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# Snake venom cardiotoxin induces G-actin polymerization

Yee-Hsiung Chen and Sin-Tak Chu

*Institute of Biochemical Sciences, College of Sciences, National Taiwan University, and Institute of Biological Chemistry, Academia Sinica, Taipei (Taiwan, China)*

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**Snake venom cardiotoxin showed the ability to induce polymerization of G-actin from rabbit skeletal muscle in a low ionic strength buffer composed of 0.2 mM $CaCl_2$/0.2 mM ATP/0.5 mM mercaptoethanol/2.0 mM Tris-HCl, pH 8.0. The activity was enhanced greatly when 0.4 mM $MgCl_2$ was present in the buffer and could be inhibited if G-actin was preincubated with deoxyribonuclease I. Furthermore, the DNAase could also partially depolymerize actin polymer previously formed by the interaction of G-actin with the toxin.**

Snake venom cardiotoxin is a basic globular protein [1]. It shows a variety of biological activities toward cells of different origins [2]. It is non-neurotoxic but can cause cardiac arrest, muscle contraction, membrane depolarization and cytolysis [2]. Thus, the toxin has been described also as a cytotoxin, a direct lytic factor, a membrane-active polypeptide and a membrane-disruptive polypeptide [3–5]. Despite many descriptions of the effects of the toxin on experimental preparations [2], its mechanism of action is far from clear. In this communication, we demonstrate that the toxin is able to induce actin polymerization under conditions in which actin is normally in the monomeric form.

Actin is in the monomeric form (G-actin) in a low ionic strength buffer composed of 0.2 mM $CaCl_2$/0.4 mM $MgCl_2$/0.2 mM ATP/0.5 mM mercaptoethanol/2.0 mM Tris-HCl, pH 8.0 [6]. The process of actin polymerization can be monitored by measuring the increase of either viscosity or absorbance of the actin solution as filaments (F-actin) are formed [7–9]. Fig. 1 shows the actin polymerization induced by the toxin. The viscosity or the absorbance of the G-actin solution (500 $\mu$g/ml) increased rapidly when 2.3 $\mu$M toxin was added. After 3 min of incubation, the viscosity or the absorbance reached a maximum level. Under similar conditions, but in the absence of $Mg^{2+}$, the polymerization was greatly reduced. The polymerization was apparently a function of the amount of toxin added (Fig. 2).

Deoxyribonuclease I (DNAase I) is known to bind tightly to G-actin [11]. We found that preexposure of G-actin to DNAase I suppressed the polymerization induced by the toxin. Very slight polymerization could be induced by the toxin when G-actin and DNAase I, in equimolar concentrations, were preincubated in the low ionic strength buffer (Fig. 3). On the other hand, the DNAase could partially depolymerize the actin polymer previously formed by the interaction of G-actin and the toxin (Fig. 3).

In conclusion, the characteristics of the toxin-induced actin polymerization are very similar to the actin polymerization induced by high ionic strength KCl, as reported previously [12]. Whether the ability of the toxin to induce actin polymeriza-

Correspondence: Y.-H. Chen, Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan, China.

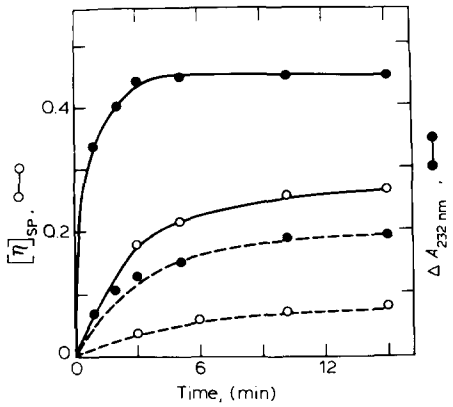


Fig. 1. Cardiotoxin-induced G-actin polymerization. Actin was prepared from rabbit skeletal muscle [7]. Cardiotoxin was purified from Formosan Cobra/*Naja naja atra* venom [10]. At 24° C, 500 μg actin in 1 ml of a low ionic strength buffer was incubated with the toxin at a final concentration of 2.3 μM. The buffer was composed of 0.2 mM $CaCl_2$/0.4 mM $MgCl_2$/0.2 mM ATP/0.5 mM mercaptoethanol/2.0 mM Tris-HCl, pH 8.0. Actin polymerization was monitored by measuring the increase of either the viscosity (○) or the absorbance of the solution (●) as reported previously [8,9]. Solid and dashed lines represent the polymerization in the presence and in the absence of 0.4 mM $MgCl_2$, respectively. A control experiment showed no evidence of polymerization in the absence of added toxin.

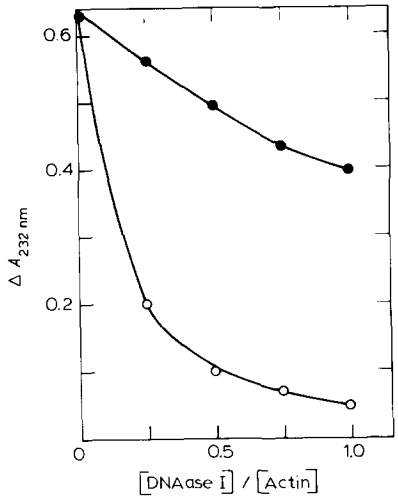


Fig. 3. DNAase I inhibited G-actin polymerization by cardiotoxin (○) and depolymerized actin polymer previously formed by the toxin (●). In the first case, G-actin and various amounts of DNAase I were incubated at 24° C for 5 min in the low ionic strength buffer as described in Fig. 1. Cardiotoxin was then added and the reaction mixture was incubated at the same temperature for another 10 min before the extent of polymerization was determined. In the second case, the actin polymer, which was previously formed by the incubation of G-actin and the toxin at 24° C for 10 min, was incubated with DNAase I at the same temperature for 5 min and the extent of actin polymerization was measured. In all experiments, the final concentrations were 8.4 μM for the toxin and 100 μg/ml for G-actin. The molar ratio of DNAase I to G-actin varied from 0 to 1.

tion plays any role in disturbing cellular activity awaits future study.

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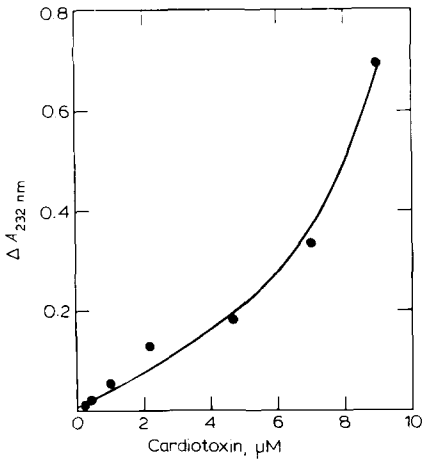


Fig. 2. Actin polymerization induced by various amounts of cardiotoxin. Cardiotoxin and 100 μg G-actin were incubated at 24° C for 15 min in 1.0 ml of the low ionic strength buffer as described in Fig. 1.

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