STABILIZATION OF F-ACTIN BY PHALLOIDIN REVERSAL OF THE DESTABILIZING EFFECT OF CYTOCHALASIN B

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1 . Introduction

The cyclic peptide phalloidin, a toxic component of the mushroom *Amanita phalloides* prevents the reduction by KI of the high viscosity of F-actin and inhibits the ATPase activity of F-actin during ultrasonic vibration [1]. Cytochalasin B (CB), a metabolite of the fungus *Helminthosporium dematioideum*, on the other hand obviously weakens the F-actin structure. This can be concluded from the fact that CB inhibits cellular functions that are believed to be linked to actin-like microfilaments (for reviews see [2] and [3]) and that it decreases the visosity of F-actin [4]. This communication describes that phalloidin is able to antagonize the weakening effect, which CB exerts on F-actin structure.

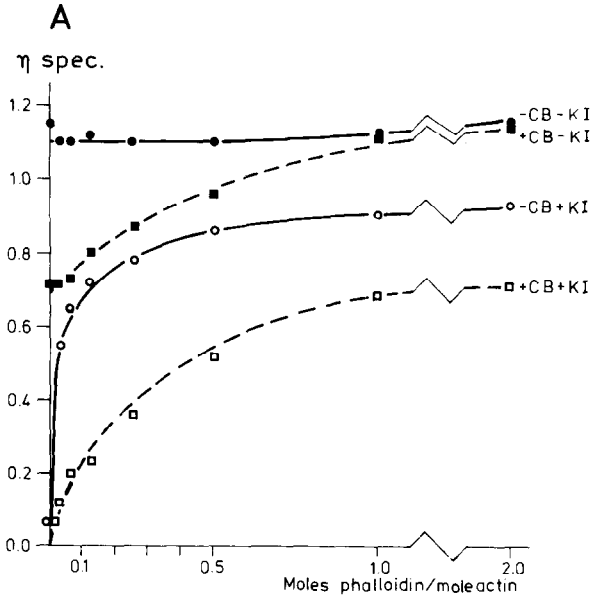
2. Material and methods

The experimental procedures applied have been described elsewhere [1] or are indicated in the legends of the figures. The actin used did not contain tropomyosin-troponin. CB was purchased from Aldrich Chemical Company, phalloidin was a gift from Dr A. Buku.

3. Results and discussion

3.1. *Influence of phalloidin on the reduction of F-actin viscosity induced by KI and/or CB*

Fig.1A shows the moderate viscosity drop of F-actin induced by CB alone and its reversal by phalloidin. This drop, however, did not occur in the presence of KCl (fig.1B). KI (either alone or in conjunction with CB) induced in all cases a nearly total viscosity reduction, which could also be reversed by phalloidin. When KI and CB were present together, more phalloidin was needed to reach a particular sub-maximal degree of viscosity increase than when KI was present alone. This was true also in the presence of KCl (fig.1B) indicating that CB exerted its weakening influence also in the presence of KCl, although in this case CB alone was not able to reduce actin viscosity. The maximal reversal of viscosity reduction, however, was reached when about 1 mole phalloidin per mole of actin



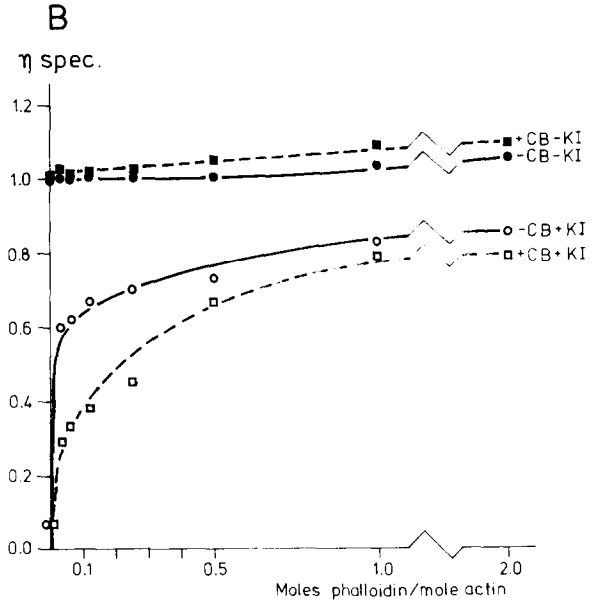


Fig.1. Reversal by phalloidin of the viscosity reduction of actin induced by KI and/or CB. A) Viscosity in the presence of 0.75 mM $MgCl_2$ without KCl. – 2.4 mg (52 nmol) G-actin in 2 ml 5 mM Tris–HCl, pH 7.4, 0.05 mM ADP were polymerized with 0.75 mM $MgCl_2$ by storing over night at +4°C. 0–80 μg (0–100 nmol) phalloidin were added to the samples about 3 hr before incubating them with or without 200 μg (0.2 mM) CB, added in 10 μl dimethylformamide for 10 min at 35°C. Viscosities were measured at 20°C in the absence or presence of 0.6 M KI with a spiral capillary viscosimeter, the flowtime of water being 30 sec. The final vol was 2.1 ml, the actin concentration 1.1 mg/ml. Specific viscosity η_{spec} means (flowtime of the sample divided by the flowtime of the medium alone) – 1. B) Viscosity in the presence of 0.09 M KCl and 0.75 M $MgCl_2$. – 2.4 mg G-actin in 2.2 ml 7.5 mM Tris–HCl, pH 7.4, 0.06 mM ADP were polymerized over night at +4°C with 0.75 mM $MgCl_2$ plus 0.09 M KCl in the presence of 0–80 μg phalloidin. The samples were then incubated for 10 min at 35°C with or without 200 μg CB and the viscosities measured at 21°C.

subunits had been added irrespective of whether KI was present alone or together with CB. (The difference which remained under the conditions of fig.1A between the viscosity in KI alone and KI plus CB did not disappear even after addition of large amounts of phalloidin.).

The course of the curves of fig.1 suggests (though does not prove) that in the presence as well as in the absence of CB the added phalloidin is completely bound to actin and that F-actin is saturated with phalloidin, when phalloidin is added in amounts equimolar to the amounts of actin subunits suggesting that each subunit of the actin filament is able to bind one molecule of phalloidin [1,5]. That the amount of added phalloidin and the viscosity are not linearly related to each other further suggests that for stabilization of the viscosity of a particular part of the actin filament against KI or KI plus CB not all subunits of that part of the filament must have been complexed with phalloidin. The stronger non-linearity in the case of KI alone suggests that in this case binding of phalloidin to any one of the actin subunits stabilizes more adjacent actin units (or a larger part of the filament) than in the case when KI and CB are present together. In other words, in order to prevent the viscosity reduction of a particular part of the actin filament, the filament need be less densely populated with phalloidin when only KI is present than when KI and CB are present together. (This kind of interpretation of the curves is derived from probabilistic considerations and more explicitely dealt within [1], compare also [6]).

3.2. *Induction of ATPase activity of F-actin by cytochalasin B and its inhibition by phalloidin*

The weakening of the F-actin structure by CB is not only revealed by its influence on actin viscosity. From fig.2 it can be seen that F-actin in the presence of CB exhibits an ATPase activity with a similar rate as that induced by sonic vibration [1,7]. This ATPase activity was not observed in the presence of KCl. From fig.2 it can be further seen that this ATPase was inhibited by phalloidin in a manner, which resembled that of the inhibition of the viscosity reduction (fig.1A and 1B) so that similar quantitative considerations can be applied. As in the case of the ATPase activity induced by sonic vibration [1] one can conclude that maximal ATPase activity in the presence of CB is only possible when all actin subunits are free from phalloidin but that in order to inhibit the contribution of about three subunits to the ATPase activity only one of them needs to be complexed with phalloidin.

4. Concluding remarks

Obviously the minimal number of phalloidin molecules that is required in order to reverse the viscocity

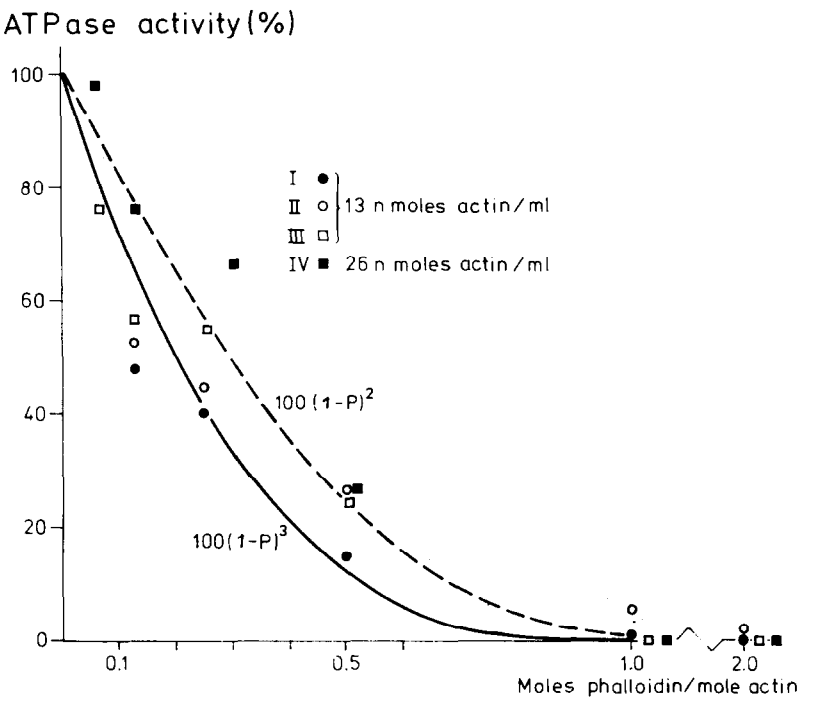


Fig.2. ATPase activity of F-actin induced by CB and its inhibition by phalloidin. – 1.2 mg G-actin in 1 ml 5 mM Tris–HCl, pH 7.4, 0.1 mM ADP (series I) or 0.025 mM ATP (series II, III and IV) were polymerized over night at +4°C with 0.75 mM $MgCl_2$ and after polymerization 0–40 μg phalloidin were added. The samples of group I, II and III were diluted with equal volumes of the Tris-buffer, the final concentrations were then 0.6 mg actin/ml and 0.38 mM $MgCl_2$. These actin-solutions were incubated with 200 μg CB and 1 mM ATP for 30 min at 35°C. The reaction was stopped with 3% TCA and the liberated P_i measured according to [8]. 100% ATPase activity corresponds to the following rates (mol P_i × mol actin^{-1} × min^{-1}): 0.17, 0.21, 0.21 (series I, II and III respectively) and 0.12 (series IV). The lines represent the functions $A = 100\,(1 - P)^2$ or $A = 100\,(1 - P)^3$ with A = % ATPase activity, P = added phalloidin, and would describe ATPase activity under the following assumptions (cf. [1,7]) (a) Each actin subunit binds phalloidin with equal affinity, (b) the degree of saturation of actin with phalloidin equals the following fraction: moles added phalloidin/moles actin subunits present, and (c) the binding of phalloidin to one actin subunit is sufficient for inhibiting the contribution to the ATPase activity of the filament of two (- - - -) or three (——) adjacent actin subunits.

reduction is larger when KI and CB are present together than when KI is present alone. This indicates that KI and CB act synergistically in destabilizing the F-actin structure. Their combined action can be reversed by phalloidin. That CB induced a viscosity reduction as well as an ATPase activity only in the presence of $MgCl_2$ alone and not in the presence of KCl reflects the strong polymerizing action of KCl and indicates a stronger adherence of the actin subunits in the presence of KCl.

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