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Concentration-Dependent Influence of Various Cytochalasins and Chaetoglobosins on the Phalloidin-Induced Polymerization of G-Actin in 0.6 M Potassium Iodide†

Theodor Wieland* and Irmentraut Löw

ABSTRACT: Phalloidin, a bicyclic peptide from the poisonous *Amanita phalloides* mushroom, stimulates the viscosimetrically determined polymerization of G-actin to F-actin in 0.6 M potassium iodide, a medium in which spontaneous polymerization does not occur. The cytochalasins B, D, E, and G (CB, CD, etc.) and the chaetoglobosins A, B, C, E, F, and J (Ch-A, Ch-B, etc.) have been found to influence the rate of polymerization in different ways, depending on their chemical structure and concentrations applied. Class I cytotoxins, e.g., CB, CG, Ch-C, Ch-E, and Ch-F, which exert on F-actin a weak degradative power (DP, "Spudich effect"), increase the polymerization rate when present in a ratio of 4 mol to 1 mol of actin. Polymerization is slightly enhanced or retarded at molar ratios ranging from 0.04:1 to 0.4:1. Class III cytotoxins of strong degradative power, i.e., high affinity for actin, such as CE, Ch-B, and Ch-J, decrease the polymerization rate at a molar ratio of 4:1 during the first 30–40 min; however, they increase it at a molar ratio of 0.4:1. Members of class II (CD, Ch-A) exert an effect that can be interpreted as a combination of the effects of class I and class III cytotoxins. An explanation of this difference in behavior is offered on the basis of experiments on F-actin degradation by Ch-J and its reconstitution by phalloidin in the presence of Ch-J.

Cytochalasin B (CB),[1] a cytotoxic mold metabolite, inhibits microfilament-dependent functions of eukaryotic cells (Wessels et al., 1971; Spudich, 1973) by destabilizing F-actin, as observed by viscosimetry. The degradation does not lead to G-actin monomers; instead the rate of G-actin polymerization is increased when CB is present at a ratio of about 1 mol to 1 mol of actin (Löw & Dancker, 1976). In concentrations 5–10 times smaller, CB lowers the increase in viscosity of a solution of G-actin as compared to the control (Dancker & Löw, 1979).

Recently, Löw et al. (1979) reported on the influence of additional cytochalasins and of several chaetoglobosins (Natori, 1977) on the rate of polymerization of G-actin from rabbit muscle. They found that in a molar ratio of 0.4:1 mol of actin each of the cytotoxins investigated, CB, CD, CE, and CG, as

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[1] Abbreviations used: CB, CD, etc., cytochalasin B, D, etc.; Ch-A, Ch-B, etc., chaetoglobosin A, B, etc.; DP, degradative power (on F-actin); PHD, phalloidin.

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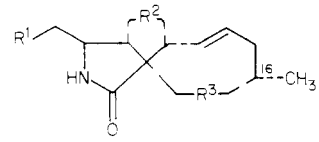
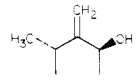
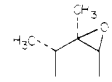
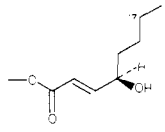
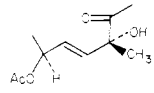
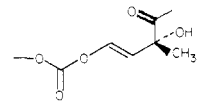
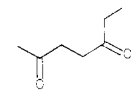
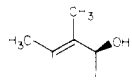
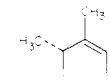
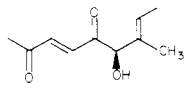
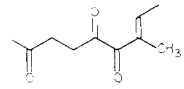
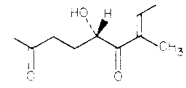
Table I: Structures of the Cytochalasins and Chaetoglobosins Used in This Study



cytochalasins

	B	D	E	G
R^1	phenyl	phenyl	phenyl	indol-3-yl
R^2		as in CB		as in CE
R^3				

chaetoglobosins

	A	B	C	E	F	J
R^1	indol-3-yl	indol-3-yl	indol-3-yl	indol-3-yl	indol-3-yl	indol-3-yl
R^2	as in CE		as in CE	as in Ch-B	as in CE	
R^3		as in Ch-A			as in Ch-E	as in Ch-A

well as Ch-A, Ch-B, Ch-C, Ch-E, Ch-F, and Ch-J (see Table I), increased the rate of polymerization, but to a different extent, and that the final viscosities of the F-actin solutions achieved in their respective presence were distinctly different from each other.

Phalloidin (PHD), one of the toxic principles of the mushroom *Amanita phalloides* (for a review, see Wieland & Faulstich, 1978), strongly stimulates the polymerization of G-actin (Dancker et al., 1975). With PHD a slow but steady increase of viscosity occurs even in 0.6 M KI, a medium in which rapid depolymerization of F-actin takes place in the absence of the drug (Löw & Wieland, 1974).

The spontaneous polymerization of G-actin can also be suppressed by changing the pH value from neutral to 9 or greater. At these higher pH values, CB had no or only a very weak stimulatory effect. On the other hand, PHD induced a rapid increase in viscosity that was further enhanced when CB was present in a 10-fold molar amount (Dancker & Löw, 1978).

We have now studied the combined effects of PHD and the cytotoxins mentioned on the polymerization rate of G-actin. To be sure that polymerization did not occur in the absence of PHD, we used the 0.6 M KI medium. Under these conditions, PHD-induced polymerization was of a velocity at which accelerating or retarding effects could be observed equally well by viscosimetry. The cytotoxins were applied in molar ratios to actin of 4:1, 0.4:1, and in some cases also at 0.04:1.

Experimental Section

F-Actin from rabbit skeletal muscle (Dancker & Hoffmann, 1973) was depolymerized in 1 mM Tris-HCl, pH 8.0, by gentle homogenization and storage at 4 °C overnight after the addition of 0.1 mM ATP. Protein concentrations were measured by spectroscopy at 280 nm with the empirical factor $E_{1cm}^{1mg/1mL} = 1.0$ (in the absence of ATP). The cytochalasins CB, CD, and CE were obtained from the Aldrich Chemical Co. (Milwaukee, WI). CG was a gift of Dr. B. Hesp (Mereside Alderly Park, England), and the chaetoglobosins were gifts from the Japanese laboratory of S. Natori (see Löw et al., 1979). The molecular weights of those drugs range between ~460 (CE) and ~500 (Ch-A). Phalloidin (mol wt ~850) was a sample from our laboratory.

The cytotoxins were added to the reaction as solutions in dimethylformamide, 10–25 μL/2.2 mL of sample; controls contained the solvent only. PHD was employed as a 5 mM aqueous solution. KI was added as a solid to a final concentration of 10% (0.6 M) just before starting the measurements, but after the addition of ATP. Viscosities were measured at room temperature in a capillary viscosimeter and are indicated as $\eta_{spec} = [(t/t_0) - 1]$, t_0 is the flow time of the buffer (30–33 s) and t is the flow time of the solution. Other details are described in the legends to the figures.

Results

Measurements at pH 9. At pH 9 in the presence of 0.2 mM Mg^{2+}, CB alone at a molar ratio of 10:1 had virtually no effect on the viscosity of a solution of G-actin. PHD produced a distinct increase in viscosity, which was exceeded when CB was also present (Figure 1).

Measurement in 0.6 M Potassium Iodide. The effects on the polymerization rate of G-actin induced by PHD (controls) of the cytochalasins and chaetoglobosins investigated are represented in Figures 2–4. In all experiments the concentration of G-actin was 1 mg/mL (0.022 mM) and that of PHD, 0.044 mM. The cytotoxins were employed in molar ratios to actin of 4:1, 0.4:1, and in some cases 0.04:1.

Figure 2 demonstrates that CB alone in the chaotropic medium did not induce polymerization of G-actin, whereas PHD alone induced a linear increase of the viscosity. In

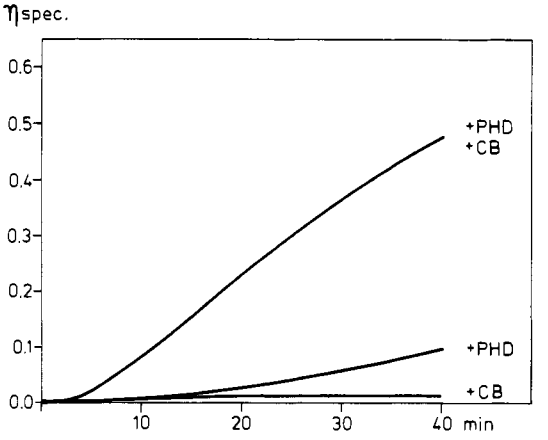


FIGURE 1: Rate of polymerization of G-actin with CB and/or PHD at 20 °C. A 1-mL sample contained 1 mg (22 nmol) of G-actin in 10 mM Tris-HCl, pH 9.0, 1 mM ATP, 0.2 mM EGTA, and 0.2 mM $MgCl_2$. The concentration of CB was 0.2 mM (10 mol/1 mol of actin); [PHD] was 44 μM (2 mol/1 mol of actin).

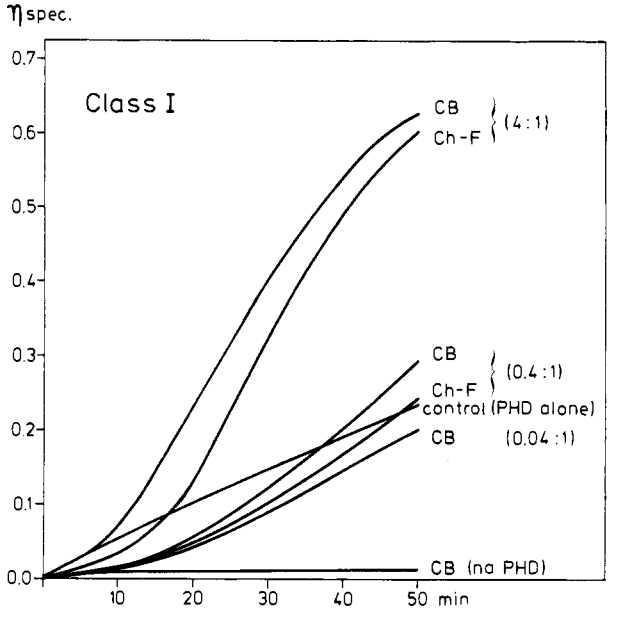


FIGURE 2: Rate of polymerization of G-actin with PHD in 0.6 M KI in the presence of CB or Ch-F. One milligram of G-actin (22 nmol) per 1 mL of 10 mM Tris-HCl, pH 8.0, 1 mM ATP, 0.2 mM EGTA, and 0.6 M KI was polymerized with 44 nmol (2 mol/1 mol of actin) of PHD and CB or Ch-F, whose concentrations are indicated in the figure, at 22.5 °C. The controls contained PHD and dimethylformamide only.

combination with PHD, CB enhanced the rate considerably when present in a molar ratio of 4 mol to 1 mol of actin. This positive effect could still be observed weakly at a ratio of 0.4:1 and turned slightly negative at 0.04:1 within the first 20 to 30 min.

The curves have sigmoid shapes at all CB concentrations applied. This is fully expressed at the highest concentration, although at lower concentrations it appears as the flat initial part, where the viscosity remains lower than the control for some time. Ch-F (Figure 2) and Ch-E (not shown) have similar effects and, in principle, also Ch-C, whose stimulatory power at a ratio of 4:1 is weaker than that of the other compounds mentioned.

The influence on the polymerization rate of G-actin of Ch-B, Ch-J, and CE at different molar ratios is depicted in Figure 3. At a ratio of 4:1 they caused a slower polymerization rate than in the control during the time of observation, whereas

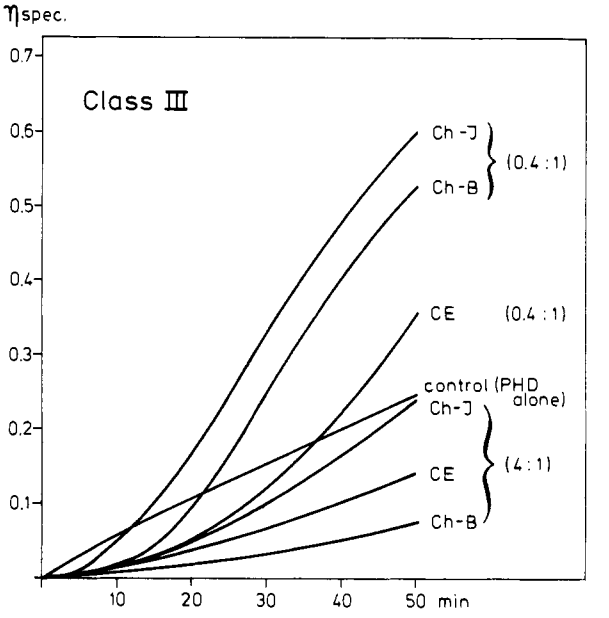


FIGURE 3: Rate of polymerization of G-actin with PHD in 0.6 M KI in the presence of CE or Ch-B or Ch-J at 22.5 °C. The solutions contained 1 mg of G-actin per 1 mL of 10 mM Tris-HCl, pH 8.0, 1 mM ATP, 0.2 mM EGTA, and 0.6 M KI. The samples were polymerized with PHD and dimethylformamide only (controls), or with solutions of CE, Ch-B, or Ch-J in dimethylformamide in addition to PHD in the concentrations indicated in the figure.

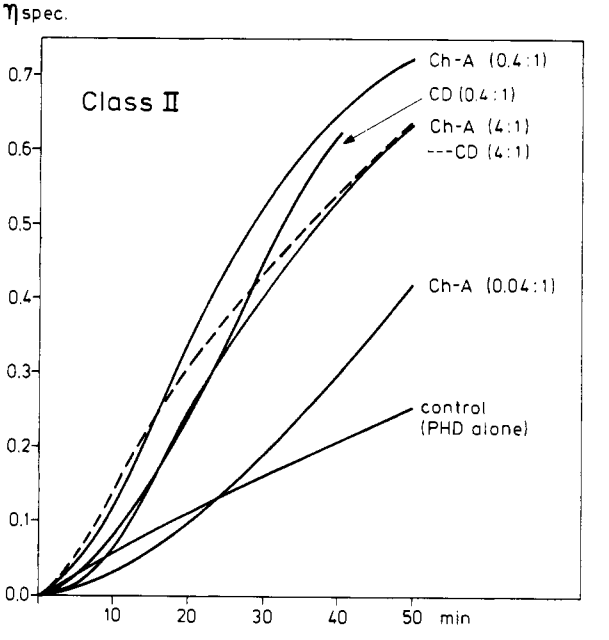


FIGURE 4: Rate of polymerization of G-actin with PHD in 0.6 M KI in the presence of Ch-A or CD at 22.5 °C. The samples contained 1 mg of G-actin per 1 mL of 10 mM Tris-HCl, pH 8.0, 1 mM ATP, 0.2 mM EGTA, and 0.6 M KI. They were polymerized with PHD (2 mol/1 mol of actin) and dimethylformamide in the control or with PHD in the presence of Ch-A or CD. The concentrations are indicated in the figure.

an increase occurred at a ratio of 0.4:1. Almost no effect was visible at a ratio of 0.04:1.

As seen in Figure 4, Ch-A shows a particularly strong cooperative effect. Its stimulation on polymerization at a ratio of 4:1 was comparable to the cytotoxins of Figure 2; however, at a ratio of 0.4:1 it was even more effective than at the higher concentration. Even at a ratio of 0.04:1, an acceleration was observed after 20 min of incubation. CD showed a behavior similar to that of Ch-A.

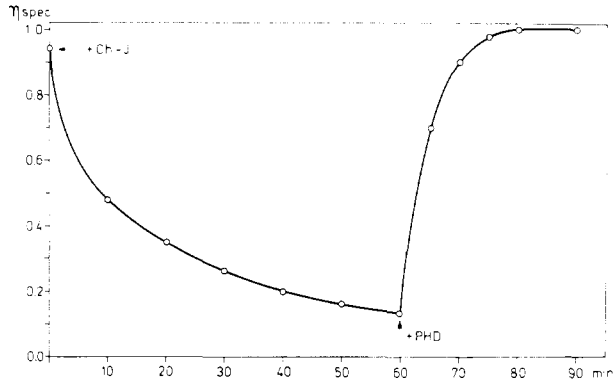


FIGURE 5: Rate of polymerization of actin with PHD after pretreatment of F-actin with Ch-J at 20.5 °C. G-Actin (1 mg/1 mL) was polymerized overnight in 10 mM Tris-HCl, pH 7.2, 1 mM ATP, and 0.2 mM EGTA with 0.19 mM $MgCl_2$. Ch-J was added (1 mol/1 mol of actin), followed 60 min later by PHD (2 mol/1 mol of actin).

Degradation and Reconstitution of F-Actin. In Figure 5 an experiment is represented in which F-actin at pH 7.2 in the presence of Mg^{2+} (no K^+) has been subjected to the destabilizing effect of Ch-J (see Löw et al., 1979). When, after 60 min, the viscosity had reached a constant low value, PHD was added. A sudden rise clearly occurred after the addition.

Discussion

The synergistic effect of CB on the rate of the PHD-induced polymerization of G-actin at pH 9, as shown in Figure 1, appeared even more clearly in 0.6 M KI (Figure 2). Similar to the activity of CB, the activities of the nine other cytotoxins investigated in this study are dependent on the molar ratios of cytotoxin to actin. From Figures 2–4, respectively, it appears that in 0.6 M KI the kinetics of the PHD-induced increase of viscosity differs from the kinetics induced by additional cytotoxins. Whereas PHD alone causes a practically linear rise (initial part of a flat hyperbolic curve), all reactions in the presence of the cytotoxins exhibited a sigmoid shape, reflecting a sequence of kinetically different events. We assume that in all cases of interaction with the cytotoxins a faster rise of viscosity will eventually occur than in the controls with PHD alone, but the initial phase of the sigmoidal curves may take a longer time than the usual experimental time of 50 min.

As recently shown by Löw et al. (1979), the viscosities reached 50 min after addition of the various cytotoxins (without PHD) were quite different. In the case of Ch-J, it was demonstrated that identical viscosity was obtained whether F-actin was reduced in size by the cytotoxin or G-actin was polymerized in its presence. We therefore conclude that, generally, the degree of viscosity of a solution of G-actin obtained in the presence of each of the cytotoxins equals that of a solution of F-actin after degradation by the respective compound. We would like to introduce the term "degradative power" (DP) for this property of the individual cytotoxins and define DP as the difference between the end viscosity expressed as a percent of control, η_{end}, obtained in the presence of the respective cytotoxin and the end viscosity of the control taken as 100%. Then DP = $(100 - \eta_{end})$%, as shown in Table II.

We attribute strong DP to a cytotoxin in whose presence at a molar ratio of 0.4:1 in 20 mM KCl after 40 min a specific viscosity (η_{sp}) will be obtained that is less than 60% of the control. Medium DP is defined by an end viscosity of 60 to 80%, and weak DP has an end viscosity of more than 80%. As is apparent from Table II, the cytotoxins can be divided into three classes. Class I (CB, Ch-E, Ch-F, CG, and Ch-C; only CB and Ch-F are shown in Figure 2) has weak DP (0–18%) and, in the presence of PHD, promotes the polymerization of G-actin in 0.6 M KI strongly at a molar ratio of 4:1, fairly at 0.4:1, and minimally, if at all, at 0.04:1. Class III compounds (CE, Ch-J, and Ch-B; Figure 3) have strong DP (45–58%) and will retard the polymerization strongly at a ratio of 4:1, and stimulate at a ratio of 0.4:1. Class II compounds (CD and Ch-A) are of medium DP (26–42%) and somewhat intermediary in their stimulatory effect on polymerization; i.e., one finds characteristic features of both classes I and III (Figure 4).

In order to interpret the results of Figures 2, 3, and 4, summarized in Table III, we suggest that (1) the cytotoxins studied display dual reactivity, i.e., retard or stimulate the polymerization of G-actin, (2) this property correlates with the individual affinity to actin (DP) and, therefore, with the concentration applied (molar ratio to actin), and (3) all of the cytotoxins in a concentration high enough (which cannot be verified in every case for reasons of poor solubility) inhibit the initial phase of the polymerization and stimulate it at lower concentrations.

The class III compounds (Tables II and III) by virtue of their strong DP, i.e., high affinity to F-actin, will retard the polymerization at a molar ratio of 4:1, whereas such an effect still cannot be observed with the class I and class II cytotoxins of weaker DP. These compounds in a 4:1 ratio already stimulate the polymerization; stimulation will also be exerted by

Table II: Final Specific Viscosities (after 40 min) and Degradative Power (DP) on Actin of Several Cytochalasins and Chaetoglobosins[a]

drug	η_{sp} after 40 min	DP (%)
Class I		
none (control)	0.78	0
CB	0.78	0
Ch-E	0.78	0
Ch-F	0.76	3
CG	0.72	8
Ch-C	0.64	18
Class II		
CD	0.58	26
Ch-A	0.45	42
Class III		
CE	0.43	45
Ch-J	0.35	55
Ch-B	0.33	58

[a] In a molar ratio of 0.4:1 mol of actin in the presence of 2 mM KCl. Data are from Figures 1b and 2 of Löw et al. (1979).

Table III: Three Classes of Cytochalasins and Chaetoglobosins According to Their Effect on the Polymerization of G-Actin in 0.6 M KI in the Presence of PHD and According to Their DP on F-Actin

class	drugs	effect on G-actin polymn during 50 min[a] at molar ratio drug:actin of 4:1	0.4:1	0.04:1	degradative power (DP)[b]
I	CB, CG	++	−+, −	−[c]	weak
	Ch-C, Ch-E, Ch-F	++	−+	*d*	
II	CD, Ch-A	++	++	+	medium
III	CE	−−	+	*d*	
	Ch-B, Ch-J	−, −+	++	−, −+	strong

[a] Enhancement of polymerization: ++, strong; +, slight. Retardation of polymerization (during the first 50 min): −−, strong; −, slight; −+, at first inhibition, after 20 to 40 min; slow acceleration. [b] See Table II. [c] Determined with CB only. [d] Not determined.

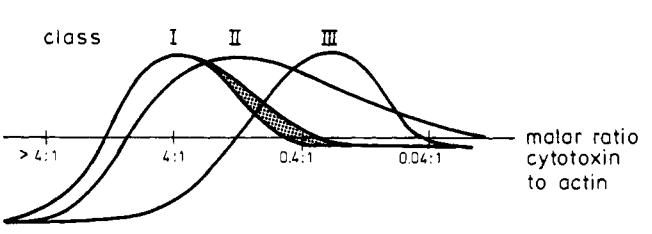


FIGURE 6: Schematic representation of the suggested dual influence of the cytotoxins of classes I, II, and III, respectively (Table II), on the rate of polymerization of G-actin depending on their molar ratio to actin.

the class III drugs, however, only at lower concentrations than by the other classes. At very low concentration (0.04:1), the compounds of class I and class III produce weak retardation of the polymerization rate during the experimental time, whereas CD and Ch-A (class II, after a slower initial phase of 25 min) induce a slightly faster polymerization than with PHD alone. Generally, at high dilution the effects of the drugs are not very prominent. Figure 6 is a schematic representation of the suggestions made.

The PHD-stimulated polymerization of G-actin does not seem to obey strictly the model proposed by Oosawa (1970) or Engel & Winklmair (1972), namely, a sequential attachment of G-actin protomers to the growing double helical chain, with the process of nucleation being the slowest. The very rapid reconstitution of F-actin by PHD from its fragments produced by Ch-J, as shown in Figure 5, suggests that most probably small- and medium-sized fragments of F-actin also assemble, and that PHD also prevents the dissociation of actin nuclei.

Conceivably, the length of the initial phase of the respective curves (Figures 2, 3, and 4), the retardation of viscosity increase, is controlled in a twofold way: cytotoxins with strong affinity to actin (class III) at a ratio of 4:1 will bind tightly to G-actin oligomers in an early stage, thus preventing further polymerization. We propose that fragments bearing cytotoxins at crucial points are unable to grow unless the toxin has dissociated away. Therefore, with a drug of strong DP present in high concentration, the formation of a sufficient number of oligomers goes very slowly. Since dissociation occurs nevertheless to a certain degree, polymerization will proceed, gaining velocity with the elongation of the oligomeric fragments which assemble (Figure 3). With cytotoxins of low affinity (small DP, class I), retardation occurs in a relatively dilute solution (ratio 0.4:1; Figure 2). Here, intervention in the growth of actin chains can be assumed at later stages (as not yet detectable by viscosimetry) by drug molecules temporarily blocking sites of polymerization.

At higher molar ratios (4:1), class I (and class II) compounds promote polymerization (Figures 2 and 4), as do class III compounds at lower concentrations (Figure 3). The stimulatory effect of higher concentrations of CB (without PHD) is attributed to its enhancement of nucleation (Dancker & Löw, 1979). We assume that this is also true in the presence of PHD in 0.6 M KI. Thus, the affinity of a compound for actin is decisive for its stimulatory effect already at low concentration (class III) or only at higher concentrations. For members of class II, this affinity may be adequate at molar ratios of 0.4:1 to promote polymerization via nucleation, while at ratios of 4:1 it interferes with the assembly of short fragments.

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