Short Communication

Gamma-Hemolysin Genes in the Same Family with LukF and lukS Genes in Methicillin Resistant Staphylococcus aureus†

Arifur RAHMAN, Kazuo IZAKI, and Yoshiyuki KAMIO*

Department of Applied Biological Chemistry, Faculty of Agriculture, Tohoku University, Aobaku, Sendai 981, Japan
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Cloning and DNA sequencing of γ-hemolysin genes from two strains of Staphylococcus aureus showed three open reading frames, transcribed serially within the sequenced 4353 nucleotide bases. In this report, from the nucleotide sequence, we demonstrate that leukocidin and γ-hemolysin share the F or Hyl component and the specificity of both toxins depends on this component.

Staphylococcal bi-component toxins, leukocidin and γ-hemolysin, consist of two protein components, i.e., F and S for leukocidin and Hyl and HyII for γ-hemolysin. We have reported the cloning and sequencing of the leukocidin genes lukS and lukF from MRSA No. 4.2,3) In a previous report4) we purified both HyI and HyII components to homogeneity from S. aureus, compared their properties with those of the F and S components of leukocidin, and suggested that the leukocidin F component is identical with γ-hemolysin HyI. In this report, by sequencing the DNA, we demonstrate that lukF acts as a common gene for the two toxins, leukocidin and γ-hemolysin and the specific activity of leukocidin and γ-hemolysin are set by the gene product of lukS or hlg2, i.e., the S or HyII component.

The 72% homology of the N-terminal 58-residue amino acid sequences from S and HyII indicated their similar DNA sequences. Therefore, we hybridized HindIII-digested chromosomal DNA of MRSA No. 4 and S. aureus 5R Smith, which is a known producer of γ-hemolysin,5) with a 1.1-kbp EcoRI–XhoI fragment (probe I) from plasmid pSA9-36.2,3) For MRSA No. 4, two fragments of 12-kbp and 3-kbp were positive, but in 5R Smith, only one 15-kbp fragment was hybridized (Fig. 1A). On the other hand, 12-kbp and 4-kbp fragments of MRSA No. 4 chromosomal DNA, digested with HindIII and HindIII–EcoRI, respectively hybridized with probe I and a DdeI–DdeI fragment (probe I) containing lukS+lukF and lukF genes portions only, respectively (Fig. 1B and C). Interestingly, in the case of probe I, another 3-kbp fragment was identified in both digestions products (Fig. 1, B). The 3-kbp fragment was inserted into the HindIII site of pUC119 vector DNA to construct the plasmid pMRSA3 and the recombinant plasmid was introduced into Escherichia coli DH5α. The 12-kbp and 15-kbp fragments were inserted into the HindIII site of Chromid 9-28 vector DNA (Wako Chemical Co., Tokyo) to construct plasmids pMRSA12 and p5R15, which were transduced into E. coli DH5α. After sequencing of all the plasmids, three open reading frames (ORF) were identified from both MRSA No. 4 and S. aureus 5R Smith strain. Alignment of the sequence containing three ORFs from both strains showed almost complete homology of the full sequences except the positions indicated in Fig. 2. The HindIII site at position 1398 bp was not found in the 5R Smith DNA sequence due to the change of G to A at position 1399 bp. The ORFs started from 1770 bp and 2719 bp, corresponding with those of the ORFs encoded by lukS and lukF genes, respectively.2,3) Upstream from the ORF at 1770 bp, another ORF started from 272 bp, the deduced amino acid sequence encoded by the nucleotide sequence from 359 bp to 538 bp exactly corresponded with the N-terminal 60 amino acids of HyII. Furthermore, the chemically analyzed C-terminal 5 amino acids sequence4) corresponded with the deduced amino acid sequence. It was

Fig. 1. Southern Hybridization of Chromosomal DNA of MRSA No. 4 and S. aureus 5R Smith with Probes from Leukocidin Genes.
(A) With Probe I (EcoRI–XhoI fragment). Lanes: 1, HindIII digested MRSA DNA; 2, HindIII-digested 5R Smith DNA. (B) With Probe II. Lanes: 1, HindIII-digested MRSA DNA; 2, HindIII–EcoRI-digested MRSA DNA; 3, HindIII–EcoRI-digested MRSA DNA. (C) With Probe II (DdeI–DdeI fragment). Lanes: 1, HindIII digested MRSA DNA; 2, HindIII–EcoRI-digested MRSA DNA. Each probe was labeled with α-32P[ATP] by using a random primer kit (Takara Shuzo Co., Kyoto). Hybridization was done at 42°C. PI, PII, and bars represent probe I, probe II, and transcription termination signals.

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* Corresponding author.

Abbreviations: MRSA, methicillin resistant Staphylococcus aureus; H, HindIII; X, XhoI; E, EcoRI; C, ClaI; D, DdeI.
Fig. 2. Nucleotide Sequence Containing Leukocidin and γ-Hemolysin Genes from MRSA and the Deduced Amino Acid Sequence.

The strand shown is from the 5' to 3' direction. Amino acid sequences analyzed chemically, putative ribosomal binding sites, signal peptide sequences, promoter sequences, and transcription termination sequence are indicated by broken lines, double underlines, single underlines, boxes, and horizontal arrows, respectively. Diversities in 5R Smith are indicated by nucleotide residues presented in those positions shown above the sequence.
The sequences were compared using the GCG program GAP using a gap weight of 8. Identical and related amino acid residues are indicated by stars and dots, respectively. The gross difference between them is indicated by a box.

evident that the first ORF was for the HyII component, the second ORF was for the leukocidin S component, and the third one, encoded by a co-transcriptional gene with \( \text{hukS} \), was for the F component of leukocidin and probably acted as HyI in hemolysis. The three genes were transcribed in the same direction. After homology study of deduced amino acid sequence from the structure genes of leukocidin S component (\( \text{hukS} \)) and \( \gamma \)-hemolysin HyII component (\( \text{hlg2} \)), a gross dissimilarity became evident at the C-terminal regions but not at the N-terminal regions (Fig. 3).

\( \gamma \)-Hemolysin and leukocidin activities were measured by using the cloned S, F, and HyII components, which were purified from periplasmic spaces of \( \text{E. coli} \) containing pSRK91,2 pFRK92,3 and pMRSA3, respectively, by the method described previously.4) Cloned HyII was found to cause hemolysis with cloned F or HyI from the third ORF, cloned S with cloned F or HyI caused leukocytolysis (data not shown). From these findings, it was demonstrated that the F component of leukocidin is identical with HyI of \( \gamma \)-hemolysin and that leukocidin or \( \gamma \)-hemolysin activity is regulated by \( \text{lukS} \) or \( \text{hlg2} \) gene products, respectively.

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**References**