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Inhibition of actin polymerization by latrunculin A

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Latrunculin A, a toxin purified from the red sea sponge *Latrunculia magnifica*, was found previously to induce striking reversible changes in the morphology of mammalian cells in culture and to disrupt the organization of their microfilaments. We now provide evidence that latrunculin A affects the polymerization of pure actin in vitro in a manner consistent with the formation of a 1:1 molar complex between latrunculin A and G-actin. The equilibrium dissociation constant (K_d) for the reaction in vitro is about 0.2 μM whereas the effects of the drug on cultured cells are detectable at concentrations in the medium of 0.1–1 μM.

Latrunculin A; Actin polymerization

1. INTRODUCTION

Recently, latrunculins, a new class of drugs isolated from the Red Sea sponge *Latrunculia magnifica*, were found to disrupt the organization of microfilaments and to have profound effects on the morphology of non-muscle cells in culture [1]. Concentrations of latrunculin A and latrunculin B as low as 90 nM were shown to alter the morphology of mouse neuroblastoma clone N1I-115 cells and to disrupt their microfilament organization within 1 h of application; similar effects on Swiss 3T3 mouse fibroblasts required about 900 nM latrunculin. Typically, affected cells rounded up in the nuclear region and arborized. As revealed by immunofluorescence microscopy using purified anti-actin antibodies, microfilaments, microfilament bundles and stress fibers disappeared and the actin was re-distributed as dots and patches within a diffuse background. All of these effects were fully reversed within 1 h after the cells were placed in fresh medium without latrunculin. Immunofluorescence microscopy with anti-tubulin antibodies showed that microtubules were unaffected by the latrunculins. To determine if the effects of latrunculin on cells can be attributed to a direct interaction with actin, we investigated the effects of latrunculin A on the polymerization of pure actin in vitro.

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2. MATERIALS AND METHODS

Latrunculin A [2] and rabbit skeletal muscle actin [3,4] were prepared as described. Actin was labeled on Cys-374 with pyrene and the polymerization of actin containing 8% pyrene-labeled actin was monitored by the increase in fluorescence [5]. The buffer used in all experiments contained 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.2 mM ATP, 0.2 mM dithiothreitol, 0.1% NaN_3 and 5 mM Tris, pH 7.8, at 25°C. Latrunculin A was added in dimethyl sulfoxide and an equivalent volume of solvent was added to the control samples. At the concentrations used, the dimethyl sulfoxide had no effect.

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3. RESULTS

In the first experiments, we determined the concentration of F-actin as a function of total actin concentration (critical concentration curves) in the absence of latrunculin and in the presence of 2 or 6 μM latrunculin A (fig.1, left). The critical concentration of the actin in the absence of latrunculin A was 0.24 μM. Parallel curves were obtained in the presence of latrunculin with apparent critical concentrations of 1.29 and 3.67 μM for 2 and 6 μM latrunculin, respectively. The simplest interpretation of these data is that latrunculin A forms a 1:1 complex with G-actin [6]. On this basis, K_d values of 0.18 and 0.22 μM can be calculated using the equation:

$$K_d = [A][L]/[LA] \quad (1)$$

where [A] is the concentration of free actin monomer (the critical concentration), [LA] is the concentration of the 1:1 complex (the increase in apparent critical concentration in the presence of latrunculin) and [L] is the concentration of free latrunculin (total latrunculin, [LA]).

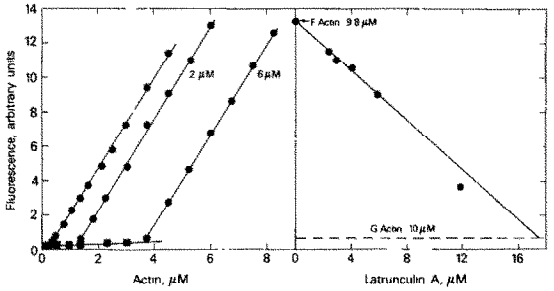

Fig.1. The effect of latrunculin A on actin polymerization at steady state. (Left) The concentration of F-actin at steady state is plotted as a function of total actin concentration in the absence or presence of 2 and 6 μM latrunculin A. The buffer conditions are given in section 2. The F-actin concentration is reported in arbitrary units of fluorescence of the pyrene-actin tracer (8% of the total actin). The apparent critical concentrations are the intercepts of the curves for F-actin with the curve (■) showing the fluorescence of G-actin at the same concentration but in the absence of 2 mM $MgCl_2$. The same results were obtained when latrunculin was added to F-actin before polymerization or to F-actin after polymerization in the absence of latrunculin had reached steady state. (Right) The steady-state fluorescence values measured for 10 μM actin are plotted as a function of the total concentration of latrunculin A. The F-actin concentration in the absence of latrunculin A was 9.8 μM.

In the second experiment, 10 μM G-actin was polymerized in the presence of 0–12 μM latrunculin A. The lag time increased progressively with increasing concentrations of drug (not shown), indicating that latrunculin inhibited the rate of nucleation, with a less pronounced effect on the apparent rate of elongation. These effects are similar to those of profilin, a protein known to form a 1:1 complex with G-actin [6]. The concentration of F-actin at steady state (fig.1, right) decreased in direct proportion to the latrunculin A concentration, which is consistent with the formation of a 1:1 molar complex with G-actin [6]. On this basis, a K_d of 0.19 μM can be calculated [6] from the slope of the line, m, using the equation:

$$m = C_c/(C_c + K_d) \quad (2)$$

where C_c is the critical concentration of pure actin (= 0.24 μM).

Thus, the effects of latrunculin A on the kinetics of actin polymerization and on the concentrations of F-actin at steady state are qualitatively similar to those of proteins such as profilin [6], which form a 1:1 complex with G-actin. In other experiments (not shown), the inhibition by latrunculin A of the rate of elongation of F-actin seeds added to solutions of G-actin and the effects of latrunculin A on the apparent critical concentration of actin in the presence of cytochalasin D (which increases the critical concentration of actin by capping the barbed end of the filament [7]) were also consistent with this interpretation.

4. DISCUSSION

The present data provide reasonable evidence for the formation of a 1:1 molar complex between G-actin and latrunculin A. But can this reaction account for the dramatic and specific effects of latrunculin A in vivo? If we knew both the concentration of free latrunculin A and the critical concentration of actin in the cell, we could calculate the concentration of the complex that would be formed in situ. If both ends of the actin filaments in the cell were free, the critical concentration of actin under cellular ionic conditions would be about 0.2 μM. If we assume that cells are freely permeable to latrunculin A (and impermeable to

G-actin and the G-actin–latrunculin complex), the intracellular concentration of latrunculin would be the same as its initial concentration in the medium (because the intracellular volume is a very small fraction of the total culture volume). Then, from eqn 1, we can calculate that the concentration of the latrunculin A–G-actin complex would be about 0.1 and 1 μM, respectively, at latrunculin A concentrations of 90 and 900 nM [1]. The concentrations of actin in N1E-115 and 3T3 cells have not been determined but, if they are similar to the actin concentrations of other cells (about 100 μM [7]), the formation of 0.1–1 μM complex would probably have only a small effect on the concentrations of polymerizable actin. However, if the critical concentration of actin were higher in situ, which would be the case if the barbed ends of filaments were capped [7], or if latrunculin A were concentrated by cells, the concentration of the non-polymerizable complex would be greater. At this time, we cannot be certain that the effects of the latrunculins on microfilament organization is a direct consequence of their interaction with G-actin. The biochemical basis of the cytotoxicity of the latrunculins may become clearer when studies in progress comparing the cytotoxicity and actin affinities of a number of derivatives of latrunculin are completed. The present data do demonstrate, however, that the latrunculins provide another useful drug for the study of actin polymerization in vitro and one whose mode of action is complementary to that of cytochalasin D.

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