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Toxicon 48 (2006) 1083–1094

TOXICON

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Structural and functional characterization of a recombinant sticholysin I (rSt I) from the sea anemone *Stichodactyla helianthus*

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Received 23 June 2006; received in revised form 31 August 2006; accepted 1 September 2006
Available online 12 September 2006

Abstract

Sticholysins I and II (Sts I and II) are two potent cytolysins from the sea anemone *Stichodactyla helianthus*. These isoforms present 13 substitutions, with three non-conservative located at the N-terminus. St II is considerably more hemolytic than St I in human red blood cells, a result explained by the smaller number of negatively charged groups present at St II's N-terminus. In the present work, we have obtained a recombinant St I (rSt I), differing from the wild type in a single amino acid residue (E16Q). This pseudo-wild type is structurally similar to St I and shows a similar capacity to interact with and form pores in model membranes. This was assessed by the intrinsic fluorescence increase in the presence of liposomes, their adsorption to bilayers (measured by SPR), their concentration at the air–water interface, their interaction with lipid monolayers and their capacity to promote the release of carboxyfluorescein entrapped in liposomes. In spite of these similarities, rSt I presents a larger hemolytic activity in human red blood cells than St I, being intermediate in activity between Sts I and II. The results obtained in the present work emphasize that even the change of one single E by Q at the N-terminal segment may modify the toxin HA and show that this functional property is the most sensitive to subtle changes in the protein primary structure.

Keywords: Actinoporins; Sticholysin; Pore-forming toxins; Surface plasmon resonance; Lipid monolayer

1. Introduction

Sticholysins I and II (Sts I and II) are two closely related pore-forming toxins from the sea anemone *S. helianthus* (Huerta et al., 2001; Lanio et al., 2001).

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0041-0101/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2006.09.004

In spite of their structural similarity (98% similarity), St II is considerably more active than St I in disrupting human red blood cells (RBC) (Martinez et al., 2001). This difference has been related to changes in the net charge of the N-terminal sequence, segment that plays a fundamental role in pore formation, as has been demonstrated for the homologous actinoporin EqT II (Hong et al., 2002; Kristan et al., 2004; Gutierrez-Aguirre et al., 2004). Comparison of the N-terminal sequence of Sts I and II reveals four substitutions, three of them non-conservative and one conservative: Glu2 (in St) to Ala, Asp9 to Ala, Glu16 to Gln, and Gly23 to Glu (Lanio et al., 2001). These substitutions render the N-terminal region of St I more negatively charged than that of St II. This, together with a slightly more compact structure (Martinez et al. 2001), could hamper its insertion into negatively charged human RBC membranes.

De los Rios et al. (2000) produced a St II recombinant variant that included a six histidine cluster at the N-terminal. This St II variant showed a reduced hemolytic activity and decreased pore formation efficiency in liposomes, in spite of an efficient binding to lipidic bilayers (Pazos et al., 2003). These results pointed to the relevance of the N-terminal on the toxin function. However, the presence of the histidine cluster at the N-terminal precluded a clear interpretation of the differences between St II and its modified variant. In order to further understand the N-terminal characteristics that favor St function and get insight on the source of the different behavior of St I and St II, we have obtained, by a recombinant procedure, a St I variant that, by replacing 16 glutamine by glutamic acid, has a primary structure very close to that of St I but with a reduced net charge in the N-terminal sequence. This change renders the charge of this segment intermediate between those of the two native isoforms. rSt I behaves as St I in several model systems: pore formation in bilayers, interaction with lipidic monolayers and bilayers, and adsorption to air/water interface. In spite of this, the pseudo-wild type toxin is significantly more lytic than St I.

2. Materials and methods

2.1. Cloning and expression of recombinant Sticholysin I (rSt I)

Total RNA from one specimen of the Caribbean Sea anemone *S. helianthus* was prepared with TRIzol reagent following the supplier's instructions (Gibco-BRL, NY, USA). Aliquots (0.5 μg) of this preparation were used as template for the synthesis of St I DNA coding sequence by RT-PCR, using the Access RT-PCR system (Promega, CA, USA) according to the manufacturer instructions. The oligonucleotide primers used for the amplification reaction were designed based on the reported St I DNA sequence (De los Rios et al., 2000): sense primer 5′-GGGCATATGTCCGAGCTCGCTGGCACCATTATTGAT-3′, and antisense primer 5′-GGGGGATCCTTAGCGTGAAATCTTAATTTGCAT-3′. For cloning purposes, the restriction sites for *Nde*I (forward primer) and *Bam*HI (reverse primer) enzymes were also included in the primers' sequence (underlined). RT-PCR products were purified from an agarose gel by using the QIAquick Gel extraction Kit (Qiagen, CA, USA) and cloned into the pMOS*Blue* plasmid according to the manufacture's protocol (pMOS*Blue* Blunt Ended Cloning Kit, Amersham Pharmacia Biotech, Uppsala, Sweden). Putative positive clones were selected by restriction analysis with appropriate enzymes and some of them were sequenced using T7 Sequencing TM Kit (Amersham Pharmacia Biotech, Uppsala, Sweden). A plasmid containing an open reading frame (ORF) of the correct size of St I DNA coding sequence was digested with *Nde*I and *Bam*HI. The 530 bp fragment, containing the recombinant St I ORF, was ligated into the pET-3a plasmid previously digested with the same enzymes, and the ligation reaction mixture was used to transform the TG1 strain of *Escherichia coli*. The resulting construction, designated as pET3a-rStI was employed for production of the recombinant protein.

Expression *of the recombinant protein. E. coli* strain BL21(DE3) pLys S transformed with pET3a-rStI plasmid was grown in LB medium supplemented with ampicillin (100 μg/mL) and chloranphenicol (34 μg/mL). Expression of the recombinant protein was induced in the middle log phase (OD_{600nm} = 0.4−0.8) by the addition of isopropyl-1Thiol-β-*D*-galactopinanoside (IPTG, 0.1 mM final concentration) and the culture was grown for 4 h at 37 °C. The cells were harvested by centrifugation, re-suspended in 0.02 M sodium phosphate buffer, pH 7, cooled on ice, and lysed by sonication in a *Soniprep150-MSE* sonicator cell disrupter (York, UK). The resulting suspension was centrifuged to separate the supernatant. The expressed protein was checked after each step by SDS–PAGE, according to Laemmli (1970).