Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Identification of matrix metalloproteinase 9-interacting sequences in staphylococcal superantigen-like protein 5

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ARTICLE INFO

Article history:
Received 9 February 2018
Accepted 15 February 2018
Available online xxx

Keywords:
Staphylococcal superantigen-like protein 5
MMP-9

ABSTRACT

Staphylococcal superantigen like 5 (SSL5) is an exotoxin produced by *S. aureus* and has a strong inhibitory effect on MMP-9 enzymatic activity. However, the mechanism of inhibition remains unclear. We sought to identify the responsible regions of SSL5 for the interaction with MMP-9 by comparing a series of domain swap and deletion mutants of SSL5. Binding analyses revealed that SSL5 had two regions for binding to MMP-9 catalytic domain, β1-3 region (^{25}SKELKNVTGY RYSKGGKHYL IFDKNRKFTR VQIFGK^{60}) in N-terminal half and α4β9 region (^{138}KELDFKLRQY LIQNFDLYKK FPKDSKIKVI MKD^{170}) in C-terminal half. The collagen binding domain and zinc-chelating histidine residues of MMP-9 were not essential for the specific binding to SSL5. The domain swap mutants of SSL5 that conserved β1-3 but not α4β9 region inhibited the gelatinolysis by MMP-9, and the mutant of SSL7 that substituted β1-3 region to that of SSL5 acquired the binding and inhibitory activity. Furthermore, the polypeptide that harbored β1-3 region of SSL5 inhibited gelatinolysis by MMP-9. Taken together, SSL5 inhibits the MMP9 activity through binding to the catalytic domain, and the β1-3 region is responsible for the inhibition of proteolytic activity of MMP-9.

1. Introduction

Staphylococcal superantigen-like proteins (SSLs) is a family of the exotoxin comprised of 14 SSLs that exhibit relatively low homology. SSLs are composed of N-terminal oligonucleotide/oligosaccharide-binding (OB)-fold and C-terminal β-grasp as similar as staphylococcal superantigens (SAgs) [1,2]. However, SSLs never ligate T cell antigen receptor and major histocompatibility complex and do not function as SAgs. Some SSLs have been revealed to bind to proteins that play a role in immunity such as IgA and C5 [3], IgG [4,5] and CXCL12 [6] and toll like receptor 2 (TLR2) [7,8]. SSLs target to physiological molecules, such as prothrombin [9], phosphatidylserine [10] and tenascin C [11]. SSL5 is a pleiotropic exotoxin as is reported to bind to P-selectin glycoprotein ligand-1 [12], chemoattractant and chemokine receptors [13] and platelet receptors (αIIbβ3, GPIb and GPVI) [14,15]. SSL5 has been reported to bind to matrix metalloproteinase 9 (MMP-9) with high affinity (KD = 1.9 nM^{-1}), and inhibits proteolytic activity of MMP-9 in a non-competitive manner and interferes the invasion of neutrophils and cleavage of proIL-8 [16,17]. So far SSL5 never shares homology with known MMP inhibitory proteins and the mechanism of the inhibitory effect of SSL5 on MMP-9 protease activity remains unclear.

In this study we sought to identify the responsible sequences of SSL5 for interacting with MMP-9 by examining a series of domain swap and deletion mutants of SSL5 with their binding to MMP-9 and the inhibition of its proteolytic activity. We also examined the requirement of zinc anion and collagen binding domain (CBD) of MMP-9 for their interaction.

Abbreviations: HAT, histidine affinity tag; HRP, Horse radish peroxidase; MMP, matrix metalloproteinase; MMP-9me, MMP-9 minienzyme; OB, oligonucleotide/oligosaccharide-binding; SAg, staphylococcal superantigen; SSL, staphylococcal superantigen-like protein.

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https://doi.org/10.1016/j.bbrc.2018.02.138
0006-291X/

2. Materials and methods

2.1. Reagents

All chemical reagents and media were purchased from, Nacalaitesque (Kyoto, Japan), SIGMA (St. Louis, MO), WAKO pure chemicals (Osaka, Japan). Restriction endonucleases and a ligase were products of Roche (Basel, Switzerland), TaKaRa (Osaka, Japan) and Toyobo (Osaka, Japan). Oligonucleotides were supplied by Eurofin Genomics (Huntsville, AL), Invitrogen (Carlsbad, CA), Nihon Gene Research Laboratories (Sendai, Japan), RIKAKEN (Nagoya, Japan) and SIGMA.

2.2. Cloning of SSLs and construction of their mutants

The cDNA of SSLs was amplified from genomic DNA of *S. aureus* ATCC27733 using KOD polymerase (Toyobo) and PrimeSTAR GxL polymerase (TaKaRa) [4]. Domain swap mutants consists of a portions of SSL5 and SSL7, depicted in Fig. 1A, were constructed by ligating DNA fragments, megaprimer method and inverse PCR as described previously [18]. Deletion mutants of SSL5 were constructed by the amplification of fragment using specific primers including restriction site at 5′ end and the cDNA of SSL5 as template. Primers for preparing the mutants are listed in supplementary Table 1. The cDNA fragment was subcloned into pGEM-T Easy (Promega, Madison, WI) and confirmed the nucleotide sequence using ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

2.3. Preparation of recombinant SSL proteins

The DNA fragment was subcloned into prokaryotic expression plasmid pGEX-5X-1 (GE Healthcare, Buckinghamshire, UK) and pCold-II (TaKaRa). In some experiment the DNA fragment was subcloned into pCold-II that 6xHis tag was replaced with histidine affinity tag (HAT, KDHLIHNVHKEEHAHAHN) and Strep-tag II (WSHPQFEK) was inserted in front of the stop codon; the plasmid expresses polypeptide that possesses HAT at N-terminus and Strep-tag II at C-terminus (Fig. 1B). The expression plasmids were transformed into *E. coli* BL21 and SHuffle Express (New England Biolabs, Ipswich, MA). The recombinant protein was induced by the treatment with 1 mM of Isopropyl β-D-1-thiogalactopyranoside with shaking at 120 rpm at 37 °C for 4 h (pGEX) or at 15 °C for 24 h (pCold). The recombinant SSLs used in this study were expressed in soluble fraction. The recombinant protein was affinity-purified using Glutathione Sepharose 4B (GE Healthcare) for GST-fused protein and Ni Sepharose 6 Fast Flow (GE Healthcare) for 6xHis and HAT-tagged proteins as described previously [16]. The purified protein was dialyzed against PBS or TBS. The concentration was determined with a Bradford's protein assay kit (Nacalaitesque) using BSA as standard. The purities of recombinant proteins were estimated to be more than 95% by SDS-PAGE and Coomassie Brilliant Blue (CBB) staining.

2.4. Preparation of MMP-9 protein and mutants

The DNA fragment corresponding to catalytic domain of MMP-9 (MMP-9me) was amplified from the cDNA of A375 melanoma cells, subcloned into pCold-II that of 6xHis was replaced to C-terminus by inverse PCR. The mutant of MMP-9me that of CBD was deleted (MMP-9meΔCBD) and three histidine residues responsible for zinc binding (His-401, His-405 and His-411) [19] were substituted to alanine residues (MMP-9me3HA) were constructed by inverse PCR. Primers used are in supplementary Table 1. The recombinant MMP-9 was induced in *E.coli* and purified under the denatured condition as described previously [16].

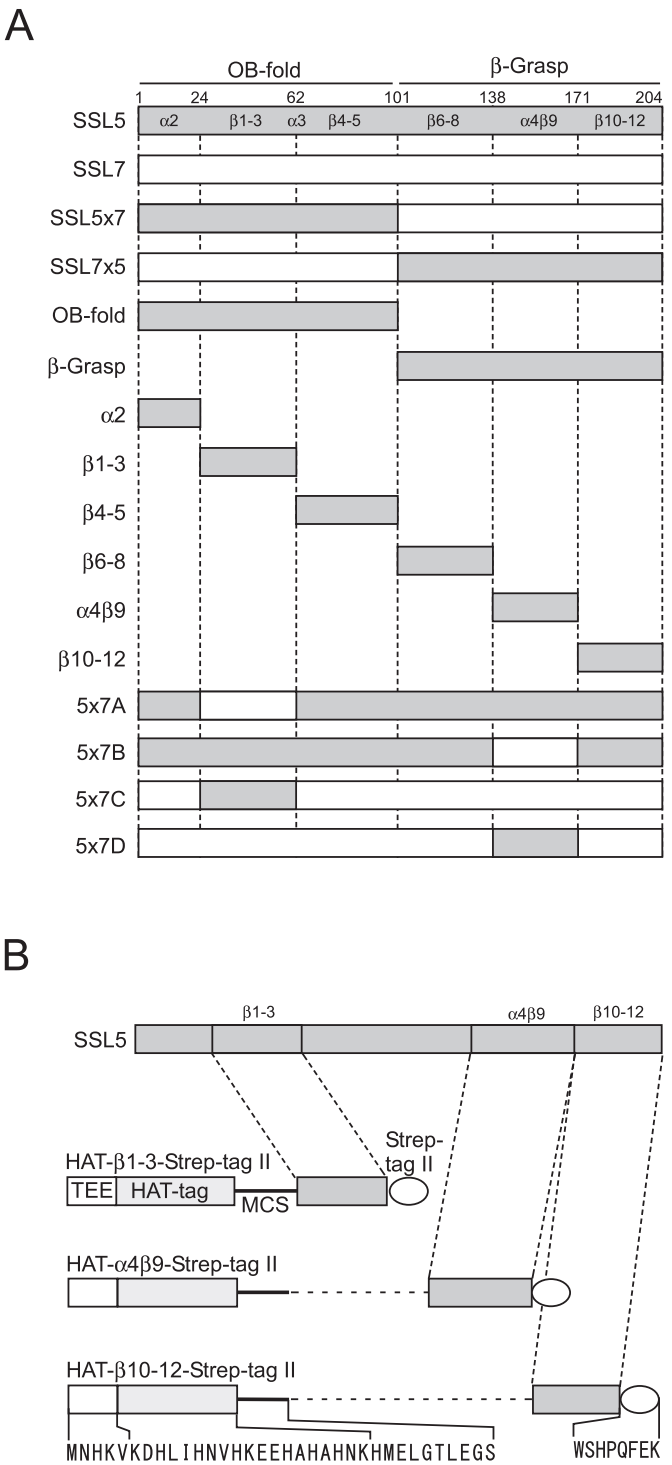

Fig. 1. SSL5 and its domain swap mutants, deletion mutants and SSL5 derived polypeptides.
A, Schematic representation of mutated SSL5 proteins prepared in this study. Gray boxes represent region derived from SSL5, and white boxes represent that from SSL7. The numbers indicate the amino acid position corresponding to wild type SSL5. B, Schematic representation of SSL5 derived polypeptides prepared in this study. HAT, histidine affinity tag; TEE, translation enhancing element; MCS, multiple cloning site.

2.5. Solid phase binding assay

Interaction between SSLs and MMP-9 was determined using solid phase binding assay [9]. MaxiSorp™ microtiter plate (Nalge

Please cite this article in press as: K. Kohno, et al., Identification of matrix metalloproteinase 9-interacting sequences in staphylococcal superantigen-like protein 5, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.02.138