Evidence that Cytochalasins Reduce the Size of Polymerization Nuclei during Actin Polymerization

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In a double-logarithmic plot of the rate of actin polymerization against monomer concentration, the slope has a lower value in the presence of the fungal metabolites cytochalasin B and D than in their absence. This suggests that actin oligomers which serve as polymerization nuclei are smaller in the presence of cytochalasins than in their absence.

Introduction

Cytochalasins are a group of fungal metabolites which strongly influence cell motility and the structure of the actin-based cytoskeleton (*cf.* [1]). They are widely considered as inhibitors of actin polymerization but this is only partially true. In fact they inhibit actin monomer incorporation into filaments or into polymerization seeds ("nuclei") [2–6]. This inhibition is produced by very low concentrations of cytochalasins supporting the view that cytochalasin binding to filament ends is responsible for inhibition of filament elongation. On the other hand, as first shown by Löw and Dancker [7] concentrations of CB which are stoichiometric to actin accelerate actin polymerization appreciably. This has been confirmed for CD by different laboratories (Frieden and coworkers [8, 9], Brenner and Korn [10], Inoué *et al.* [11]).

When cytochalasins accelerate overall actin polymerization but retard filament elongation, they must favor the initial nucleation step of actin polymerization thus overcompensating the inhibition of elongation (Dancker and Löw [12]).

Oligomers can function as nuclei when their dissociation into monomers is slower than elongation to filaments. Since larger oligomers are more stable than smaller ones (due to increased subunit interaction in larger oligomers) oligomers must have a certain "critical size" in order to be an effective nucleus. One way of facilitating nucleation could be to stabilize shorter oligomers which otherwise would dissociate too fast. Since in this case fewer actin-actin encounters are necessary, functional nuclei are formed faster. This should influence the dependence on monomer concentration of polymerization rate. We have shown [13] that the mushroom peptide phalloidin accelerates actin polymerization by this mechanism. In this communication we show that cytochalasin B and D act in a similar way.

Abbreviations: CB, cytochalasin B; CD, cytochalasin D.

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Materials and Methods

Actin from rabbit skeletal muscle was prepared after ref. [14]. Actin concentration was determined by absorption at 290 nm using an extinction coefficient $E_{1\,mg/ml} = 0.63$.

Actin polymerization at various actin concentrations was monitored by measuring increasing light scattering intensity in a Shimadzu RF 520 Double Beam Fluorescence Spectrophotometer at 22 °C. Excitation and emission monochromators were both at 400 nm. Polymerization velocity at each actin concentration was deduced from the slope (light scattering increment per minute) of the linear part of the registrations of polymerization at this particular actin concentration. Accordingly, polymerization rate is expressed in arbitrary units but this does not influence the slope of a double-logarithmic plot as that Fig. 1. Cytochalasins were obtained from Serva, Heidelberg. Stock solutions of 10 mM in dimethyl formamide were prepared.

Results and Discussion

The dependence of the rate of actin polymerization on monomer concentration can be approximated by Eqn. (1) (*cf.* [15])

$$v = k\, c_1^{\,n} \qquad (1)$$

or, in logarithmic terms

$$\log v = \log k + n \log c_1 \qquad (1\,a)$$

where v is polymerization rate, k is a rate constant, c_1 is monomer concentration (equivalent to total actin concentration at the beginning of polymerization). The exponent n allows for the possibility that the reaction can be of higher than first order.

The figure showing a double-logarithmic plot of polymerization rate *versus* actin concentration (thus corresponding to Eqn. (1a)) confirms (*cf.* [15]) that actin polymerization is higher than first order (the values of the slopes which yield n of Eqn. (1) are larger than 1). One can further see that in the presence of both CB and CD polymerization rate is higher at all actin concentrations. From the data presented in the figure one can deduce that at an actin concentration of 1 mg/ml polymerization is two- to threefold faster in the presence of cytochalasins than in their absence. Particularly important is that the slope has a lower value in the presence of cytochalasins than in their absence (1.85 in CD, 2.1 in CB as compared to 2.8 in the absence of cytochalasins).

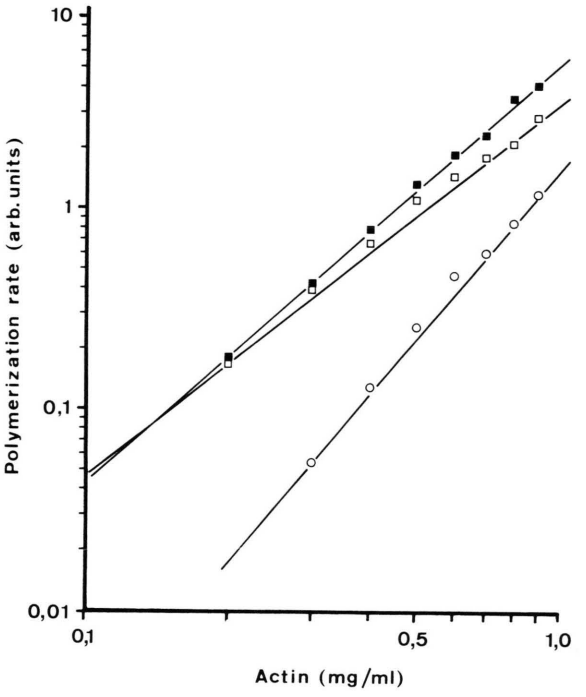

Fig. 1. Rate of actin polymerization as dependent on actin concentration. – The rates of polymerization were measured as rate of change of light scattering intensity at various actin concentrations. Actin was in 5 mM Tris-HCl, pH 8.0, 0.1 mM ATP, 1 mM $MgCl_2$. — ○ — ○: No cytochalasins; □——□: 20 μM cytochalasin D; ■——■: 20 μM cytochalasin B.

The fact that the rate of actin polymerization is proportional not to the first power of monomer concentration but to higher powers suggests that nucleus formation (requiring the simultaneous contact of several monomers thus giving rise to a multimolecular reaction) is rate-limiting during actin polymerization. The size of the power (measured as the slope in diagrams like those of the figure) is most probably related to the size of the nucleus (*cf.* Nishida and Kasai [15] and Wendel and Dancker [13]). The slopes measured in this communication suggest that average nucleus size is about 3 subunits per nucleus in the absence and about 2 subunits per nucleus in the presence of cytochalasins. This is probably related to the observation of Frieden and colleagues (Goddette and Frieden [16, 17], Goddette *et al.* [18]) that CD stimulates the formation of actin dimers.

In conclusion: The observations of this communication suggest that cytochalasins accelerate actin polymerization by just the same mechanism as phalloidin does, namely by reducing the size of polymerization nuclei thereby facilitating the formation of such nuclei.

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