) The same as (○) except that actin was omitted. (×) The same as (○) except that actin and myosin were omitted (= MAPs alone).

we should use the conditions where interacting proteins are free in solution. On the other hand, we have been tempted to speculate that myosin possesses two tubulin-binding sites on its molecule from the evidence mentioned in the previous paragraph. To ascertain the speculation, we should carry out an experiment with myosin fragments such as light meromyosin (LMM), heavy meromyosin (HMM), subfragment-1 (S1), and subfragment-2 (S2). For the two purposes, we have carried out tubulin–binding experiments with these myosin fragments. However, results of our preliminary experiments showed that neither LMM nor HMM could bind to tubulin: (i) no coprecipitation occurred between LMM and tubulin at the low ionic strength; (ii) little or no tubulin–HMM binding occurred when examined by gel filtration; (iii) acto-HMM Mg^{2+}-ATPase was little affected by tubulin even when tubulin was preincubated with HMM before F-actin addition. Although we are still continuing such experiments, the results obtained so far are seemingly incompatible with the reproducible, positive data of tubulin–myosin interaction as revealed by the coprecipitation method (Figures 1–4), by the ATPase measurement (Figure 5 and 6), by the appearance of the superprecipitation-like phenomenon (Mohri & Shimomura, 1973; Hayashi et al., 1980), and by the electron microscopic observation suggesting that the head portions of each myosin in myosin threads were decorated with tubulin particles (Hayashi et al., 1980). Under the circumstances, we now consider that a whole myosin molecule might be needed for the interaction with tubulin dimer. Stafford et al. (1979) recently reported that actin-activated Mg-ATPase of S1 from scallop myosin lacked calcium sensitivity, which was exhibited in the case of intact myosin molecule, although S1 retained regulatory and essential light chains. This seems to be a result analogous to ours.

The present study showed that the tubulin–myosin binding is relatively independent of pH, relatively insensitive to low temperature, and not affected by colchicine or Ca^{2+}. Therefore, the ability of tubulin to bind to myosin appears to be distinguishable from the ability of tubulin to form microtubules. The tubulin–myosin binding is also different in its characteristics from actin–myosin binding because Mg-ATP cannot bring about dissociation of tubulin–myosin complex.

Use of PC-tubulin and MAPs made it possible to separate microtubule protein into an inhibitory component and accelerative component against actomyosin Mg^{2+}-ATPase: tubulin exerted only an inhibitory effect whereas MAPs exerted only an accelerative effect. With regard to the effect of MAPs, we elucidated that a protein factors(s) within MAPs is responsible for the activation and the activation reached a maximum at about fourfold (results will be published elsewhere). Griffith & Pollard (1978) reported the binding between F-actin and MAPs. This F-actin–MAPs interaction might be related to the cause eliciting the acceleration phenomenon. At any rate, a rise in ATPase by MAPs is surely due to the activation of myosin ATPase but not to that of MAPs ATPase, since (1) MAPs ATPase activity was extremely low as compared with actomyosin ATPase, (2) intact MAPs could not activate acto-*N*-ethylmaleimide–myosin ATPase prepared as described by Meeusen & Cande (1979), and (3) ATPase activity of MAPs was considerably decreased by *N*-ethylmaleimide (NEM) treatment but NEM-treated MAPs could stimulate the ATPase activity of actomyosin solution just as intact MAPs did (results will be published elsewhere).

In the present paper, we point out the interactions between the components contained in the actomyosin system and microtubule system, tubulin–myosin and MAPs–actomyosin interactions. The actual roles of such interactions in cell motility remain to be elucidated.

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Redox and Spectroscopic Properties of Oxidized MoFe Protein from *Azotobacter vinelandii*†

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ABSTRACT: The MoFe protein from *Azotobacter vinelandii* undergoes a six-electron oxidation by various organic dye oxidants with full retention of initial activity. Reduction of the oxidized protein by $S_2O_4^{2-}$ and by controlled potential electrolysis indicates the presence of two reduction regions at −290 and −480 mV, each requiring three electrons for complete reaction. Control of the oxidation conditions provides a means for preparing two distinct MoFe protein species selectively oxidized by three electrons. Selective reduction of the redox region at −290 mV causes development of the EPR signal associated with fully reduced MoFe protein while reduction at −480 mV produces a change in the visible spectrum but has no effect on the EPR signal intensity. Kinetic differences for reduction of the two redox regions indicate that the cofactor region undergoes a more rapid reaction with reductant than the other metal redox sites.

The redox properties of the MoFe protein from different bacterial sources have been investigated by a number of workers [see Zumft & Mortenson (1975) and Mortenson & Thorneley (1979) for a review; Orme-Johnson et al., 1977; Watt & Bulen, 1976; O'Donnell & Smith, 1978; Watt et al., 1980]. Two groups of redox values for the MoFe protein have been reported in these studies, one occurring near −100 mV and the other near −450 mV vs. the normal hydrogen electrode (NHE).[1] In a recent comprehensive study by O'Donnell & Smith (1978), the redox potentials (in parentheses) for MoFe proteins from the organisms *Azotobacter vinelandii* (−42 mV), *Clostridium pasteurianum* (0 mV), *Bacillus polymyxa* (−95 mV), *Azotobacter croococcum* (−42 mV), and *Klebsiella pneumonia* (−180 mV) were measured by an EPR–potentiometric technique under similar conditions, thus giving a self-consistent set of data for these proteins. The redox reactions were all reported to be essentially reversible, except for the clostridial protein which could only be reduced at potentials −250 mV more negative than that required for oxidation. In all cases studied by O'Donnell and Smith and in all but one of the previously reported studies [Albrecht & Evans (1973) being the exception], a single redox region was observed by this EPR technique with $n = 1$.

It is important to note that the total number of electrons involved in the redox reaction(s) affecting the EPR signal was not measured in the EPR–potentiometric experiments. The $n = 1$ value was obtained from an analysis of the redox curve only, and the total number of electrons involved in the redox reaction was left unevaluated.

A spectrophotometric method was used to measure both a redox potential of −70 mV for the MoFe protein from *C. pasteurianum* (Walker & Mortenson, 1973) and the total number of electrons required for oxidation of the protein in this redox process. A single redox region was observed involving about four electrons during this optical titration performed at 460 nm.

These two spectroscopic techniques follow different properties of the protein and while both could report on the same redox events, it is conceivable that they report on quite different and even independent redox events. Evidence that this latter situation might be occurring comes from the oxidative EPR titrations reported by Orme-Johnson et al. (1977) and Zimmerman et al. (1978) and the electrochemical reductive titrations of Watt & Bulen (1976) and Watt et al. (1980). The oxidative EPR titrations show that three to four electrons are removed from $S_2O_4^{2-}$-free (but otherwise reduced) MoFe protein by thionine oxidation before the EPR signal intensity at $g = 3.65$ is affected. The next two to three electron equivalents of added thionine nearly abolish the EPR signal, producing a MoFe protein oxidized by approximately six electrons. The redox experiments of Watt et al. (1980) using polarographic and potentiostatic techniques clearly indicate two separate redox regions where dye-oxidized MoFe protein undergoes a methyl or benzyl viologen mediated reduction at various potentials at a carefully controlled platinum electrode.

The oxidative results of Orme-Johnson et al. (1977) and Zimmerman et al. (1978) and the reductive results of Watt et al. (1980) start from completely different oxidation states of the MoFe protein but are consistent in showing two separate redox regions, only one of which is responsible for the EPR signal. We report here details of our reduction measurements and include a more detailed description of the spectroscopic and electrochemical properties of the oxidized forms of the MoFe protein.

Experimental Section

MoFe Protein. This protein with activities ranging from 1800 to 2700 nmol of H_2 min^{-1} mg^{-1} was prepared by the method of Bulen & LeComte (1972) and Shah & Brill (1973) or a modification of this latter procedure by Burgess et al.

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[1] Abbreviations used: NHE, normal hydrogen electrode; SCE, saturated calomel electrode; EPR, electron paramagnetic resonance.

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