

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 $\gamma 1$ and $\gamma 2$ components, respectively. The HlgB protein ($\gamma 2$) 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 Pantone-Valentine leukocidin.

Staphylococcus aureus can express five cytolytic toxins, the alpha-, beta-, delta-, and gamma-toxins and a toxin that acts on leukocytes, the Pantone-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 $\gamma 1$ 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 $\gamma 1$ component of gamma-toxin (1) was identified. This demonstrates unambiguously that $\gamma 1$ is specified by *hlgA*. The HlgA ($\gamma 1$) and HlgC ($\gamma 2$) 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 *Sau3A* 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- $\gamma 1$ serum in Western immunoblots (data not shown). No immunoreactive

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1 TGTCTCTCAATACATGTTGATAGTAATTAACCTTTTAAACGAACAGTTAAATTCGAAACCGCTTACAAATGGATTATTATATATATGAACCTAAAATAAAATAGAAAGAAAGTGATTTCTAT
 121 GATTAAAAATAAAAATTAACAGCAACTTTAGCAGTGGTTAATAGCCCTTTAGCCAATCCATTTATAGAAAATTTCTAAGCAGAAAAAAGATAGAAGATATCGGCCAAGGTGCAGA
hlgA→
 241 AATCATCAAAAGAACACAAGACATTACTAGCAAACGATTAGCTATAACTCAAACATTCAAATTTGATTTTGTAAAAGATAAAAAATAACAAAGATGCCCTAGTTGTTAAGATGCAAGG
 361 CTTCAATAGCTCTAGAACAACATATTAGCAGCTTAAAAAATATCCATATATTAAGAAGATGATATGGCCATTTCAATATAATATCAGTTTGAAGAACGAAAGACTCTAATGTTGATTTAAT
 481 TAATTATCTTCCTAAAAATAAAATGATTTCAGCAGATGTTAGTCAGAAATTAGGCTATAATATCGGCGGAACTTCCAATCAGCGCCATCAATCGGAGGAGTGGCTCATTCAACTACTC
 601 TAAAACAATTAGTTATAATCAAAAAACTATGTTACTGAAGTAGAAAGTCAGAACTCTAAAGGTGTTAAATGGGGAGTGAAGCAAAATTCATTGTTACACCGAATGGTCAAGTATCTGC
 721 ATATGATCAATACTTATTGTCACAAGACCACTGGTCCAGCAGCAGAGACTATTTGCTCCAGATAATCAACTACCTCCTTTAATTCAAAGTGGCTTTAATCCATCATTATTACAAAC
 841 ATTGTCACACGAAAGAGGTAAAGGTGATAAAAGCGAGTTTGAATCACTTACGGCAGAAACATGGATGCTACATATGCTTACGTGACAAGACATCGTTTAGCCGTGATAGAAAACATGA
 961 TGCTTTTAAAAACCGAAACGTTACAGTTAAATATGAAGTGAACCTGGAACACATGAAGTAAAAATTAAGCATCACACCTAAGTAAACAGTTCAATCATCTTAAAAATCCCTGGGACA
 1081 CTTCACTTCTCTCAGGATTTTAAACAAATGAAATCAGCCTCATAACATTAATTTATTTATCGTACATTAATTTAATAATAACAACCTGATTTTATAAGAATAAAGATATCGAACCA
 1201 TAGTAGATACACAAATAATACAAATGAAACATTTAACTTGAAGCTTAAATAAATATTATCAAGTTAATAAACAATTAATTTTATAGTGGATTATCAAAAATCGTAAAAAGCACAATT
 1321 TGTATTTTACAACATTAATTAAGAAAGAAAGCAAGACATTCGTGCAATCGTTACCTTAAATGTTTACAACCTGTCAACAATACCAAGGTTTTATTAAGTATATTCTCACAAAATTAG
 1441 CTTTATAGCATTCCAACAAAAAGGTTAAATCGAACGGAATTTATGGCATTTTTAACTTAATTTGTAAGAAAGTGGATAATGGTCAATTGTTAATGAACAGTTAATTTAATAACGCCCAA
 1561 AATATATTATTATTAATTAAGTTAAATAAAATTTATAGAAAGAAAGTGAACCTTATGCTTAAAAATAAATATTAACTACAACCTTTATCTGTGAGCTTACTTGCCCTCTTGCCAATCCG
 1681 TTATTAGAAAATGCTAAAGCTGCTAACGATACTGAAGACATCGGTAAGAAAGCGGATATAGAAAATTATCAAAAGGACAGAAAGATAAAAACAAGTAAATAAATGGGGCGTGACTCAAATATT
 1801 CAATTTGATTTTGTAAAGGATAAAAAATAACAAAGATGCTTTGATATTAAGATGCAAGGATTCATTAGCTCTAGAACAACATATTACAACATAAAAAAACTAATCATGTTAAAGCT
 1921 ATGCGATGGCCATTCCAATATAATATGTTTAAAAACAATGATAAATATGTTCTTTAATTAATTTTACCTAAAAATAAATGAACTTACAAACGTGAGTCAGACATTAGGATAC
 2041 AATATCGGTGGTAAATTCCAATCAGCCCATCACTCGGTGGTAAATGGATCATTAACTATTCTAAATCGATTAGCTATACACAACAAAATTATGTAAGTGAAGTAGAACACAAAACCTCA
 2161 AAAAGTGGTTTTATGGGGCGTCAAAGCGAATTCATTCCGCCACTGAAATCAGGTCAAATAACAGCCCTTGGATAGCGATTATTTGTAGGCTACAACCTCATAGTAAAGATCCTAGAGATTAT
 2281 TTCGTTCCAGACAGTGAAGTACACCTCTTGTACAAAGTGGATTAAACCTTCAATTTATCGCCACAGTATCTCATGAAAAGGTTCAAGCGATACAAGCGAATTTGAAATTAACCTACGGA
 2401 AGAAACATGGATGTCACCTCATGCCATTAAAGATCAACGCATTTATGGCAACAGTTATTATTAGACGGACATAGATCCATAATGCATTGTAATAGAACTATACTGTGAAATACGAGGTC
 2521 AATGGAAGACTCATGAAATCAAGGTGAAAGGACAGAAATGATATGAAATGAATAAATAGTCAAATCATCCGTTGCTACATCTATGGCATTATTACTTCTGCTACTGCTAATGC
 2641 TGAAGTAAAAAACACCAGTCAGCGTAAAAAAGTCGATGACAAAGTTACTTTATACAAAACAACAGCCACAGCAGATTCTGATAAATTTAAAATTTACAGATTTAACATTTAATTT
 2761 CATCAAAGATAAAAGTTATGATAAAGATACTTTAGTACTTAAAGCTACTGGGAATATAAATCAAGGCTTTGTGAAACCTAATCCTAATGACTATGACTTTTCAAATATATATGGGGAGC
 2881 TAAATACAATGTATCTATAAGCTCACAACTAATGATTCAAGTAAACGTCGTTGATTTATGCAACAAAAATCAAATGAAGAGTTTCAAGTTCAAATACTTTTAGGCTATACATTTGGTGG
 3001 TGACATTAGTATCTCTAATGGTTTATCTGGTGGACTTAATGGAATACAGCTTTTCTGAAACAATTAATATAAACAAGAAAGTTACAGAACAACATTAAGTCGCAACACAAATATATA
 3121 AAATGTTGGCTGGGAGTTGAAGCACATAAAATTTAATAAATGGTTGGGACCTTTATGGAAGAGATAGCTCCACCCAACATATGGTAATGAACTCTTCTTACTGTCAGACAAAGCAG
 3241 TGCATACGCTGGCCAAAACCTCATAGCGCAACCAATGCCATTATTATCTAGAAGTAACTTCAATCCAGAATTTTAAAGCGTACTATCACAGACAAGATGGCGCTAAAAATCTAA
 3361 AATTACAGTAACTTATCAACGTGAAATGGATTTATACCAAAATTCGTTGGAATGGCTTCTACTGGGCAGCGCAAAATATAAAAACCTTAAAACCTAGAACATTTAAATCAACATATGAAAT
 3481 TGATTGGGAAAATCACAAAGTGAATGTTAGATACAAAAGAACTGAAACAATAAATAGCTAATCCAAAACAGGTCGAACAGTAATTTGTGACGACCGTGTGTTGATTTATATCTTA
 3601 GTAATACTGCCATCTTTTCTCAATGTGAGATATAAAGGAATAGCTACAATTAAGTGAATATTACGCTCGGAATCGCGTTTAAACAACACTCCACACAGGTAATTTAAAATAATAGT
 3721 AAATAGTAGCTAGATACCAACTGCCTAATACACTTGCTAACTAATGATAGTACATTTATTTTCAATAAATAACA

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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H1a 1 ADSDI---NIKTGTTDIGSNTTVKTDGLVYTDKENGMMHKVYFSYFIDDKNAIKKLLVIRTKGTIAG--QYRVYSEEGANKSGLAWPSAFKVLQLPNDNEV
H1gA 1 EN-----KIEDIGQGA--EIKRTQDITS--KRLAITQNIQDFVKDKKYNKDALVVKMQGFISRRTTYSDLKKYPY-IKRMWPFQYNISLKT-KDSN
H1gB 1 AEGKITPVSVKKVDKVTLYKTATADSDKFK----ISQILTFNFIKDKSYDKDTLVLTKATGNINS--GFVVKPNPNDYDFSKLYWGAKYNVISSSQSND
H1gC 1 AN-----DTEDIGKSDIEIIKRTEDKTS--NKGWVTQNIQDFVKDKKYNKDALILKMQGFISRRTTYNYKKTNH-VKAMRWPFFQYNIGLKT-NDKY
. . . . . * * . . . . * * * * . . . . . * * . . . . .
H1a 96 AEISDYYPNSIDTKEYMSTLYGFNGNVTGDDTGKIGGLIGANVSIGHTLKYVQPDFKTILESPTD-KKVGWVKVIFNNMVNQNWGPYDRDSWNVPVYGNQ
H1gA 89 VDLINYLPKNKIDSADVSQKLGYNIGGNF--QSAPSIGG--SGSFNYSKTIISYNQKNYVTEVESQNS-KGVKVGWKANSFVTPN-----GQVSAYDQY
H1gB 95 VNVVDYAPKNQNEEFQVQNTLGYTFGGDISISN-GLSGGLNG-NTAFSETINYSYRTTSLRNTNYKNVGVGVEAHKIMNNGWGPYGRDSFHPHTYGN
H1gC 91 VSLINYLPKNKIESTNVSETLGYNIGGNF--QSAPSLGG--NGSFNYSKISISYQQNYVSEVEQQNS-KSVLVGKWKANSFATES-----GQKSAFDS
. . * . * . . . * . * . . . . * . . . . . * * * * . . . . .
H1a 191 LFMKTRNGSMKAADNFDPNKASSLLSSGFSPDFATVITMDRKASKQQTNIIDVIYERVRRD-YQL----HWTSTNWKGNTTKD-KWTRDRSSERYKIDWEK
H1gA 178 LFAQ-DPTGPAARDYFVDPNQLPPLIQSGFNPSFITLTS-HERGKGDKSEFEITYGRNMDATYAYVTRHR-----LAVDRKHDFAKRNRTVKYEWNWKT
H1gB 194 LFLAGRQSSAYAGQNFIAQHQMPLLSRSNFNPEFLSVLS-HRQDGAKKSKITVTYQREMDL-YQI----RWNGFYWAGANYKN-FKTRTFKSTYEIDWEN
H1gC 180 LFVGYKPHSKDPRDYFVDPSELPLVQSGFNPSFIATVS-HEKGSSTSEFEITYGRNMDVTHAIKRSTHYGNSYLDGHRVHNAFVNRNYTVKYEWNWKT
** . . . . . * * * . . . . . * . . . . * * . . . . .
H1a 286 EEMT-----N
H1gA 272 HEVKIKSITP----K
H1gB 288 HKVKLLDVKETENNK
H1gC 280 HEIKVKG--Q----N
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FIG. 2. Comparison of the amino acid sequences of the processed forms of H1a (*S. aureus* alpha-toxin), H1gA, H1gB, and H1gC. 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-hemolysin (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

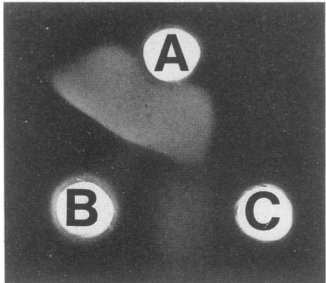


FIG. 3. Synergistic hemolysis between components of gamma-hemolysin. Extracts of *E. coli* cells harboring pHLGA (well A), pHLGB (well B), or pHLGC (well C) were applied to wells in agarose plates containing rabbit blood.

whether gamma-toxin-producing strains also express leukocidin activity.

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

This work was funded by grants from the Health Research Board of Ireland to T.J.F., from the Medical Faculty of Lund University to J.C., and from the Swedish Medical Research Council to P.O.

We thank M. Clyne for generously supplying antisera. J.C. gratefully acknowledges Lars Björck for outstanding support.

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