Peptides 38 (2012) 1–7

Contents lists available at SciVerse ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

# Inhibition of emetic and superantigenic activities of staphylococcal enterotoxin A by synthetic peptides

Edward K. Maina[a], Dong-Liang Hu[a,b], Krisana Asano[a], Akio Nakane[a,*]

[a] Department of Microbiology and Immunology, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori 036-8562, Japan
[b] Laboratory of Zoonoses, Kitasato University School of Veterinary Medicine, Higashi 23-35-1, Towada, Aomori 034-8628, Japan

ARTICLE INFO

*Article history:*
Received 26 June 2012
Received in revised form 17 August 2012
Accepted 17 August 2012
Available online 28 August 2012

*Keywords:*
Staphylococcal enterotoxin
Superantigen
Emesis
Synthetic peptide
House musk shrew

ABSTRACT

*Staphylococcus aureus* is a major human pathogen producing different types of toxins. Enterotoxin A (SEA) is the most common type among clinical and food-related strains. The aim of the present study was to estimate functional regions of SEA that are responsible for emetic and superantigenic activities using synthetic peptides. A series of 13 synthetic peptides corresponding to specific regions of SEA were synthesized, and the effect of these peptides on superantigenic activity of SEA including interferon γ (IFN-γ) production in mouse spleen cells, SEA-induced lethal shock in mice, spleen cell proliferation in house musk shrew, and emetic activity in shrews were assessed. Pre-treatment of spleen cells with synthetic peptides corresponding to the regions 21–40, 35–50, 81–100, or 161–180 of SEA significantly inhibited SEA-induced IFN-γ production and cell proliferation. These peptides also inhibited SEA-induced lethal shock. Interestingly, peptides corresponding to regions 21–40, 35–50 and 81–100 significantly inhibited SEA-induced emesis in house musk shrews, but region 161–180 did not. These findings indicated that regions 21–50 and 81–100 of SEA are important for both superantigenic and emetic activities of SEA molecule while region 161–180 is involved in superantigenic activity but not emetic activity of SEA. These regions could be important targets for therapeutic intervention against SEA exposure.



## 1. Introduction

Staphylococcal enterotoxins (SEs) produced by *Staphylococcus aureus* possess superantigenic activity, they can directly bind to major histocompatibility complex class II molecules on antigen presenting cells. They stimulate a large population of T cells bearing specific variable β elements, and subsequently trigger release of pro-inflammatory cytokines from these cells, including interferon γ (IFN-γ), tumor necrosis factor α, interleukin-1 (IL-1), IL-2 and IL-6, and induce toxic shock syndrome [1,7,8,40,41]. In addition, SEs are recognized as the most common causative agents of human food poisoning worldwide [3,23,24,35] that results from consumption of foods containing sufficient amounts of preformed SEs. SE-induced food poisoning represents a considerable burden in terms of loss of working times productivity, hospital expenses, and economical losses in food industries, catering companies and restaurants [2,19,27]. Control of this disease is also of social and economic importance.

Many studies have been conducted on the nature of SEs and the molecular basis of the superantigenic activities of SEs has been extensively explored [10,21,29,34,36]. However, little progress has been made in studying the mechanism of toxins in staphylococcal food poisoning, and relation of superantigenic and emetic activities of these toxins are little known. Since the commonly used animal models such as mice, rats and rabbits do not display emetic reflex to these toxins [11,17], the delay of progress in these studies can be largely attributed to the lack of a suitable animal model that mimics the clinical features of SEs intoxication [8,11,17]. Our recent studies demonstrated that house musk shrews, a small animal model, show emetic response to peroral and intraperitoneal administration of SEs and they are suitable for studying the emetic activity of SEs [17,18]. To shed some light on the structural relation of superantigenic and emetic activities of SEA molecule, in this study we investigated regions of SEA which are responsible for superantigenic and emetic activity in house musk shrew using synthetic peptide approach. Our results suggested that the regions responsible for superantigenic and emetic activities of SEA are not identical because only some regions of SEA are responsible for both superantigenic and emetic activities.

*Abbreviations:* DSG, disuccinimidyl glutarate; GST, glutathione S-transferase; IFN, interferon; IL, interleukin; LPS, lipopolysachharide; SE, staphyococcal enterotoxin; TFA, trifluoroacetic acid; TSAT, tris-succinimidyl aminotriacetate.

* Corresponding author. Tel.: +80 172 39 5033; fax: +80 172 39 5034.
*E-mail address:* a27k03n0@cc.hirosaki-u.ac.jp (A. Nakane).

0196-9781/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.peptides.2012.08.013

**Table 1**
Primary sequences of synthesized peptides.

| Peptide | Corresponding SEA fragment | Primary sequence |
|---|---|---|
| A-1 | 1–20 | SEKSEEINEKDLRKKSELQG |
| A-2 | 21–40 | TALGNLKQIYYYNEKAKTEN |
| A-3 | 35–50 | KAKTENKESHDQFLQH |
| A-4 | 41–60 | KESHDQFLQHRILFKGFFTD |
| A-5 | 61–80 | HSWYNDLLVDFDSKDIVDKY |
| A-6 | 81–100 | KGKKVDLYGAYYGYQCAGGT |
| A-7 | 101–120 | PNKTACMYGGVTLHDNNRLT |
| A-8 | 121–140 | EEKKVPINLWLDGKQNTVPL |
| A-9 | 141–160 | ETVKTJKKNVTVQELDLQAR |
| A-10 | 161–180 | RYLQEKYNLYNSDVFDGKVQ |
| A-11 | 181–200 | RGLIVFHTSTEPSVNYDLFG |
| A-12 | 201–220 | AQGQYSNTLLRIYRDNKSIN |
| A-13 | 221–233 | SENMHIDIYLYTS |

## 2. Materials and methods

### 2.1. Preparation of SEA

SEA was expressed as recombinant SEA by the *Escherichia coli* expression system as described previously [17]. Briefly, genomic DNA was isolated from *S. aureus* FRI 722. To construct the SEA expression plasmid, the *sea* gene was amplified by PCR and the amplified DNA fragments were digested with *Eco*RI and *Bam* HI. The fragments were inserted into the pGEX-6p-1 (Amersham Pharmacia Biotech), a glutathione S-transferase (GST) fusion expression vector. The resultant plasmid named pKAX1 was then introduced into *E. coli* DH5α cells. Expression and purification of GST-fused SEA and removal of the GST tag were performed as described previously [16]. The purified proteins were quantified by Bradford assay (BIO-RAD) and analyzed by sodium dedocyl sulfate polyacrylamide gel electrophoresis. This recombinant protein was shown to posses the same biological activities as native toxin [17,28].

### 2.2. Synthetic peptides of SEA

According to the amino acid sequence of SEA (Accession number: P0A0L2.1) reported previously [5,34], short peptides (13–20 amino acid residues in length) corresponding to the fragment of the primary sequence of SEA were synthesized with a Shimadzu PSSM-8 automated peptide synthesizer (Shimadzu, Kyoto, Japan) by standard 9-fluorenylmethoxycarbonyl method. The monomer was dissolved in dimethylsulfoxide and pH was adjusted to 9 with triethyl-amine. Cross-linking reactions were performed using disuccinimidyl glutarate (DSG) for obtaining a dimeric peptide (RGD-dimer) and tris-succinimidyl aminotriacetate (TSAT) for a trimeric peptide (RGD-trimer). The monomer was reacted with 1/2 molar ration of DSG or with 1/3 molar ratio of TSAT for 16 h at room temperature. The peptides were cleaved from the resins with trifluoroacetic acid (TFA) cocktail solution (125 ml TFA, 0.25 ml $H_2O$, 0.375 g phenol, 0.125 ml ethanedithiol and 0.25 ml thioanisole). HPLC analysis showed the purity of each peptide was >96%. The amino acid sequences of the peptides are presented in Table 1. Peptides were measured aseptically and dissolved in distilled water, mixed and kept at −81° C untill use.

### 2.3. Animals

Specific pathogen-free C57BL/6 mice (6–8 weeks old) and house musk shrews (*Suncus murinus*, Jic-SUN) were purchased from Clea Japan, Inc., Tokyo, Japan. Mice were maintained under specific pathogen-free conditions at the Institute for Animal Experimentation, Hirosaki University Graduate School of Medicine. Animals were kept on a cycle consisting of 12 h of light and 12 h of darkness, and food and water were available at all times. Shrews were housed in plastic cages, and fed on commercial *S. murinus* formula (Clea Japan) as well as being provided water ad libitum. All animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of Hirosaki University.

### 2.4. Spleen cell culture and IFN-γ production

Spleens were removed aseptically from naïve mice, and the spleen cells were obtained by squeezing the organs in RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) and filtered through stainless steel mesh (100–200 μm). After lysis of erythrocytes with 0.83% $NH_4Cl$, the cells were washed 3 times and then resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum. To investigate superantigenic activity of SEA and synthetic peptides, mouse spleen cells were placed in a 24-well tissue culture plate at a density of $1 \times 10^6$ cells per well in the presence of SEA or each peptide. Cells were incubated at 37 °C for 72 h in a humidified 5% $CO_2$ atmosphere. IFN-γ production in the cell culture supernatants was quantified by a double-sandwich ELISA as previously described [25]. To investigate the effect of synthetic peptides on SEA-induced IFN-γ production, cell culture was incubated with each peptide (100 μg/ml) for 1 h and thereafter SEA (1 μg/ml) was added. The cells were incubated for 72 h and IFN-γ titers in the cell culture supernatants were quantified as above.

### 2.5. Cell proliferation assay

Spleens were removed aseptically from naïve house musk shrews and spleen cells were obtained by squeezing the organs in RPMI 1640 medium and filtered through stainless steel mesh (100–200 μm). After lysis of erythrocytes with 0.83% $NH_4Cl$, the cells were washed 3 times and then resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum, 10 μM sodium pyruvate (Wako Pure Chemical, Osaka, Japan) and 50 μM 2-mercaptoethanol (Wako). Cells were placed in round-bottomed 96-well microplates at a density of $1 \times 10^6$ cells per well and incubated for 48 h with 1 μg/ml SEA, 100 μg/ml synthetic peptides or peptide/SEA. The cells were then pulsed for 24 h with 25 kBq/well of [$^3$H] thymidine (ICN Biomedicals, PA) and then harvested on glass fiber filters. The amount of incorporated [$^3$H] thymidine was measured using liquid scintillation counting. Each sample was tested in duplicate in at least two separate assays.

### 2.6. Lethal toxic shock model

For SEA-induced toxic shock, lipopolysaccharide (LPS)-potentiated mouse lethality model was employed [39]. Mice were injected intraperitoneally with 10 μg SEA diluted in 0.2 ml PBS, or a mixture of 10 μg SEA and 200 μg each synthetic peptide in 0.2 ml PBS. Then, they were intraperitoneally injected with 80 μg LPS from *E. coli* O111:B4 (Sigma–Aldrich) 4 h later and their lethality was recorded over 72 h-period. Controls included animals given SEA or LPS alone. Two independent experiments with ten to fifteen mice in each group were carried out.

### 2.7. Emetic assay

House musk shrews were intraperitoneally injected with 100 μg SEA, or a mixture of 100 μg SEA and 200 μg each synthetic peptide. Emesis of animals were observed for 3 h after injection. Numbers of vomiting (vomiting episode is defined by retching with the expulsion of saliva and gastric juices) and any behavioral changes were real-time recorded by digital video camera.