# Binding of Adenosine Triphosphate to Myosin, Heavy Meromyosin, and Subfragment 1*

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ABSTRACT: The number of sites for ATP on myosin, heavy meromyosin, and subfragment 1, in the presence of excess magnesium ions, was determined by means of a kinetic analysis.

This analysis was based on the use of the ATPase rate as a measure of the fraction of enzyme which was saturated with ATP under conditions where all of the added nucleotide was maintained as ATP by the creatine kinase–phosphocreatine system. Analysis of the data by the method of Scatchard showed that under conditions where ATP is actually being hydrolyzed, myosin and heavy meromyosin have two ATP binding sites per molecule whereas subfragment 1 has only a single site for ATP. Since subfragment 1 has been shown to retain the major functional properties of heavy meromyosin, namely, activation of its MgATPase by actin and dissociation of its actin complex by ATP, it appears that these properties do not depend on a relationship between the two sites for substrate on heavy meromyosin.

One of the most important questions concerning the enzymatic activity of myosin and its tryptic digestion products, heavy meromyosin and subfragment 1, is the number of sites each of these proteins has for ATP in the presence of $Mg^{2+}$. Structural studies have shown that myosin and heavy meromyosin are probably composed of two subunits which, upon relatively extensive tryptic digestion, yield two subfragment-1 molecules (Stracher and Dreizen, 1966; Slayter and Lowey, 1967; Lowey *et al.*, 1969). On this basis, it would be expected that myosin and heavy meromyosin would have two sites for substrate whereas subfragment 1 would have one site. However, in previous work with inorganic pyrophosphate and ADP as well as ATP, the number of sites reported for myosin or heavy meromyosin has varied from one to three (Nanninga and Mommerts, 1960; Imamura *et al.*, 1966; Young, 1967; Sekiya and Tonomura, 1967; Schliselfeld and Bárány, 1968; Nauss *et al.*, 1969; Lowey and Luck, 1969; Morita, 1969; Kiely and Martonosi, 1968, 1969; Murphy and Morales, 1970).

In the present study we have approached this problem by a kinetic analysis, based on the method of Straus and Goldstein (1943), in which we use the ATPase rate as a measure of the amount of enzyme which is bound to ATP. Although such an analysis is necessarily indirect, it has the advantage of allowing us to estimate the number of binding sites for substrate under conditions where actual hydrolysis of the substrate is occurring, *i.e.*, under conditions similar to those *in vivo*. Analysis of our data by the method of Scatchard (1949) shows that, as the structural studies would suggest, myosin and heavy meromyosin have two ATP binding sites whereas subfragment 1 has only a single site.

## Methods

*Protein Preparations.* Myosin, heavy meromyosin, and subfragment 1 were prepared as we described previously (Eisenberg and Moos, 1967; Eisenberg *et al.*, 1968), using Sephadex G-200 chromatography for purification of the subfragment 1. Creatine kinase was prepared from rabbit muscle by the method of Kuby *et al.* (1954) with the addition of a final treatment with Norit A charcoal to remove contaminant nucleotide (Moos, 1964). All protein concentrations were determined as previously by ultraviolet absorption at 280 mμ (Eisenberg *et al.*, 1968). The extinction coefficient used for creatine kinase was 890 $cm^2/g$ (Noda *et al.*, 1954).

*ATPase Activity.* In all experiments the ATP was maintained by the creatine kinase–phosphocreatine system. In this coupled system, the rate of ATP hydrolysis is equal to the rate of breakdown of phosphocreatine, and this reaction in turn consumes about 0.3 mole of $H^+$/mole of phosphocreatine degraded at pH 7 so that we could measure the rate of the reaction using the pH-Stat with HCl as the titrant (Eisenberg and Moos, 1970). Since, in all cases, we were interested in the ratio of the ATPase rate at a given ATP concentration to the ATPase rate at a saturating level of ATP, it was not necessary to determine the absolute value of the ATPase activity. The temperature in all experiments was 25°.

*Creatine Kinase Activity.* To determine the activity of our creatine kinase preparation as well as the effective binding constants of ATP, ADP, and phosphocreatine under the conditions of our experiments, we measured the creatine kinase activity at varied ATP, ADP, and phosphocreatine concentrations. The transfer of 1 mole of phosphate from phosphocreatine to ADP consumes 1 mole of $H^+$ at pH 7; therefore we were able to measure the absolute rate of this reaction directly by using the pH-Stat with a standard solution of HCl as titrant.

*Reagents.* All ordinary reagents were of analytical grade,

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and glass-distilled water was used for all solutions. The ATP and phosphocreatine were purchased from Sigma and used without further purification.

## Theory

It has previously been shown that Lineweaver–Burk plots of myosin and heavy meromyosin ATPase are linear (Tokiwa and Tonomura, 1965; Sekiya *et al.*, 1967; Schliselfeld and Bárány, 1968) from which it follows that

$$\frac{v}{V_{max}} = \frac{[E\text{–}ATP]}{[E]_T} \quad (1)$$

and

$$K_m = \frac{[E][ATP]}{[E\text{–}ATP]} \quad (2)$$

where $v$ is the measured ATPase rate, $V_{max}$ is the ATPase rate at a saturating level of ATP, [ATP] is the concentration of free ATP, and [E], [E–ATP], and $[E]_T$ are the molar concentrations of free, substrate-bound, and total enzyme, respectively. As we shall show below, eq 3 and 4 are also true in our system

$$[ATP]_{added} = n[E\text{–}ATP] + [ATP] \quad (3)$$

$$[E]_T = [E] + [E\text{–}ATP] \quad (4)$$

Here, $[ATP]_{added}$ is the total nucleotide concentration, and $n$ is the number of ATP binding sites on each enzyme molecule. From eq 1 to 4 we can easily derive eq 5, which corresponds to the binding equation of Scatchard (1949).

$$\frac{\bar{\nu}}{[E]} = \frac{1}{K_m} - \frac{n}{K_m}\bar{\nu} \quad (5)$$

where $\bar{\nu} = [E\text{–}ATP]/[ATP]_{added}$. We determined [E–ATP] from eq 1 by measuring $v/V_{max}$, and [E] is then obtained from eq 4. According to eq 5, a plot of $\bar{\nu}$ *vs.* $\bar{\nu}/[E]$ will yield an ordinate intercept equal to $1/K_m$, and an abscissa intercept equal to $1/n$, the reciprocal of the number of ATP binding sites on the enzyme.

This analysis requires that eq 3 and 4 be valid. In fact, however, the complete conservation equation for nucleotide in our system is

$$[ATP]_{added} = [ATP] + [ADP] + n[E\text{–}ATP] + n[E\text{–}ADP] + [CK\text{–}ATP] + [CK\text{–}ADP] \quad (6)$$

where CK is creatine kinase. We must therefore show that in our system, the creatine kinase does not bind a significant amount of nucleotide but nevertheless maintains all of the added nucleotide as ATP. To do this we independently measured the effective binding constants of creatine kinase for ATP, ADP, and phosphocreatine, as well as its $V_{max}$. Our values for these constants were essentially the same as those of Morrison and James (1965) with the exception that our value for $V_{max}$ (380 μmoles/mg min) was higher, probably because of the lower pH of our system. Using these constants in the steady-state rate equation for the creatine kinase system (Morrison and James, 1965), we can easily show that, at the creatine kinase concentrations used in our experiment, the ratios, [CK–ADP]/[E–ATP], [CK–ATP]/[ATP], and [ADP]/[E–ATP], are negligible. Then, assuming that the binding constant of ADP to myosin, heavy meromyosin, or subfragment 1 is $4 \times 10^5$ $M^{-1}$ or weaker (Sekiya and Tonomura, 1967; Young 1967; Lowey and Luck, 1969; Kiely and Martonosi, 1969), we can also show that the ratio, [E–ADP]/[E–ATP], is quite small. Therefore, eq 3, and 4 as well, do hold under the conditions of our experiment, *i.e.*, the creatine kinase binds a negligible fraction of the nucleotide but does maintain all of the added nucleotide as ATP.

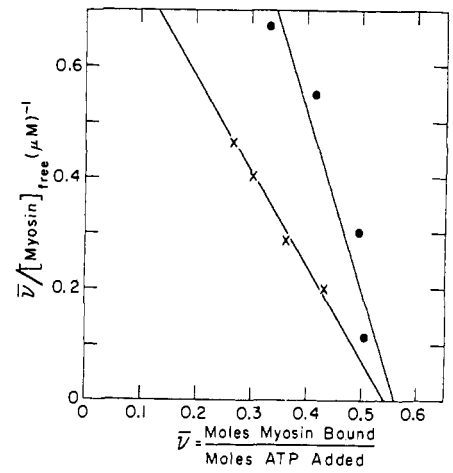


FIGURE 1: Scatchard plot of myosin–ATP binding. All samples contained 0.3 M KCl, 5 mM phosphocreatine, and 1.6 mg of creatine kinase per ml. The $V_{max}$ of the myosin ATPase (see Theory section) was measured at 0.5 mM ATP, 1.5 mM $MgCl_2$, and 2.2 mg of myosin per ml. For the binding plots, the samples contained 1.0 mM $MgCl_2$ and 9.0 μM ATP, and the myosin concentration was varied between 1.4 and 4.4 mg per ml. × and ○ represent two different preparations of myosin. For the calculations, the molecular weight of myosin is taken to be 500,000.

## Results

*Binding of ATP to Myosin.* As we described in the Theory section, under conditions where the ATP is maintained by the creatine kinase–phosphocreatine system, measurement of $v/V_{max}$ at a constant total ATP concentration but with varying myosin concentrations provides enough information for a Scatchard plot which should yield both the $K_m$ of myosin ATPase and the number of ATP binding sites per myosin molecule. Figure 1 shows such Scatchard plots for two preparations of myosin. The plots appear to be linear and, although there is some difference in the $K_m$ values obtained from the lines for the two preparations (0.55 and 1.09 μM, respectively), both are in the range of other reported values (Tokiwa and Tonomura, 1965; Schliselfeld and Bárány, 1968). As described in the Theory section, the abscissa intercepts of these plots give the reciprocal of the number of ATP binding sites per myosin molecule. For both plots this intercept is near 0.55 assuming a molecular weight for myosin of 500,000 (Lowey *et al.*, 1969), suggesting that there are two ATP binding sites on each myosin molecule.

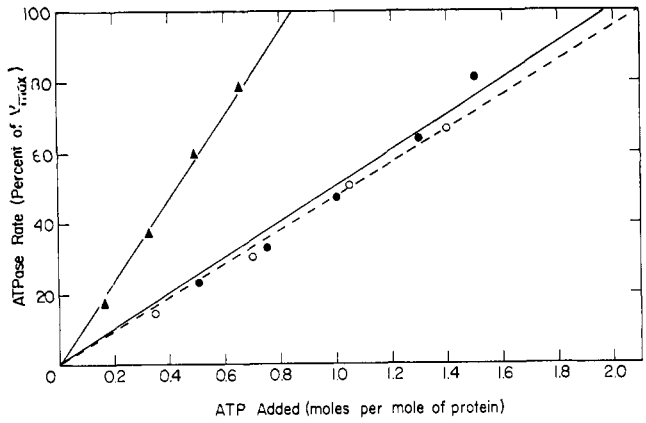


FIGURE 2: Titration plots of ATP binding to myosin, heavy meromyosin, and subfragment 1. Ordinate: ATPase rates expressed as percentages of $V_{max}$, the measured ATPase rate under the same conditions except for the presence of 1.5 mM $MgCl_2$ and 0.5 mM ATP. Abscissa: added ATP expressed as moles per mole of myosin, heavy meromyosin, or subfragment 1, using molecular weights of 500,000, 350,000, and 120,000, respectively. (●) Myosin, at a concentration of 20 mg/ml, with 0.4 M KCl, 10 mM phosphocreatine, 1.0 mM $MgCl_2$, 1.6 mg of creatine kinase per ml, and ATP varied from 20 to 60 μM. O, heavy meromyosin at a concentration of 10 mg/ml, with 0.1 M KCl, 10 mM phosphocreatine, 0.8 mg of creatine kinase per ml, 1 mM $MgCl_2$, and ATP varied from 10 to 40 μM. (▲) Subfragment 1 at a concentration of 7.3 mg/ml, with 0.1 M KCl, 10 mM phosphocreatine, 1.6 mg of creatine kinase per ml, 1 mM $MgCl_2$, and ATP varied from 10 to 40 μM.

An alternative way of determining the stoichiometry of binding of ATP to myosin is a simple titration plot. If we work at a myosin concentration much higher than $K_m$, then nearly all of the added ATP should bind to the myosin and the measured ATPase should increase in direct proportion

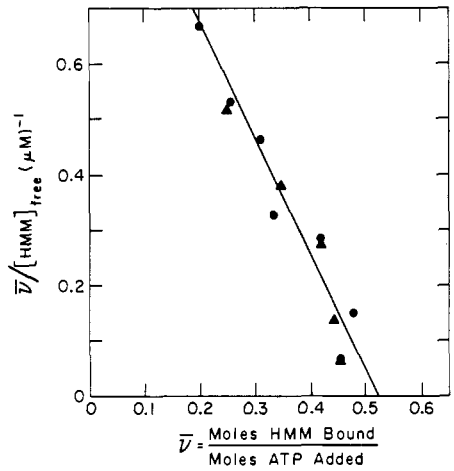


FIGURE 3: Scatchard plot of heavy meromyosin ATP binding. All samples contained 0.1 M KCl, 5 mM phosphocreatine, and 0.4 mg of creatine kinase per ml. The $V_{max}$ of the heavy meromyosin ATPase (see Theory section) was measured at 0.5 mM ATP, 1.5 mM $MgCl_2$, and 1.0 mg of heavy meromyosin per ml. For the binding plot, the samples contained 1.0 mM $MgCl_2$ and 6.0 μM ATP, and the heavy meromyosin concentration was varied between 0.5 and 3.1 mg per ml. ▲ and ● represent two different preparations of heavy meromyosin. For the calculations, the molecular weight of heavy meromyosin was taken to be 350,000.

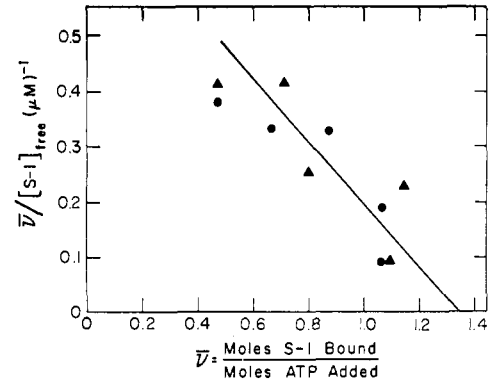


FIGURE 4: Scatchard plot of subfragment-1 ATP binding. All samples contained 0.1 M KCl, 5 mM phosphocreatine, and 0.8 mg of creatine kinase per ml. The $V_{max}$ of the subfragment-1 ATPase (see Theory section) was measured at 0.5 mM ATP, 1.5 mM $MgCl_2$, and 0.74 mg of subfragment 1 per ml. For the binding plot, the samples contained 1.0 mM $MgCl_2$ and 6.0 μM ATP, and the subfragment-1 concentration was varied between 0.5 and 2.2 mg per ml. ▲ and ● represent two different preparations of subfragment 1. For the calculations, the molecular weight of subfragment 1 was taken to be 120,000.

to the added ATP concentration. Figure 2 shows the results of such experiments with myosin, heavy meromyosin, and subfragment 1. Extrapolation of these plots to an ATPase rate equal to $V_{max}$, the measured ATPase with excess ATP, gives values on the abscissa for the total number of ATP binding sites per molecule. From the plot for myosin in Figure 2 (solid circles), this value was found to be 1.9, again indicating that there are two binding sites for ATP on each myosin molecule.

*Binding to ATP to Heavy Meromyosin.* Figure 3 shows Scatchard plots for two different preparations of heavy meromyosin obtained in the same manner as Figure 1 for myosin above. As in the case of myosin, the plot is linear, and the value of $K_m$ obtained from this plot is 0.92 μM, which is similar to other reported values for heavy meromyosin ATPase (Sekiya *et al.*, 1967; Schliselfeld and Bárány, 1968). The intercept on the abscissa, which gives the reciprocal of the number of ATP binding sites per heavy meromyosin molecule, is 0.52, assuming a molecular weight of 350,000 (Mueller, 1964). Therefore the Scatchard plot suggests that there are two ATP binding sites per heavy meromyosin molecule. In confirmation of this finding, the titration plot for heavy meromyosin in Figure 2 (open circles) gives a stoichiometry of 2.1 moles of ATP binding sites/mole of heavy meromyosin.

*Binding of ATP to Subfragment 1.* Figure 4 shows Scatchard plots for two different preparations of subfragment 1, which again appear to be linear and give a $K_m$ of 1.3 μM for subfragment 1 ATPase. In this case, however, the abscissa intercept gives a value of 1.34 for the reciprocal of the number of ATP binding sites per subfragment 1, assuming a molecular weight of 120,000 (Young *et al.*, 1965). Therefore, in contrast to our finding of two ATP binding sites on myosin and heavy meromyosin, we find a binding stoichiometry of 0.75 mole of ATP/mole of subfragment 1. This value for the stoichiometry is confirmed by the titration plot in Figure 2 (triangles), which shows a stoichiometry of 0.84 mole of ATP/mole of

subfragment 1. The fact that these values are less than one may indicate that about 20% denaturation of the subfragment 1 occurred during its rather extensive preparation procedure. At any rate, it appears that, in contrast to myosin and heavy meromyosin, subfragment 1 has only a single binding site for ATP.

## Discussion

Our kinetic data clearly suggest that under conditions where significant hydrolysis of MgATP occurs, myosin and heavy meromyosin have two sites for substrate whereas subfragment 1 has only a single site. Recognizing that kinetic studies are necessarily an indirect measure of the number of substrate binding sites on an enzyme, they are nevertheless of interest because direct equilibrium studies are essentially impossible under conditions where hydrolysis of nucleotide is occurring. Our conclusions agree well with several other recent binding studies of various types (Schliselfeld and Bárány, 1968; Nauss *et al.*, 1969; Lowey and Luck, 1969; Morita, 1969; Kiely and Martonosi, 1968, 1969; Morita and Shimizu, 1969; Murphy and Morales, 1970) as well as with the finding that myosin and heavy meromyosin are apparently composed of two subunits (Stracher and Dreizen, 1966; Slayter and Lowey, 1967; Lowey *et al.*, 1969). Furthermore, the finding that subfragment 1 has only a single site for ATP goes along with the suggestion that each of the two subunits of heavy meromyosin contains one subfragment-1 molecule (Slayter and Lowey, 1967; Lowey *et al.*, 1969). The presence of only one site for ATP on subfragment 1 under conditions where ATP hydrolysis occurs at low ionic strength is of further interest in the light of our earlier finding that under these conditions subfragment 1 retains two of the major functional properties of heavy meromyosin; first a MgATPase which is activated by actin, and second, a weakening of the actin subfragment-1 binding by ATP (Eisenberg *et al.*, 1968). We have previously proposed that both of these properties involve the binding of ATP to the hydrolytic site of subframgent 1 or heavy meromyosin, rather than to two separate sites, a hydrolytic site and a nonhydrolytic "dissociating" site (Eisenberg and Moos, 1968; Eisenberg, *et al.*, 1968). The present data for subfragment 1 also argue against the presence of such a dissociating site on subfragment 1. If subfragment 1 did have a nonhydrolytic ATP binding site with an affinity stronger than that of the hydrolytic site, the titration plot in Figure 2 would have shown a lag rather than rising linearly from the origin, whereas if there were a nonhydrolytic dissociating site with a binding constant equal to that of the hydrolytic site, both the Scatchard plot in Figure 4 and the titration plot in Figure 2 would have indicated two sites per subfragment-1 molecule. While the presence of a dissociating site for ATP with a binding constant much weaker than that of the hydrolytic site is not directly ruled out by the data shown here, it is essentially precluded for heavy meromyosin by our observation that double-reciprocal plots of heavy meromyosin ATPase against ATP concentration are linear over a wide range of actin and ATP concentrations and show no evidence of substrate inhibition (Eisenberg and Moos, 1970) and it does not seem likely that subfragment 1 would have a separate weak binding site for ATP if heavy meromyosin does not. Hence we can probably safely conclude that the dissociating effect of ATP on the acto subfragment 1 system is due to the binding of ATP to the hydrolytic site rather than to any separate dissociating site; and since the dissociating effect of ATP on acto heavy meromyosin and acto subfragment 1 appear to be similar, we can extend this conclusion to the acto heavy meromyosin system as well.

Our finding that subfragment 1 has only a single site for ATP but still retains the major functional properties of heavy meromyosin also leads to the conclusion that those properties of heavy meromyosin which are shown by subfragment 1, such as actin activation of the MgATPase, do not require the presence of two sites for substrate on the enzyme molecule. Further work will be required to determine if more subtle differences exist betweeen the enzymatic properties of heavy meromyosin and subfragment 1.

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## References

Eisenberg, E., Barouch, W. W., and Moos, C. (1969), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 28*, 536.
Eisenberg, E., and Moos, C. (1967), *J. Biol. Chem. 242*, 2945.
Eisenberg, E., and Moos, C. (1968), *Biochemistry 7*, 1486.
Eisenberg, E., and Moos, C. (1970), *J. Biol. Chem. 245*, 2451.
Eisenberg, E., Zobel, C. R., and Moos, C. (1968), *Biochemistry 7*, 3186.
Imamura, K., Tada, M., and Tonomura, Y. (1966), *J. Biochem. (Tokyo) 59*, 280.
Kiely, B., and Martonosi, A. (1968), *J. Biol. Chem. 243*, 2273.
Kiely, B., and Martonosi, A. (1969), *Biochim. Biophys. Acta 172*, 158.
Kuby, S. A., Noda, L., and Lardy, H. A. (1954), *J. Biol. Chem. 209*, 191.
Lowey, S., and Luck, S. M. (1969), *Biochemistry 8*, 3195.
Lowey, S., Slayter, H. S., Weeds, A. G., and Baker, H. (1969), *J. Mol. Biol. 42*, 1.
Moos, C. (1964), *Biochim. Biophys. Acta 93*, 85.
Morita, F. (1969), *Biochim. Biophys. Acta 172*, 319.
Morita, F., and Shimizu, T. (1969), *Biochim. Biophys. Acta 180*, 545.
Morrison, J. F., and James, E. (1965), *Biochem. J. 97*, 37.
Mueller, H. (1964), *J. Biol. Chem. 239*, 797.
Murphy, A. J., and Morales, M. F. (1970), *Biochemistry 9*, 1528.
Nanninga, L. B., and Mommaerts, W. F. H. M. (1960), *Proc. Nat. Acad. Sci. U. S. 46*, 1155.
Nauss, K. M., Kitagawa, S., and Gergley, J. (1969), *J. Biol. Chem. 244*, 755.
Noda, L., Kuby, S. A., and Lardy, H. A. (1954), *J. Biol. Chem. 209*, 203.
Scatchard, G. (1949), *Ann. N. Y. Acad. Sci. 51*, 660.
Schliselfeld, L. H., and Bárány, M. (1968), *Biochemistry 7*, 3206.
Sekiya, K., Takeuchi, K., and Tonomura, Y. (1967), *J. Biochem. (Tokyo) 61*, 567.
Sekiya, K., and Tonomura, Y. (1967), *J. Biochem. (Tokyo) 61*, 787.
Slayter, H. S., and Lowey, S. (1967), *Proc. Nat. Acad. Sci.*

*U. S. 58*, 1611.
Stracher, A., and Dreizen, P. (1966), *Curr. Top. Bioenerg. 1*, 153.
Straus, O. H., and Goldstein, A. (1943), *J. Gen. Physiol. 26*, 559.
Tokiwa, T., and Tonomura, Y. (1965), *J. Biochem. (Tokyo) 57*, 616.
Young, M. (1967), *J. Biol. Chem. 242*, 2790.
Young, M., Himmelfarb, S., and Harrington, W. F. (1965), *J. Biol. Chem. 240*, 2428.

# Aggregation of Microtubule Subunit Protein. Effects of Divalent Cations, Colchicine and Vinblastine*

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ABSTRACT: The self-association of calf brain microtubule subunit protein (tubulin) has been studied. Divalent cations (Mg or Ca) induce the reversible aggregation of tubulin. Depending upon the divalent ion concentration, aggregation may result in an increase in sedimentation rate, formation of a 30S peak, or formation of a fibrous precipitate. Divalent cation induced precipitation is specific for the active, colchicine binding protein and is a useful step in the purification of tubulin. Colchicine alone has no apparent effect on the aggregation of tubulin, but it *increases* significantly the divalent ion induced aggregation. Vinblastine induces a strong, well-defined aggregation of tubulin and also increases and alters the effect of divalent cations. Low concentrations of vinblastine and divalent cations will precipitate tubulin. The observed differences between the various colchicine- or vinblastine-induced ultrastructural change may be related to the effects of divalent cations on the actions of these drugs.

The isolation from porcine brain of a protein, which is believed to be a subunit of microtubules, has been reported recently (Weisenberg *et al.*, 1968). This protein, tubulin (Mohri, 1968), is characterized by its ability to bind the antimitotic drug colchicine, by its ability to bind guanosine nucleotides and by its physical and chemical properties. Native tubulin has a sedimentation coefficient of 5.8 S and a molecular weight close to 120,000. Its amino acid composition is grossly similar to that of actin (Weisenberg *et al.*, 1968; Shelanski and Taylor, 1968), although marked differences both in the amino acid composition (Stephens and Linck, 1969) and in peptide maps (Stephens, 1970) exist between the two proteins from the same species.

Although identified as a subunit of microtubules (Weisenberg *et al.*, 1968), native tubulin has not yet been successfully repolymerized into microtubules. It has been found, nevertheless, that tubulin can undergo reversible aggregation (Weisenberg *et al.*, 1968; Weisenberg and Timasheff, 1969). The present work describes the results of studies on the effects of divalent cations and the antimitotic drugs, colchicine and vinblastine, on the aggregation of tubulin. Vinblastine, which has been observed to induce the formation of "microtubule crystals" *in vivo* (Bensch and Malawista, 1969) and to precipitate tubulin *in vitro* (Marantz *et al.*, 1969), induces the reversible aggregation of tubulin. Colchicine, which dissociates microtubules *in vivo*, increases the divalent ion-induced aggregation of this protein.

## Materials and Methods

All chemicals (unless otherwise noted) were reagent grade. GTP was grade II-S from Sigma Chemicals; colchicine was from Fisher Chemical Co. Vinblastine sulfate was a gift from Eli Lilly Co.

Protein concentration was determined by absorption at 278 nm using an absorptivity of 1.2 l./g. This value includes the contribution from the tightly bound guanosine nucleotide.

Colchicine binding was determined (unless otherwise noted) by the DEAE filter assay (Weisenberg *et al.*, 1968) using tritiated colchicine obtained from New England Nuclear Corp. Vinblastine binding was determined by gel filtration on Sephadex G-100 and followed by analysis of the fractions for protein and vinblastine; in these experiments, vinblastine concentration was determined spectrophotometrically using a molar extinction coefficient of $6.06 \times 10^3$ at 310 nm, while the protein concentration was determined by the Lowry procedure (Lowry *et al.*, 1951).

Analytical ultracentrifugation was performed in a Spinco Model E instrument equipped with an electronic speed control and the RTIC temperature control unit. All runs were done in 12-mm, 2° sector Kel-F cells. Sedimentation

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