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Nonmuscle actin ADP-ribosylated by botulinum C2 toxin caps actin filaments

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The effect of nonmuscle actin ADP-ribosylated by botulinum C2 toxin on the polymerization of nonmuscle actin was investigated in order to clarify whether nonmuscle actin is converted into a capping protein by ADP-ribosylation. ADP-ribosylated actin was found to decrease the rate of polymerization of actin filaments which are free at both ends. ADP-ribosylated actin turned out to have no effect on the rate or extent of polymerization at the pointed ends of actin filaments the barbed ends of which were capped by gelsolin. The monomer concentration reached at the final stage of polymerization was similar to the critical concentration of the pointed ends of actin filaments. The results suggest that nonmuscle actin ADP-ribosylated by botulinum C2 toxin acts as a capping protein which binds to the barbed ends to inhibit polymerization.

Botulinum C2 toxin; ADP-ribosylation; Actin; Capping protein

1. INTRODUCTION

Botulinum C2 toxin is one of eight known toxins produced by different types of *Clostridium botulinum* [1]. Among these toxins, botulinum C2 toxin is unique in its structural and functional properties [2]. Botulinum C2 toxin is binary in structure and consists of two components [3]. Apparently, component II is involved in binding of the toxin to the eukaryotic cell membrane [4], while component I possesses ADP-ribosyltransferase activity [5] and modifies actin [6,7]. The substrate of botulinum C2 toxin is monomeric nonmuscle G-actin but not polymerized F-actin. In contrast to botulinum neurotoxins A–G, botulinum C2 toxin is not neurotoxic but induces cytopathic effects [8–10]. The toxin destroys the microfilament network and causes rounding up of intact cells [9]. These effects were correlated with toxin-induced ADP-ribosylation of actin. Recently it has been shown that ADP-ribosylated skeletal muscle actin which was modified by *Perfringens* iota toxin, caps actin filaments [11]. Skeletal muscle actin, which is highly homologous to nonmusle actin [12], is a poor substrate of botulinum C2 toxin [6]. In order to gain further insight into the mechanism of the cytopathic effects of botulinum C2 toxin on nonmuscle cells and to determine whether ADP-ribosylated nonmuscle actin behaves also as a capping protein, we have investigated the effect of ADP-ribosylation of nonmuscle actin on the polymerization of liver actin.

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2. MATERIALS AND METHODS

2.1. *Preparation of proteins*

Component I of botulinum C2 toxin was purified from culture medium of *C. botulinum* type C strain 9213 essentially as described [3]. Actin was prepared from pig liver according to [13] with the following modification. Before chromatography the protein was dialyzed vs buffer A (0.5 mM ATP, 0.2 mM $CaCl_2$, 5 mM triethanolamine-HCl, pH 7.5, and 3 mM NaN_3). The protein was then applied to a Sephacryl S-200 column (2.5 × 90 cm) equilibrated with buffer A. The concentration of actin was determined photometrically at 290 nm using an absorption coefficient of 24900 $M^{-1} \cdot cm^{-1}$ [14]. Fluorescently labeled actin was produced by modification of actin with

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7-chloro-4-nitro-2-oxa-1,3-benzdiazole [15]. ADP-ribosylated actin was prepared by incubation of 20 μM monomeric actin in a medium containing 4 μg component I of botulinum C2 toxin, 0.1 mM NAD, 0.45 mM ATP, 0.18 mM $CaCl_2$, 0.02 mM $MgCl_2$, 9.5 mM triethanolamine-HCl (pH 7.5), 1 mM dithiothreitol and 2.7 mM NaN_3 for 1 h at 37°C in a total volume of 1 ml. The amount of ADP-ribosylated actin was determined as described [16]. About 50% of the given actin was ADP-ribosylated. Gelsolin-actin complex was prepared from human platelet concentrate. Platelets were purified according to [17]. Gelsolin-actin complex was isolated as in [18] with some modifications [19]. The concentration of gelsolin-actin complex was determined according to [19].

2.2. *Experimental design*

Actin polymerization was followed by a 2.2–2.5-fold greater fluorescence intensity of polymeric actin vs that of monomeric actin [15]. 5% of fluorescently labeled actin was copolymerized with unmodified actin. The excitation wavelength was 480 nm, and the fluorescence intensity was measured at 540 nm. Changes in fluorescence intensities were evaluated in terms of concentrations of monomeric or polymeric actin. Fluorescence intensities of monomeric or polymeric actin were calibrated by measuring those of a dilution series of monomeric or polymeric actin. Fluorescence samples were prepared by mixing buffer A, a salt solution (10 mM $MgCl_2$, 500 mM KCl) and various protein solutions. A 4 μM polymeric actin solution was obtained by addition of 100 mM KCl and 2 mM $MgCl_2$ to monomeric actin. Actin filaments capped at the barbed ends were formed by polymerizing 4 μM actin onto 50 nM gelsolin-actin complex in the presence of 100 mM KCl and 2 mM $MgCl_2$. The solutions were mixed in a ratio such that the final composition of the samples was 100 mM KCl, 2 mM $MgCl_2$, 0.18 mM $CaCl_2$, 0.45 mM ATP, 4.5 mM triethanolamine-HCl (pH 7.5), 2.7 mM NaN_3 and the desired concentrations of ADP-ribosylated actin, monomeric actin, polymeric actin or gelsolin-capped actin filaments. In every case, the salt solution and buffer A were first combined and then ADP-ribosylated actin, monomeric actin and finally polymeric actin or gelsolin-capped filaments were added. All experiments were carried out at 37°C.

3. RESULTS

3.1. *Effect of ADP-ribosylated actin on polymerization of barbed and pointed ends of actin filaments*

1 μM monomeric actin was polymerized onto 0.5 μM polymeric actin in the presence of various concentrations of ADP-ribosylated actin. The time course of polymerization is depicted in fig.1. The rate of polymerization and the final concentration of polymerized actin were decreased by ADP-ribosylated actin. In order to test the specificity of the effect of ADP-ribosylated actin for the two ends of actin filaments, actin polymerization onto the pointed ends was investigated in the presence of ADP-ribosylated actin. Actin filaments capped at the barbed ends were prepared by combining gelsolin-actin complex with monomeric actin. Monomeric actin was polymerized onto the pointed ends of these gelsolin-capped filaments in the presence of various concentrations of ADP-ribosylated actin. The results depicted in fig.2 demonstrate that even 1 μM ADP-ribosylated actin neither retarded polymerization at the pointed ends nor decreased the final concentration of polymerized actin. Thus, ADP-ribosylated actin did not affect the pointed ends. Retardation of nucleated actin polymerization and the decrease in final polymeric actin concentration depicted in fig.1 must be attributed to specific inhibition by ADP-ribosylated actin of the actin assembly at the barbed ends. The results suggest that ADP-ribosylated actin caps the barbed ends of actin filaments to inhibit monomer binding at these ends.

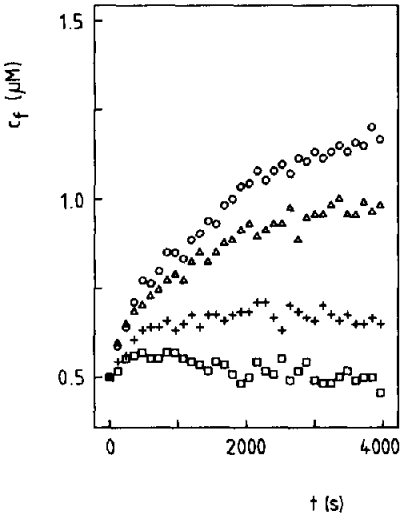

Fig.1. Nucleated polymerization of actin in the presence of ADP-ribosylated actin. c_f, concentration of polymerized actin. 1 μM monomeric actin was added to 0.5 μM polymeric actin in the presence of the following concentrations of ADP-ribosylated actin: (○) 0 μM, (△) 0.2 μM, (+) 0.8 μM, (□) 2.0 μM.

3.2. *Effect of ADP-ribosylated actin on monomer concentration of actin*

Capping activity of ADP-ribosylated actin was investigated by its effect on the concentration of monomers which coexist with filaments [20]. Under the given experimental conditions (100 mM

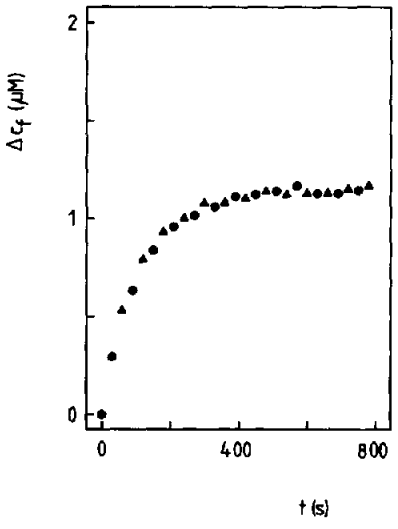

Fig.2. Polymerization onto gelsolin-capped actin filaments in the presence of ADP-ribosylated actin. Δc_f, change in concentration of polymerized actin. Gelsolin-capped filaments, which were prepared by combining 0.1 μM gelsolin-actin complex with 2 μM monomeric actin, were added to 2 μM monomeric actin in the presence of the following concentrations of ADP-ribosylated actin: (●) 0 μM, (▲) 1 μM.

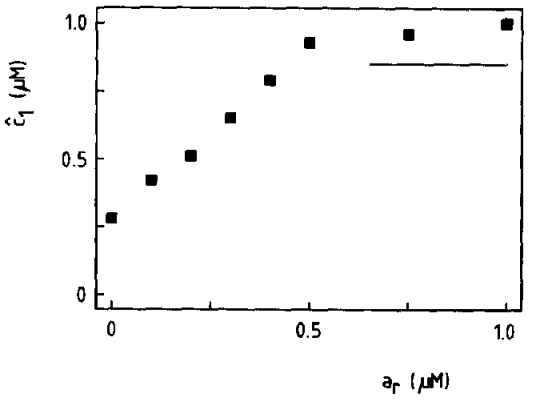

Fig.3. Plot of the final steady-state monomer concentration c_1 vs the concentration of ADP-ribosylated actin a_r. The monomer concentration was determined following nucleated polymerization of actin (0.5 μM polymeric actin plus 1.0 μM monomeric actin). (——) Critical concentration of the pointed end.

KCl, 2 mM $MgCl_2$, pH 7.5, 37°C) actin filaments treadmill, i.e. the barbed ends polymerize while the pointed ends depolymerize, leading to a flux of subunits along actin filaments from the barbed to the pointed ends [14]. Thus, capping proteins which bind to the barbed ends and inhibit polymerization at these ends are expected to increase the monomer concentration, since monomer consumption at the barbed ends is inhibited, however, monomers are still produced by depolymerizing pointed ends [19,20]. In fig.3 a plot of the monomer concentration reached at the final stage of nucleated polymerization vs concentration of ADP-ribosylated actin is presented. Maximally about 1 μM actin was in the monomeric state. Fig.2 shows that on addition of 2 μM monomeric actin to gelsolin-capped filaments 1.1–1.2 μM actin polymerizes onto the pointed ends of gelsolin-capped filaments while 0.8 or 0.9 μM actin remains in the monomeric state. Thus, the critical monomer concentration of the pointed ends of gelsolin-capped filaments is about 0.85 μM. This value corresponds approximately to the maximal monomer concentration reached by addition of ADP-ribosylated actin. Furthermore, the plot in fig.3 displays a hyperbolic shape which is typical for capping proteins [20].

4. DISCUSSION

In this paper, we have demonstrated that non-muscle liver actin which is ADP-ribosylated by botulinum C2 toxin exhibits capping activity similar to that of skeletal muscle actin ADP-ribosylated by *Perfringens* iota toxin. Thus, the present data suggest a likely explanation for the cytopathic effects to be, for example, the disorganization of the microfilament network observed after treatment of intact cells with botulinum C2 toxin [9]. ADP-ribosylation by botulinum C2 toxin converts actin into a capping protein which blocks polymerization at the barbed ends of actin filaments but does not affect pointed ends. During the course of actin treadmilling, capped actin filaments depolymerize at the pointed end. The released G-actin, which is a substrate of the toxin, accumulates since ADP-ribosylated actin is not able to polymerize. Finally, all these events cause destruction of the microfilament network.

In addition to botulinum C2 toxin, other microbial toxins such as *Perfringens* iota toxin [16], *C. spiroforme* toxin [21] and an ADP-ribosyltransferase produced by *C. difficile* [22] belong to the novel class of actin-modifying ADP-

ribosyltransferases. Recent reports indicate that these agents most likely modify actin at an identical amino acid [21–24]. Thus, it is feasible that all these ADP-ribosylating toxins affect actin-dependent cellular functions by capping of actin filaments and removal of actin from the polymerizable pool.

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