# Cytochalasin B-Induced ATPase Activity of Actin: Dependence on Monomer Concentration

Peter Dancker and Agnes Kliche

Max-Planck-Institut für medizinische Forschung, Abteilung Physiologie, Jahnstr. 29, D-6900 Heidelberg, Bundesrepublik Deutschland

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We simultaneously measured polymerization and ATPase activity of actin induced by cytochalasin B. It was found that under all conditions tested, ATPase activity was proportional to the concentration of actin which was unpolymerized. In addition, it was found that conditions increasing ATPase activity also increase the velocity of polymerization. From this we conclude that CB-induced ATPase activity is a property of the actin monomers and that the "readiness" of the monomers to hydrolyze ATP is correlated with an increased capacity of the monomers to polymerize.

## Introduction

We have recently described [1, 2] that the fungal metabolite cytochalasin B induces an ATPase activity of actin. This ATPase activity was comparable in magnitude to that induced by sonic vibration (about 0.3 mol ATP hydrolyzed per mol of actin and minute). ATPase activity exhibited its optimal values at moderately polymerizing conditions. It was depressed both when polymerization occurred slowly (very low salt concentrations) or when polymerization was strongly promoted (by high concentrations of KCl or $MgCl_2$ or by the mushroom toxin phalloidin).

The interpretation of the induced ATPase activity in relation to the monomeric or polymeric form of actin remained unclear. Three general mechanisms seem possible:

(1) ATPase activity is a property of the monomers.
(2) ATPase activity is a property of the polymers.
(3) Since it is known that in the presence of ATP incorporation of actin monomers into an actin filament is accompanied by the hydrolysis of one molecule of ATP per monomer incorporated, cytochalasin B-induced ATPase activity could reflect a frequent exchange of monomers with the polymers, each exchange step being associated with the splitting of one molecule of ATP.

*Abbreviations:* CB, cytochalasin B; EGTA, ethyleneglycol-bis-(2-aminoethylether)-N,N′-tetraacetic acid; PHD, phalloidin; $P_i$, inorganic orthophosphate; TCA, trichloroacetic acid.

Reprint requests to Dr. P. Dancker.

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In the present study we investigated this problem by comparing simultaneously the time course of phosphate production and the time course of polymerization. We will show that cytochalasin B-induced ATPase activity is proportional to monomer concentration and will discuss possible relationships between ATPase activity and actin polymerization.

## Methods

Actin was prepared from rabbit skeletal muscle according to Spudich and Watt [3]. ATPase activity and viscosity were measured at 30 °C, 30 μM cytochalasin B, 5 mM Tris-HCl, pH 8.0, 1 mM ATP and, if not otherwise stated 0.6 mg/ml actin (= 14 μM). Further conditions (*i.e.* $MgCl_2$ concentrations) are specified in the figures and their legends. Due to the high affinity of $Mg^{2+}$ for ATP, the free $Mg^{2+}$ concentrations are appreciably lower than the concentrations of added $MgCl_2$. The relevant values are given in the figure legends assuming an association constant for the MgATP complex of $10^4\ M^{-1}$. The concentrations of free $Mg^{2+}$ were in the range from $2.17 \times 10^{-5}$ M (at 0.2 mM $MgCl_2$) to $6.36 \times 10^{-4}$ M (at 1.5 mM $MgCl_2$).

The ATPase reactions were stopped after the desired time by transferring aliquots of the splitting assay into 10% trichloroacetic acid (TCA), the end concentration of TCA was 3.3%. Inorganic phosphat ($P_i$) was measured according to Lowry and Lopez (*cf.* [4]). For the measurements of light scattering intensity, aliquots of the splitting assays were taken and the intensity of scattered light was measured in

a fluorescence spectrophotometer with both monochromators set at 400 nm. Viscosity was measured in a viscosimeter with a spiral-formed capillary. The outflow time of water was about 45 s at 30 °C. $\eta_{sp}$ denotes the expression $(t/t_0)-1$ with $t$ = outflow time of the sample and $t_0$ = outflow time of the solvent.

Cytochalasin B was from Serva, Heidelberg, FRG and phalloidin was a gift of Prof. Th. Wieland, Heidelberg.

## Results

Fig. 1 shows ATPase activity and light scattering intensity at various concentrations of $MgCl_2$. ATPase activity was determined after a preincubation time of 20 min, light scattering intensity was measured after the ATPase activity measurements. The figure shows that increasing actin polymerization (indicated by increasing light scattering intensity values) the ATPase activity decreased.

In order to further elucidate the possible relationship between ATPase activity and actin polymerization, we measured the time course of $P_i$ production under various ionic conditions, *e. g.* at various $MgCl_2$ concentrations. At 0.5 mM $MgCl_2$ (= 74 μM free $Mg^{2+}$), with G-actin as starting material, $P_i$ production increased linearly with time (Fig. 2B), whereas, with F-actin as starting material, a high ATPase activity was reached only after a lag phase. The time course at 1.5 mM $MgCl_2$ (= 0.64 mM free $Mg^{2+}$) with G-actin (Fig. 2B) is a mirror image of that shown in Fig. 2A: after an initial fast $P_i$ production, a much slower phase followed.

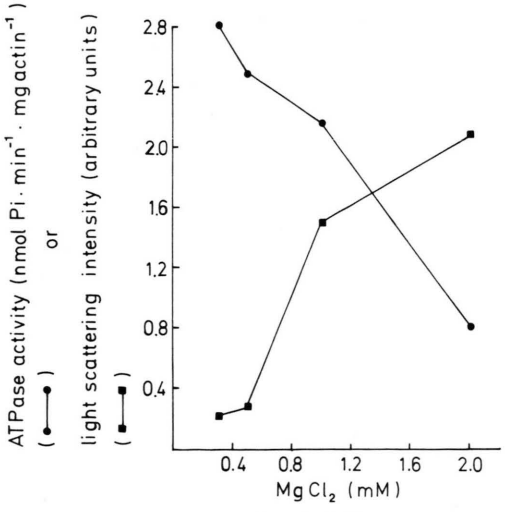


Fig. 1. Cytochalasin B-induced ATPase activity and light scattering intensity of actin at various concentrations of $MgCl_2$. The starting material was F-actin. The assays were incubated for 50 min, the ATPase activity indicated is that of the last 30 min. The concentration of free $Mg^{2+}$ varied between $3.6 \times 10^{-5}$ M and $1.08 \times 10^{-3}$ M.

The simplest explanation of these results is that the non-linear time courses of Fig. 2 reflect an increase in monomer concentration due to a slow depolymerization of the initial F-actin at low concentration of free $Mg^{2+}$ (Fig. 2A) or decrease in monomer concentration at 1.5 mM $MgCl_2$ due to polymerization of the initial G-actin (Fig. 2B).

In order to clarify this situation, we simultaneously measured the time course of $P_i$ production and the time course of polymerization by following light scattering intensity. At the end of each experiment (Fig. 3) the concentration of $MgCl_2$ was brought to 2 mM, and the now reached light scattering intensity was assumed to correspond to 100% polymerization. Thus, from the increase of light scattering intensity the increase of polymer concentration (and therefore the decrease in monomer concentration) could be deduced. In Fig. 3A the increase in light scattering intensity and in Fig. 3B the simultaneously measured $P_i$ production are shown. The symbols of Fig. 3B indicate the measured amounts of $P_i$. The lines of Fig. 3B were constructed on the assumption that ATPase activity is proportional to monomer concentration. For this purpose monomer concentration (deduced from light scattering measurements) was plotted against time and the integral of such a plot over a given time period was taken as being proportional to the amount of $P_i$ produced during that time period. Therefore, curves representing monomer concentration as dependent on time were numerically integrated over increasing time intervalls (between 0 and 2 min, 0 and 4 min etc.). The lines in Fig. 3B represent the values of such integrals between zero time and the time indicated on the abscissa. The integrals were expressed in terms of $P_i$, such that for a certain time period (usually 16 min) the numerical relationship between the amount of phosphate actually measured and the corresponding integral value (originally in arbitrary units) was determined as a scaling factor. Then the entire integral was scaled according to this factor.

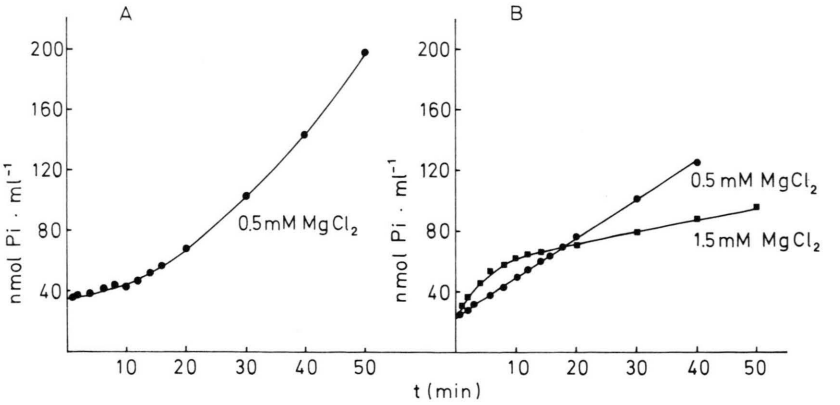


Fig. 2. ATP hydrolysis catalyzed by actin in the presence of cytochalasin B. In A, actin was initially F-actin; in B, actin was initially G-actin. The concentration of free $Mg^{2+}$ was $7.42 \times 10^{-5}$ M (at 0.5 mM $MgCl_2$) or $6.36 \times 10^{-4}$ M (at 1.5 mM $MgCl_2$).

In Fig. 3B a good fit between the determined ATPase activity and the expectation that ATPase activity is proportional to monomer concentration can be seen. In the experiment of Fig. 3 different scaling factors had to be used in order to express the integral curves in terms of $P_i$. This reflects a higher ATPase activity of monomers at higher concentrations of $MgCl_2$.

The experiments of Figs. 2 and 3 show that under conditions where polymerization velocity is high (*e.g.* at higher concentrations of $MgCl_2$) the initial ATPase activity is also high. This correlation is even more striking when EGTA-containing and EGTA-deficient assays were compared. We have previously reported [2, 5], that EGTA (*i.e.* absence of $Ca^{2+}$) stimulates both ATPase activity (induced by either cytochalasin B or ultrasonication) and polymerization. Fig. 4 shows that in the presence of EGTA a higher ATPase activity was found ($\sim 8$ nmol $P_i \times min^{-1} \times$ mg actin$^{-1}$) than in all other experiments. At these low concentrations of free $Mg^{2+}$ (22 μM) little polymerization occurred in the absence of EGTA but polymerization was significant in its presence.

Fig. 5 shows that the relationship between monomer concentration and ATPase activity also remains valid in the presence of the filament-stabilizing mushroom toxin phalloidin. Thus, the reported

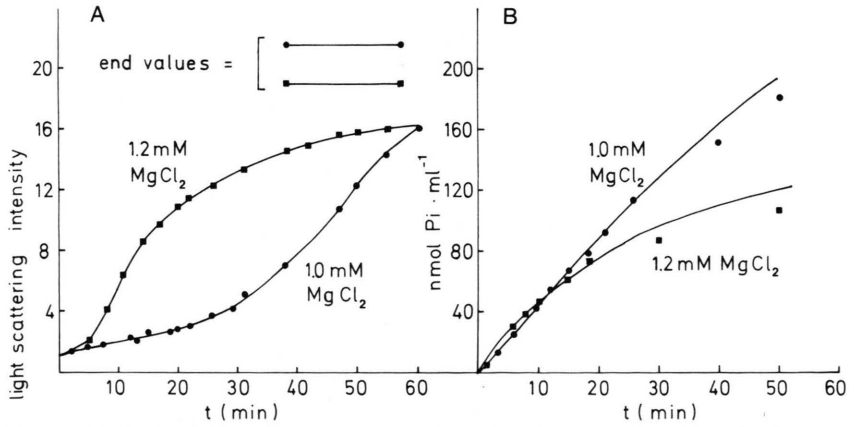


Fig. 3. ATP hydrolysis and light scattering intensity during actin polymerization at two concentrations of $MgCl_2$ and in the presence of cytochalasin B. In A, the values for light scattering (including the final values at the end of polymerization and measured at 2 mM $MgCl_2$) are indicated in arbitrary units. The concentration of unpolymerized actin is proportional to the difference between the respective end value and the actually measured intensity at a given time. In B, the symbols indicate the actually measured $P_i$ values. The lines in B represent the expectation that ATPase activity is proportional to the concentration of monomers. The concentrations of free $Mg^{2+}$ were $2.7 \times 10^{-4}$ M (at 1 mM $MgCl_2$) and $4.0 \times 10^{-4}$ M (at 1.2 mM $MgCl_2$).

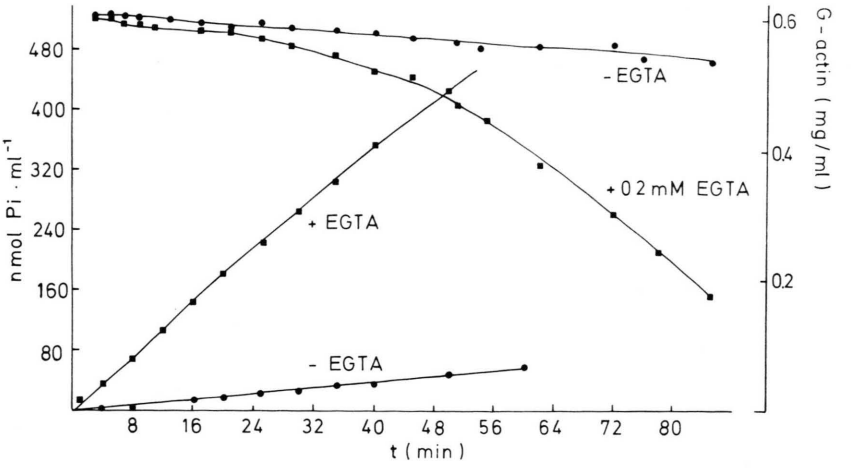


Fig. 4. ATP hydrolysis and concentration of monomers (deduced from light scattering measurements) during polymerization of actin in the presence or absence of EGTA and in the presence of cytochalasin B. The declining curves in the upper part of the figure represent the decreasing G-actin concentration (right ordinate). The increasing curves represent $P_i$ production (left ordinate). The concentration of $MgCl_2$ was 0.2 mM, the concentration of free $Mg^{2+}$ was $2.17 \times 10^{-5}$ M.

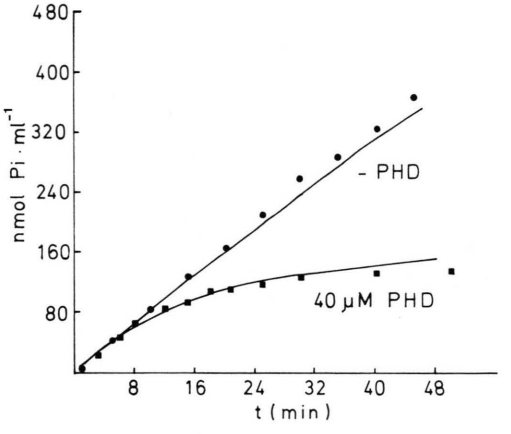


Fig. 5. ATP hydrolysis in the presence of cytochalasin B during the polymerization of actin, with and without phalloidin (PHD). The concentration of $MgCl_2$ was 0.2 mM, the concentration of free $Mg^{2+}$ was $2.17 \times 10^{-5}$ M, in addition, 0.2 mM EGTA were present. The lines were drawn on the same assumption as in Fig. 3.

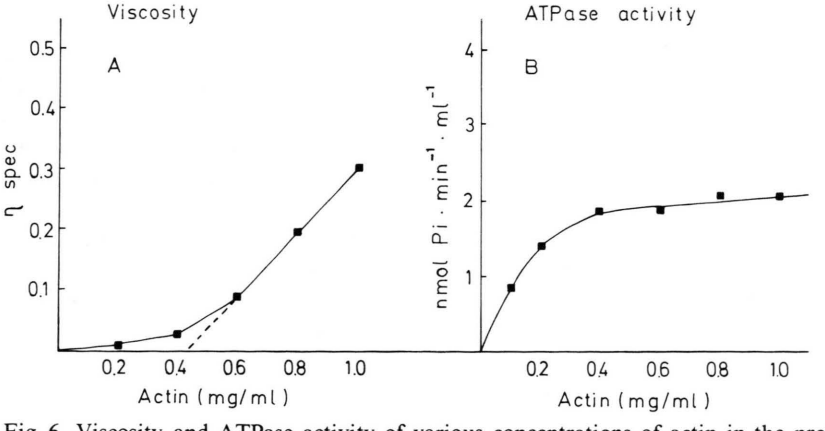


Fig. 6. Viscosity and ATPase activity of various concentrations of actin in the presence of cytochalasin B. The various actin concentrations were incubated for 50 min, the ATPase activity indicated is that of the last 30 min. Viscosity was measured after the 50 min incubation time, *i.e.* after the ATPase activity measurements. The concentration of $MgCl_2$ during the incubation time was 0.7 mM, the concentration of free $Mg^{2+}$ was $1.32 \times 10^{-4}$ M. At the beginning of the incubation time, actin was initially F-actin.

inhibition of cytochalasin B-induced ATPase activity by phalloidin [1] is due to the fact that the monomers disappear faster and to a higher extent in the presence of phalloidin.

The results reported so far suggest that CB-induced ATPase activity is proportional to monomer concentration. In order to investigate if also polymers are needed for ATPase activity to occur, ATPase activity of actin which was originally F-actin was measured at different actin concentrations. This allowed us to determine (by simultaneous viscosity measurements) the "critical concentration". (When total actin concentration is below the critical concentration, all actin is unpolymerized, therefore viscosity begins to incresase only when total actin concentration exceeds the critical concentration.) Fig. 6 shows that maximal ATPase activity is more or less complete at the critical concentration, *i. e.* in the absence of filaments.

## Discussion

Our experiments have shown that cytochalasin B-induced ATPase activity of actin is proportional to the concentration of non-polymerized actin. The question to be answered is: Can this be explained by a frequent incorporation (each single incorporation step associated with the hydrolysis of one molecule of ATP) of monomers into filaments or must be assumed that the monomers themselves are the true enzyme?

The time course of polymerization and of the ATPase activity resulting from monomer incorporation can be written in the following way (*cf.* Wegner [6]):

$$-\frac{dc_1}{dt} = c_1 c_p k_1 - c_p k_2 , \qquad (1)$$

$$-\frac{d[ATP]}{dt} = c_1 c_p k_1 , \qquad (2)$$

with $c_1$ and $c_p$ being monomer and polymer concentrations, respectively, and $k_1$ and $k_2$ being the sum of the "on" or "off" rate constants.

According to this mechanism, ATPase activity could be linearly dependent on monomer concentration only if $c_p$ would remain essentially constant during the course of polymerization; with other words: the final number of filaments must be reached immediately after the onset of polymerization with polymerization being essentially only elongation of a few filaments.

That such a mechanism could explain cytochalasin B-induced ATPase activity is unlikely on the following grounds:

(1) There is no experimental basis for the assumption that the number of filaments do not increase during actin polymerization.
(2) ATPase activity should occur only above the critical concentration of actin because only then filaments exist, compare however the experiment of Fig. 6.
(3) The total number of ATP molecules hydrolyzed in the presence of cytochalasin B exceeds the number of actin monomers about 10 fold (see Fig. 3: about 150 nmol of ATP per 14 nmol of actin). If ATPase activity would be due to monomer incorporation into filaments, cytochalasin B had to increase the exchange between filament-bound and free monomers. However, Simpson and Spudich [7] observed that in Physarum actin cytochalasin D decreases rather than increases the ATP-driven exchange between filament-bound and free monomers. On this basis one cannot expect that cytochalasin B increases the exchange in rabbit actin. If, however, the number of filament ends is higher in the presence of CB, there could be an increased rate of polymerization and ATP turnover. The total amount of ATP hydrolyzed during polymerization, however, would not be higher than in the absence of CB and would therefore not exceed the number of actin subunits.

In conclusion: Several assumptions must be introduced in order to explain cytochalasin B-induced ATPase activity of actin as due to a frequent monomer-polymer exchange, and available experimental evidence (Fig. 6 and [7]) argue against this interpretation. Therefore, the simpler explanation is that the monomers themselves are the true enzyme, a conclusion which was also reached by Brenner and Korn [8] in the case of cytochalasin D-induced ATPase activity. A slow ATPase activity of G-actin has been also observed in the presence of protamin [9].

Our ATPase measurements were performed at stoichiometric concentrations of cytochalasin B where actin polymerization is faster than it would have been in the absence of cytochalasin B [10].

This, together with the observation that the ATPase activity of monomers is higher under conditions where polymerization velocity is also higher (*cf.* Figs. 3 and 4) suggests that the capacity to hydrolyze ATP is related to the "readiness" of the momomers to polymerize.

According to Wegner's concept of head-to-tail polymerization (Wegner [11], Bergen and Borisy [12], Kirschner [13]) the purpose of ATP hydrolysis associated with actin polymerization is to ensure that the actin units inside the filaments contain ADP and that the free monomers contain ATP. In order to reduce wastage of ATP, the hydrolysis of ATP should be strictly coupled to polymerization such, that only one molecule of ATP is split per monomer incorporated. This would be best accomplished if, under normal conditions (without cytochalasin B) the monomers would hydrolyze ATP only when interacting with the polymer ends. Thus, the stimulation of monomer ATPase activity by cytochalasin B can be viewed as an "uncoupling" (see Brenner and Korn [8]) of ATP hydrolysis from polymerization so that ATP hydrolysis proceeds without contact between monomers and polymers.

The following question immediately arises: Is head-to-tail polymerization still possible in the presence of cytochalasin B? With an ATPase activity in the range of 0.2–0.5 mol $P_i \times$ mol actin$^{-1} \times$ min$^{-1}$ one monomer hydrolyzes one molecule of ATP every 2 to 5 min. If the monomers contain during most of this time ATP, head-to-tail polymerization should be possible. If, on the other hand, hydrolysis proper is very rapid with the release of ADP and $P_i$ being the rate-limiting step (which is postulated to be the case in protamin-stimulated ATPase activity of actin monomers [14]), then the monomers should contain ADP and head-to-tail polymerization should be no longer possible. The results of MacLean-Fletscher and Pollard [15] that cytochalasin B inhibits the addition of monomers to the fast-growing (the "barbed") end of the filaments are difficult to evaluate in the present context because they were obtained in the absence of added ATP.

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