

ADP-ribosylation of cell membrane proteins by staphylococcal α -toxin and leukocidin in rabbit erythrocytes and polymorphonuclear leukocytes

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Staphylococcal α -toxin resulted in ADP-ribosylation of the 37 and 41 kDa proteins of a membrane preparation from rabbit erythrocytes. In the presence of 100 μ M GTP, the toxin ADP-ribosylated proteins of 54 and 59 kDa and potentiated ADP-ribosylation of the 37 and 41 kDa forms. GTP had no effect on ADP-ribosylation of membrane proteins in the absence of α -toxin. Incubation of a membrane preparation of rabbit polymorphonuclear leukocytes with the S and F components of staphylococcal leukocidin resulted in ADP-ribosylation of the 37 and 41 kDa proteins, respectively. Furthermore, the 37, 41, 54 and 59 kDa proteins were ADP-ribosylated by leukocidin in the presence of GTP. The ADP-ribosylation of these proteins was observed to be dependent on the incubation time and toxin dose and was abolished by prior boiling. Addition of agmatine did not attenuate ADP-ribosylation of these proteins. These results demonstrate that staphylococcal α -toxin and leukocidin possess ADP-ribosyltransferase activities which are potentiated by GTP and suggest that ADP-ribosylation reactions are responsible for development of the cytolytic activities of these staphylococcal toxins.

ADP-ribosylation; Toxin, α -; Leukocidin; (Polymorphonuclear leukocyte, Erythrocyte)

1. INTRODUCTION

Staphylococcal α -toxin is produced as a water-soluble 34 kDa polypeptide chain by most strains of *Staphylococcus aureus* and is one of the strongest bacterial hemolytic toxins known. In previous papers, we reported that after binding of α -toxin to the receptor of the erythrocyte membrane, the toxin firstly induces the perturbation of the erythrocyte membrane through stimulation of membrane-associated phospholipase A₂ and C activities [1,2].

Staphylococcal leukocidin consists of two protein components designated S (31 kDa) and F (32 kDa) [3,4]. Neither component shows leukocytolytic activity when tested alone, however, in combination they act synergistically to induce

leukocytolysis [4]. In previous studies, we reported that component S specifically bound to ganglioside GM₁ of the rabbit polymorphonuclear leukocyte (leukocyte) membrane [5,6] and subsequently activated phospholipase A₂, resulting in the stimulation of arachidonic acid metabolism [7]. In our recent experiments, leukocidin also stimulated the phospholipase C activity of phosphatidylinositol metabolism in rabbit leukocytes [8].

Several bacterial toxins modify the functions in eukaryotic cells by ADP-ribosylation of cellular target proteins. One of the authors of the current article and Honjo et al. [9] discovered that diphtheria toxin possesses ADP-ribosyltransferase activity catalyzing ADP-ribose transfer from NAD to elongation factor-2, thereby inhibiting protein synthesis. Cholera and pertussis toxins also ADP-ribosylate and modify GTP-binding proteins involved in the transmembrane signal transduction system [10].

Here, we show that the staphylococcal cytolytic

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toxins, α -toxin and leukocidin, possess ADP-ribosyltransferase activities that catalyze the transfer of the ADP-ribose moiety from NAD predominantly to target-cell membrane proteins.

2. MATERIALS AND METHODS

2.1. Materials

GTP, NAD, dithiothreitol (DTT) and agmatine were purchased from Sigma, GTP γ S from Boehringer Mannheim and [adenylate- 32 P]NAD (250 Ci/mmol) from ICN. Other chemicals used were of reagent grade.

2.2. Toxins

Staphylococcal α -toxin was purified from culture media of *S. aureus* Wood 46 strain according to Kato [11]. Staphylococcal leukocidin was purified from *S. aureus* V8 strain as described by Noda et al. [4].

2.3. ADP-ribosylation

Assay samples (total volume 300 μ l) contained 1 μ M [32 P]-NAD (2 μ Ci), 10 mM thymidine, 1 mM EDTA, 5 mM MgCl₂, 2 mM DTT, 100 mM Tris-HCl (pH 7.5) and other additions as indicated. Rabbit erythrocyte membranes (25 μ g protein/assay) were incubated with α -toxin (5 μ g). Rabbit leukocyte membranes (50 μ g protein/assay) were incubated with α -toxin (5 μ g) or leukocidin (5 μ g S and F components). After incubation at 37°C for 40 min, 2 ml cold 7.5% trichloroacetic acid and 10 μ g bovine serum albumin were added, and samples kept on ice for 30 min. Precipitated proteins were pelleted by centrifugation and dissolved in 1% SDS/5% mercaptoethanol (60°C, 10 min). Samples were subjected to electrophoresis in 12% polyacrylamide gels by the method of Laemmli [12]. Gels were exposed to Kodak X-Omat AR film.

2.4. ADP-ribosylagmatine assay

The formation of ADP-ribosylagmatine was determined as described by Moss et al. [13,14]. Assay samples contained 50 mM potassium phosphate (pH 7.5), 5 mM MgCl₂, 100 μ M GTP, 100 μ M [adenine-U- 14 C]NAD ($\sim 6 \times 10^4$ cpm), 10 mM agmatine, ovalbumin (0.1 mg/ml), and other additions as indicated (total volume 300 μ l). Reactions were initiated with toxins. After 60 min at 30°C, two 50- μ l samples were transferred to columns of AG1-X2 which were washed four times with 1.25 ml water. Eluates containing [adenine-U- 14 C]ADP-ribosylagmatine were collected for radioassays.

3. RESULTS AND DISCUSSION

As shown in fig.1, when a membrane preparation (25 μ g protein) from rabbit erythrocytes was incubated without (fig.1, lane 1) or with staphylococcal α -toxin (5 μ g) in the presence of [32 P]NAD and 5 mM MgCl₂ at 37°C for 40 min, the 37 and 41 kDa proteins were ADP-ribosylated by α -toxin (fig.1, lane 2). The intensity of radioactivity incorporated into the 37 kDa protein was stronger vs

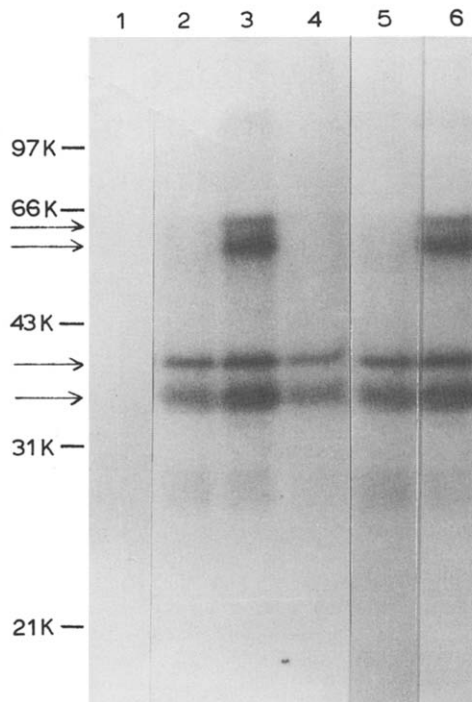


Fig.1. ADP-ribosylation of rabbit erythrocyte membrane proteins by staphylococcal α -toxin. A rabbit erythrocyte membrane preparation (25 μ g protein) and 1 μ M [32 P]NAD (2 μ Ci) were incubated without (lane 1) or with 5 μ g staphylococcal α -toxin (lanes 2-6) in the presence of 100 μ M GTP (lanes 3,6), 100 μ M GTP γ S (lane 4), or 10 mM agmatine (lanes 5,6) for 40 min at 37°C as described in section 2. The autoradiogram of the SDS-polyacrylamide gel (12%) of the labeled proteins is shown. Arrows denote positions of ADP-ribosylation substrates of 37, 41, 54 and 59 kDa.

that of the 41 kDa protein. Addition of 100 μ M GTP potentiated ADP-ribosylation of the 37 and 41 kDa proteins and ADP-ribosylated proteins of 54 and 59 kDa which were not ADP-ribosylated in the absence of GTP (fig.1, lane 3). The intensity of ADP-ribosylation for the 37 kDa protein was similar to that of the 54 kDa protein, which was stronger than those of the 41 and 59 kDa proteins. Although ADP-ribosylation of the 37 and 41 kDa proteins was caused by α -toxin, the 54 and 59 kDa proteins were not ADP-ribosylated in the presence of 100 μ M GTP γ S, a nonhydrolyzable analog of GTP, and 5 mM MgCl₂ (fig.1, lane 4).

GTP γ S-dependent inhibition of bacterial toxin-catalyzed ADP-ribosylation is a characteristic property of the GTP-binding proteins, G_s, G_i and G_b (botulinum toxin substrates) [15,17]. Therefore,

it is strongly suggested that the 54 and 59 kDa forms are GTP-binding proteins themselves or components involved in the GTP-dependent regulatory system. ADP-ribosylation of membrane proteins caused by α -toxin was not affected by the addition of other nucleotides such as GDP, ATP, ADP, CTP, CDP, UTP and UDP (1 μ M mM). The addition of 10 mM agmatine, an arginine analog, lacking the carboxyl group, had no effect on ADP-ribosylation of membrane proteins induced by α -toxin in the absence (fig.1, lane 5) or presence of 100 μ M GTP (fig.1, lane 6). When agmatine was incubated with α -toxin in the presence of [adenine-U- 14 C]NAD at 30°C for 60 min, no [adenine-U- 14 C]ADP-ribosylagmatine was detectably formed (table 1). These results suggest that α -toxin differs in catalytic properties from cholera toxin and is not an NAD:arginine ADP-ribosyltransferase [14,18]. α -Toxin ADP-ribosylated specific membrane proteins of molecular masses 37, 41, 54 and 59 kDa in a dose- and time-dependent manner (not shown). Prior boiling of α -toxin completely abolished both hemolytic and ADP-ribosyltransferase activities.

As shown in fig.2, when a membrane preparation (50 μ g protein) from rabbit polymorphonuclear leukocytes was incubated without (fig.2, lane 1) or with staphylococcal leukocidin (5 μ g of S and F components) in the presence of [32 P]NAD and 5 mM MgCl₂ at 37°C for 40 min, the 37 and 41 kDa membrane proteins were ADP-ribosylated by leukocidin (fig.2, lane 2). In the presence of 100 μ M GTP, leukocidin ADP-ribosylated the 37, 41, 54 and 59 kDa forms (fig.2, lane 3). ADP-ribosylation of the 54 and 59 kDa species did not occur in the presence of 100 μ M GTP γ S (fig.2, lane 4). The S and F components ADP-ribosylated the 37 and 41 kDa membrane proteins, respectively (fig.2, lanes 5,8). However, ADP-ribosylation of the 54 and 59 kDa forms was not caused by these components when tested alone in the presence of GTP or GTP γ S (fig.2, lanes 6,7,9,10). ADP-ribosylation of the 37, 41, 54 and 59 kDa membrane proteins by leukocidin was found to take place in a dose- and time-dependent manner (not shown). Agmatine had no effect on ADP-ribosylation of these proteins by leukocidin. The formation of [adenine-U- 14 C]ADP-ribosyl-

Table 1

NAD:agmatine ADP-ribosyltransferase activity of bacterial toxins			
Additions		Formation of [adenine-U- 14 C]-ADP-ribosylagmatine (pmol/assay)	
		- DTT	+ 20 mM DTT
None		< 10	< 10
Cholera toxin A subunit (1 μ g)		40	1320
α -Toxin (1 μ g)		< 10	< 10
(3 μ g)		< 10	< 10
(10 μ g)		< 10	< 10
Leukocidin			
S component	(1 μ g)	< 10	< 10
	(3 μ g)	< 10	10
	(10 μ g)	< 10	10
F component	(1 μ g)	< 10	< 10
	(3 μ g)	< 10	< 10
	(10 μ g)	< 10	< 10
S + S components	(1 μ g + 1 μ g)	< 10	10
	(3 μ g + 3 μ g)	< 10	20
	(10 μ g + 10 μ g)	< 10	20

NAD:agmatine ADP-ribosyltransferase activity was assayed as described in section 2

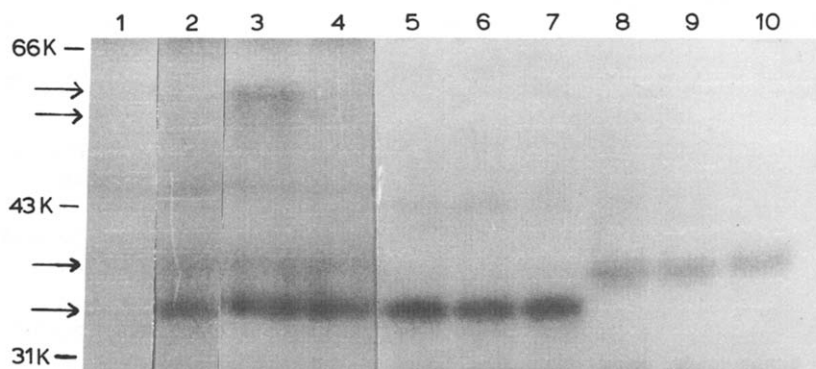


Fig.2. ADP-ribosylation of rabbit leukocyte membrane proteins by staphylococcal leukocidin. A rabbit leukocyte membrane preparation (50 µg protein) and 1 µM [32 P]NAD (2 µCi) were incubated without (lane 1) or with 5 µg S and F components of leukocidin (lanes 2-4), 5 µg S component (lanes 5-7), or 5 µg F component (lanes 8-10) in the presence of 100 µM GTP (lanes 3,6,9), or 100 µM GTP γ S (lanes 4,7,10) for 40 min at 37°C as described in the text. The autoradiogram of the SDS-polyacrylamide gel (12%) of the labeled proteins is shown. Arrows denote the positions of ADP-ribosylation substrates of 37, 41, 54 and 59 kDa.

agmatine was not observed when agmatine was incubated with leukocidin in the presence of [adenine- 14 C]NAD at 30°C for 60 min (table 1). Prior boiling of leukocidin abolished ADP-ribosyltransferase activity. It is clear that staphylococcal α -toxin and leukocidin possess ADP-ribosyltransferase activity but they may differ in catalytic properties from cholera toxin, and NAD:arginine ADP-ribosyltransferase. ADP-ribosylation of the 37, 41, 54 and 59 kDa membrane proteins of cells such as ESK cells that are insensitive to α -toxin and leukocidin, was not observed. Therefore, it is suggested that ADP-ribosylation of the membrane proteins of 37, 41, 54 and 59 kDa, resulting from α -toxin or leukocidin, may play an important role in cytolysis by these staphylococcal cytolytic toxins. Further detailed study on the role of ADP-ribosylation of these membrane proteins by staphylococcal toxins is now underway.

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