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The Gamma-Hemolysin Locus of *Staphylococcus aureus* Comprises Three Linked Genes, Two of Which Are Identical to the Genes for the F and S Components of Leukocidin

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The *Staphylococcus aureus* gamma-hemolysin comprises two polypeptides, whereas the gamma-hemolysin locus (*hlg*) contains three open reading frames. The *hlgA* and *hlgB* genes encode the γ1 and γ2 components, respectively. The HlgB protein (γ2) has 27% residue identity with *S. aureus* alpha-toxin. Surprisingly, *hlgB* and *hlgC* are 98.5 and 99.1% identical to the *lukF* and *lukS* genes, respectively, encoding the F and S components of the Panton-Valentine leukocidin.

Staphylococcus aureus can express five cytolytic toxins, the alpha-, beta-, delta-, and gamma-toxins and a toxin that acts on leukocytes, the Panton-Valentine leukocidin (9, 14). The leukocidin is composed of two proteins, the F and S components (10). There is firm evidence that gamma-toxin is also composed of two proteins (1, 5, 6, 13). We previously reported the cloning and analysis of the *S. aureus* gamma-toxin locus (2). Nonhemolytic transposon insertion mutants fell into two groups on the basis of in vitro complementation tests. The corresponding genes *hlgA* and *hlgB* expressed proteins with molecular weights of 32,000 and 36,000, respectively, in minicells.

(A preliminary report of part of this work has been presented previously [4].)

In this report we present the DNA sequence of the *hlg* locus. Deletions in *hlg* plasmids were generated with BAL 31. Sequencing reactions on plasmid templates employed T7 polymerase. Sequence analysis and data base searches were performed with the University of Wisconsin Genetics Computer Group package (3) on a VAX computer. For amplification by the polymerase chain reaction, standard reaction conditions (7) for 30 cycles and AmpliTaq polymerase were used. The assays for gamma-hemolysin, polyacrylamide gel electrophoresis, and immunoblotting were as described previously (2). Antiserum to the γ1 component of gamma-hemolysin (1) was donated by M. Clyne, and antileukocidin serum was obtained from C. Adlam.

The 3,797-bp sequence of the *hlg* locus was determined from pJC01 and pJC08 (2). Surprisingly, three open reading frames, designated *hlgA*, *hlgB*, and *hlgC*, were identified. Each is preceded by a potential ribosome binding site and encodes a protein with a putative signal sequence. The mature forms of the HlgA, HlgB, and HlgC proteins have molecular weights of 31,925, 34,123, and 32,551, respectively, and pI values of 9.43, 9.08, and 9.01. The position of the *hlgB* gene corresponds to the region that encodes the HlgB protein (2). The putative ribosome binding site lies within an open reading frame, designated *hlgC*, upstream from *hlgB*. The *hlgC* gene has a potential ribosome binding site and also a promoter (Fig. 1). It is likely that these two genes are cotranscribed. The *hlgA* gene spans a region defined by several transposon insertions. A putative promoter and a ribosome binding site are located 5′ to the coding sequence, and a transcriptional termination sequence occurs 3′ to the coding sequence. Thus, *hlgA* is likely to be monocistronic.

Beginning at residue 30 of the translation product of *hlgA*, a sequence of amino acids that corresponds exactly to the amino-terminal sequence of the γ1 component of gamma-toxin (1) was identified. This demonstrates unambiguously that γ1 is specified by *hlgA*. The HlgA (γ1) and HlgC (γ2) proteins have 70% residue identity, and each has approximately 30% identity with HlgB (Fig. 2). Additionally, there is 27% residue identity between HlgB and *S. aureus* alpha-toxin, and a lower level of similarity was noted between alpha-toxin and either HlgA or HlgC (Fig. 2). Surprisingly, the *hlgB* and *hlgC* genes were found to be 98.5 and 99.1% identical to the recently published sequences of the leukocidin F and S components, respectively (Fig. 1). The *luk* genes were cloned by using oligonucleotide probes derived from amino-terminal sequences of the purified F and S components (11, 12). Furthermore, antileukocidin serum inhibited the gamma-hemolytic activity expressed by strains PG23 (a toxin shock syndrome isolate [1]) and Smith 5R in agarose diffusion tests (data not shown). The reported pI values of 9.08 and 9.39 for the leukocidin F and S components, respectively, are similar to the values predicted from the *hlgB* and *hlgC* sequences presented here.

The three *hlg* open reading frames were cloned separately into plasmid vectors. The *hlgB* gene was cloned on a *Sau*3A fragment into pK18, forming plasmid pHLGB. The *hlgA* and *hlgC* genes were cloned after specific amplification by polymerase chain reaction primers that incorporated *Bam*HI recognition sites, and the recombinant plasmids were verified by sequence determination. The amplified products were cloned in pK18, forming pHLGA and pHLGC, respectively. Lysates of cells harboring pHLGA contained a 32,000-M_r protein that reacted with the anti-γ1 serum in Western immunoblots (data not shown). No immunoreactive

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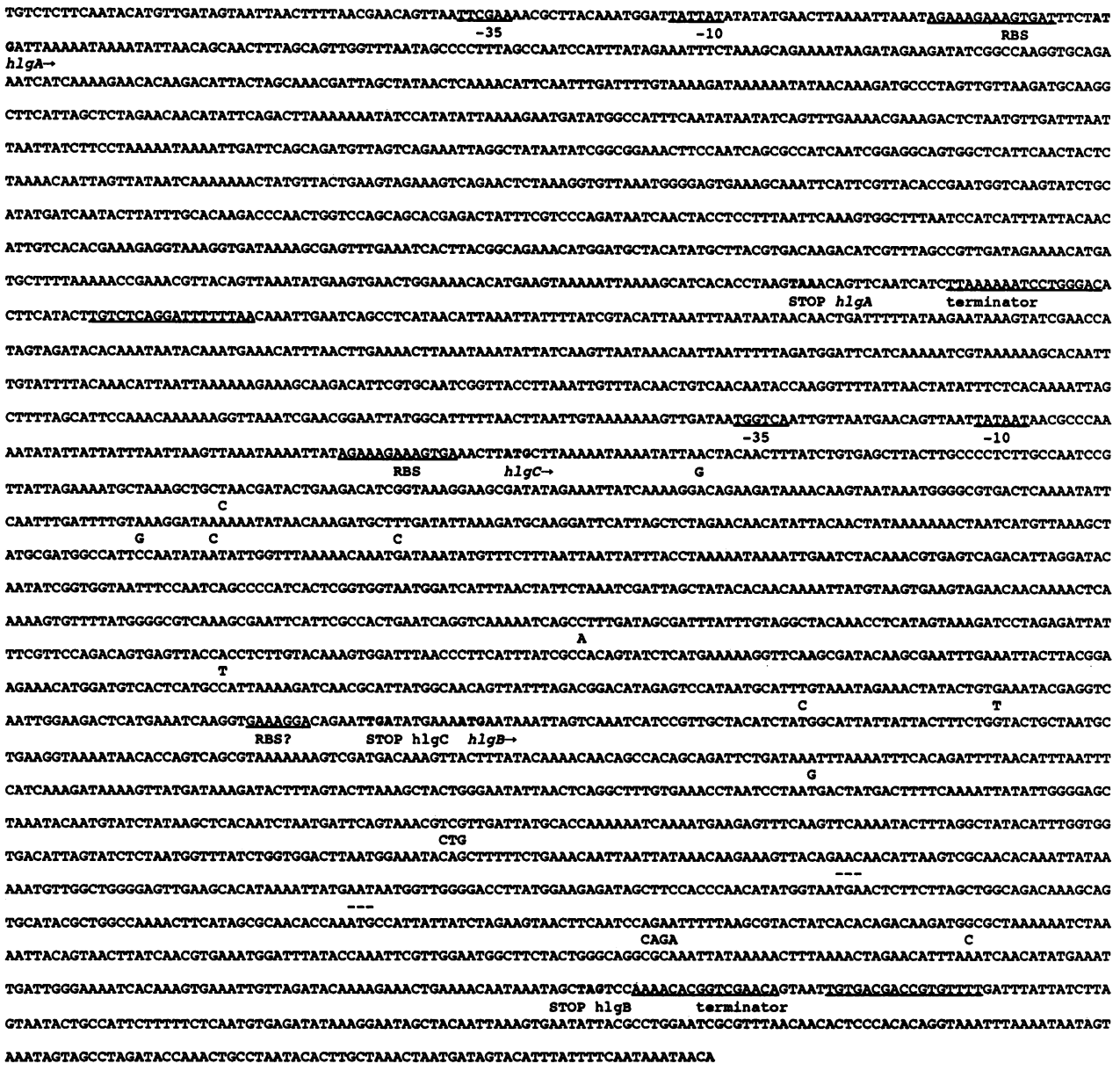

FIG. 1. Nucleotide sequence of the gamma-hemolysin locus of *S. aureus* Smith 5R. Possible promoter and terminator sites and ribosome binding sites (RBS) are indicated. The translation initiation codon and termination codon for each open reading frame are in boldface type. Divergences between *hlgC* and *lukS* and between *hlgB* and *lukF* are indicated by the residues present in these positions in the *luk* genes shown below the *hlg* sequence. Residues of *hlg* not present in the *luk* sequence are represented by hyphens.

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Hla   1    ADSDI---NIKTGTTDIGSNTTVKTGDLVTYDKENGMHKKVFYSFIDDKNAIKKLLVIRTKGTIAG--QYRVYSEEGANKSGLAWPSAFKVQLQLPDNEV
HlgA  1    EN------KIEDIGQGA--EIIKRTQDITS--KRLAITQNIQFDFVKDKKYNKDALVVKMQGFISSRTTYSDLKKYPY-IKRMIWPFQYNISLKT-KDSN
HlgB  1    AEGKITPVSVKKVDDKVTLYKTTATADSDKFK----ISQILTFNFIKDKSYDKDTLVLKATGNINS--GFVKPNPNDYDFSKLYWGAKYNVSISSQSNDS
HlgC  1    AN------DTEDIGKGSDIEIIKRTEDKTS--NKWGVTQNIQFDFVKDKKYNKDALILKMQGFISSRTTYYNYKKTNH-VKAMRWPFQYNIGLKT-NDKY

Hla   96   AEISDYYPRNSIDTKEYMSTLTYGFNGNVTGDDTGKIGGLIGANVSIGHTLKYVQPDFKTILESPTD-KKVGWKVIFNNMVNQNWGPYDRDSWNPVYGNQ
HlgA  89   VDLINYLPKNKIDSADVSQKLGYNIGGNF--QSAPSIGG--SGSFNYSKTISYNQKNYVTEVESQNS-KGVKWGVKANSFVTPN-------GQVSAYDQY
HlgB  95   VNVVDYAPKNQNEEFQVQNTLGYTFGGDISISN-GLSGGLNG-NTAFSETINYKQESYRTTLSRNTNYKNVGWGVEAHKIMNNGWGPYGRDSFHPTYGNE
HlgC  91   VSLINYLPKNKIESTNVSETLGYNIGGNF--QSAPSLGG--NGSFNYSKSISYTQQNYVSEVEQQNS-KSVLWGVKANSFATES-------GQKSAFDSD

Hla   191  LFMKTRNGSMKAADNFLDPNKASSLLSSGFSPDFATVITMDRKASKQQTNIDVIYERVRDD-YQL----HWTSTNWKGTNTKD-KWTDRSSERYKIDWEK
HlgA  178  LFAQ-DPTGPAARDYFVPDNQLPPLIQSGFNPSFITTLS-HERGKGDKSEFEITYGRNMDATYAYVTRHR-----LAVDRKHDAFKNRNVTVKYEVNWKT
HlgB  194  LFLAGRQSSAYAGQNFIAQHQMPLLSRSNFNPEFLSVLS-HRQDGAKKSKITVTYQREMDL-YQI----RWNGFYWAGANYKN-FKTRTFKSTYEIDWEN
HlgC  180  LFVGYKPHSKDPRDYFVPDSELPPLVQSGFNPSFIATVS-HEKGSSDTSEFEITYGRNMDVTHAIKRSTHYGNSYLDGHRVHNAFVNRNYTVKYEVNWKT

Hla   286  EEMT----------N
HlgA  272  HEVKIKSITP----K
HlgB  288  HKVKLLDTKETENNK
HlgC  280  HEIKVKG--Q----N
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FIG. 2. Comparison of the amino acid sequences of the processed forms of Hla (*S. aureus* alpha-toxin), HlgA, HlgB, and HlgC. The multiple alignment was produced by the Clustal V program (8) with the default parameters. Identical residues are indicated by asterisks, and conserved substitutions are indicated by dots. Spaces have been introduced to maximize alignment.

product was detected in extracts of cells harboring pHLGB or pHLGC with antisera against two purified components of gamma-hemolysin (1), possibly because of the low level of expression. Whole-cell lysates of *Escherichia coli* containing these plasmids were tested for hemolytic activity in diffusion tests in rabbit erythrocyte agarose plates. None was hemolytic alone, but the pHLGB extract acted synergistically with pHLGA or pHLGC samples to lyse the erythrocytes (Fig. 3). Hemolysis produced between pHLGA and pHLGC was slower to develop and resulted in a zone of lysis that was more opaque than that formed between pHLGB and pHLGA lysates. Dextran and dextran sulfate inhibited both synergistic reactions. This inhibition is a characteristic of gamma-hemolysis (9). In addition, hemolysis did not occur in agar incorporating the erythrocytes, presumably because of inhibition by the sulfonated polymers present.

All available evidence suggests that gamma-hemolysin and leukocidin are encoded by the same locus. The relative proportions of the polypeptide components in culture supernatants could be crucial in determining the type of cytolytic activity expressed by a particular strain. It is not yet known whether the strain from which the leukocidin genes were cloned has a functional *hlgA* gene and expresses gamma-hemolysin as well as leukocidin. Similarly, it is not known whether gamma-toxin-producing strains also express leukocidin activity.

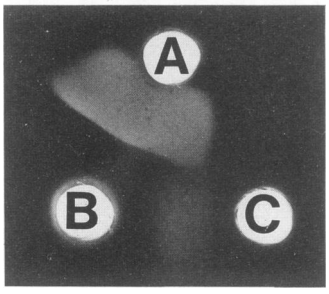

FIG. 3. Synergistic hemolysis between components of gamma-hemolysin. Extracts of *E. coli* cells harboring pHLGA (well A), pHLGB (well B), or pHLGC (well C) were applied to wells in agarose plates containing rabbit blood.

The nucleotide sequence reported herein has been submitted to GenBank under accession number L01055.

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