Complex Influence of Cytochalasin B on Actin Polymerization

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In the presence of very low concentrations (about 2 × 10⁻⁷ M) of cytochalasin B (CB) the time course of actin polymerization is much more sigmoidal when followed by viscosity measurements than when followed by light scattering measurements. This suggests that under these conditions actin polymers do not immediately reach their final length but only via short “bent” polymers which can be detected only by light scattering but not by viscosity measurements. At higher CB concentrations (about equimolar to those of actin) CB reduces the average degree of polymerization and favors the nucleation step necessary for polymerization.

1. Introduction

The mold metabolite cytochalasin B (CB) is known to impair microfilament-dependent functions in eukaryotic cells and to reduce the viscosity of polymeric actin [1–3]. CB does not depolymerize actin [2], instead, it favors actin polymerization as indicated by a reduction of the “critical concentration” necessary for polymerization and by an acceleration of polymerization [3]. This communication describes that during actin polymerization the increase in light scattering intensity and the increase in viscosity follow different time courses and that this difference is accentuated by low concentrations of CB. Therefore CB exerts a rather complex influence on actin polymerization.

2. Experimental procedure

Actin, free of tropomyosin-troponin, was extracted from an acetone powder of rabbit skeletal muscle and purified by partial polymerization in 0.7 mM MgCl₂ as described in [4]. Viscosity was measured at room temperature in a viscosimeter the capillary of which was formed as a spiral. The outflow time of water was 40 s. Light scattering intensity perpendicular to the incident beam was measured in a Hitachi-Perkin-Elmer Fluorescence Spectrophotometer MFP-2A at 400 nm and at 22 °C.

3. Results

The figure compares light scattering increase and viscosity increase during polymerization of actin. The samples for the measurements of light scattering and viscosity were always taken from the same actin mixtures. The following points are remarkable:

1. The viscosity curves are clearly sigmoidal whereas in the light scattering curves sigmoidicity is not discernible at this time resolution.
2. Low concentrations (as low as 3 × 10⁻⁷ M) of CB increase the sigmoidicity of the viscosity curves so that early during polymerization viscosity is lower in the samples with CB than in those without CB. These low CB concentrations have no effect on light scattering.
3. At higher CB concentrations (beginning with about 10⁻⁶ M) this viscosity-depressing effect begins to disappear and at CB concentrations about equimolar to that of actin both viscosity increase and light scattering increase are faster than in the absence of CB. This acceleration is associated with a lower end viscosity without affecting the final light scattering value.

4. Interpretation

4.1. Influence of low CB concentrations

The sigmoidal increase of viscosity as compared to the more or less exponential increase of light scattering intensity suggests that the filaments which can be observed early during polymerization contribute less to the viscosity of the actin solution than those filaments which can be observed later. Light scattering, on the other hand, does not distinguish between early and late filaments. Because long asym-
metric particles are more effective in increasing the viscosity of a solution than shorter and more symmetric ones whereas light scattering is less sensitive to length differences, the observed viscosity might indicate that early during polymerization the filaments are shorter and less asymmetric than later on. If so, the viscosity curve does not only reflect the appearance of polymers but also alterations of the filament dimensions whereas light scattering reflects only the appearance of polymers. Accordingly, the increased sigmoidicity of the viscosity curve in the presence of low concentrations of CB means that under these conditions the filaments are initially shorter than in the absence of CB although at the end of polymerization the average filament length should be the same in both cases. Since the intensity of light scattering is not decreased by these low concentrations of CB, the amount of monomers incorporated into filaments is the same both in the absence and presence of low concentrations of CB.

A simple explanation for this behavior could be that at the beginning of polymerization, when the polymers are necessarily short, a length change due to the addition of monomers results in a smaller viscosity increase than later, when the monomers are bound to longer polymers. If this would be true, the lower initial viscosity in the presence of CB would imply that the polymerization velocity is smaller in the presence of CB. This should be also reflected by the light scattering curves, which, however, is not the case. Therefore, we do not think that this simple explanation is true.

Then we are left with two further possibilities which could explain why the filaments are initially shorter in the presence than in the absence of low concentrations of CB: 1. There could be more filaments with less monomers per filament, or 2. the average filament length has changed by some kind of "coiling" without changing the amount of filaments and the number of subunits incorporated into each filament. From possibility (1) one would predict that in the presence of low concentrations of CB the higher number of filament ends would accelerate polymerization and that the average filament length should remain shorter than in the absence of CB. Both predictions are not fulfilled (remember that at the end of polymerization the viscosity is the same both in the absence and presence of low concentrations of CB), so that possibility (2) seems more reasonable. Therefore we conclude that polymerization does not proceed in one step from dimeric (or oligomeric) "nuclei" to the final filaments but that there is some intermediate state. It looks as if the filaments are first polymerized into a "coiled" or "bent" form which then, in a subsequent step, extends to the final length:

\[ G \rightarrow N \stackrel{k_3}{\rightarrow} F' \rightarrow F \]  

with G, N, F', and F representing G-actin, nuclei, intermediate short filaments and final filaments, respectively. Light scattering "sees" both F' and F whereas viscosity measurements detect only F. In the absence of CB \( k_3 \) is large enough to prevent appreciable accumulation of F' and to allow fast attainment of F whereas low CB concentrations reduce \( k_3 \) leading to a transitory increase of F' so that \((F'+F) > F\) and hence light scattering intensity is higher than viscosity at the beginning of polymerization. It should be borne in mind that according to our view the lower viscosity of F' is not due to a
lower degree of polymerization but due to an altered arrangement of subunits.

4.2. The action of higher concentrations of CB

Consider actin polymerization as a cooperative linear aggregation [5] with an unfavorable nucleation step $A + A \xrightarrow{2} A_2$ with $0 < \sigma < 1$ and propagation steps $A_{i-1} + A \xrightarrow{i \geq 2} A_i$ (with $i > 2$ ($K$: equilibrium constant of propagation). The smaller $\sigma$, the more unfavorable nucleation is as compared to propagation. At equilibrium the parameters $K$, $\sigma$, $c_1$ (concentration of free monomers) and $c_t$ (total monomer concentration) are connected by the following equation (cf. [6])

$$c_t = c_1 + \sigma \sum_{i=2}^{\infty} iK^{-1}(Kc_t)^i.$$  \hspace{1cm} (2)

This expression requires that $Kc_t < 1$, otherwise the arithmetico-geometric series in (2) would not converge. Then, $c_t$ can be approximated by the expression

$$c_t \approx c_1 + \sigma c_1(1 - Kc_t)^2.$$  \hspace{1cm} (3)

The average degree of polymerization $c_p/m$ with $c_p$: total number concentration of monomers incorporated into polymers and $m$: number concentration of polymers, is [6]

$$c_p/m = 1/(1 - Kc_t).$$  \hspace{1cm} (4)

The higher polymerization velocity (visible both by light scattering and viscosity) and the lower end viscosity in the presence of CB concentrations which are about equimolar to that of actin, suggest that one of the two earlier mentioned alternatives (more filaments and therefore more “ends” but each filament with less monomers incorporated) is probably valid. Moreover, the concentration of monomers remaining in equilibrium with the filaments (“critical concentration”) is lower at these CB concentrations [3].

In terms of the equilibrium model just outlined this means that both $c_p/m$ (average degree of polymerization) and $c_1$ (free monomer concentration) have decreased. The reduction of $c_p/m$ (Eqn (4)) implies that $Kc_t$ must become smaller. From Eqn (3) it can be deduced that only an increase of $\sigma$ (with $K$ remaining essentially constant) leads to a decrease of both $Kc_t$ and $c_1$. (If one lowers $Kc_t$ by decreasing $K$ at fixed $\sigma$, one obtains an increase of $c_1$ rather than a decrease.) Increasing $\sigma$ means that the difference between the equilibrium constant of nucleation and that of propagation becomes smaller. Therefore we think that high concentrations of CB favor the nucleation step of actin polymerization.

5. Conclusion

We deduce from our experiments that in the presence of low concentrations of CB actin polymerizes first into short “bent” polymers with low viscosity which then extend to long straight filaments. It is remarkable that a very small ratio of CB to actin is sufficient to retard the transition from the short to the final filaments. Therefore, these low amounts of CB induce highly cooperative effects in the actin polymer.

At higher CB concentrations (about equimolar to that of actin) the prominent effect of CB (increase in polymerization velocity and decrease in end viscosity) is interpreted as being due to a facilitation of the nucleation step and to a reduction to the average degree of polymerization.

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