Staphylococcus aureus Expresses a Major Histocompatibility Complex II Analog*

(Received for publication, June 14, 1995)

Klas J önsson§, Damien McDevitt, Mary Homonylo McGavin, Joseph M. Pattit, and Magnus Höök||

From the Center for Extracellular Matrix Biology and Department of Biochemistry and Biophysics, Institute of Biosciences and Technology, Texas A & M University, Houston, Texas 77030-3303 and the University of Mannitoba, Winnipeg, Manitoba R3E 0W2, Canada

Staphylococcus aureus expresses various surface proteins which specifically recognize and bind to different host molecules. We have previously identified a bacterial protein that exhibits a broad specificity and binds to several mammalian extracellular proteins. The gene encoding this bacterial component has now been cloned and sequenced. The deduced protein consists predominantly of six repeated domains of 110 residues. Each of the repeated domains contains a subdomain of 31 residues that share striking sequence homology with a segment in the peptide binding groove of the \( \beta \) chain of the major histocompatibility complex (MHC) class II proteins from different mammalian species. The purified recombinant bacterial protein bound several mammalian proteins, including recombinant osteopontin, suggesting a protein-protein interaction and also specifically recognized a 15-amino acid residue synthetic peptide. Taken together, these results suggest that the bacterial protein resembles mammalian MHC class II molecules with respect to both sequence similarities and peptide binding capabilities.

Pathogenic bacteria have evolved in close association with their hosts and have developed sophisticated mechanisms to increase their chances of survival. Some of these mechanisms exploit normal host processes and signaling systems thus illustrating the powerful adaptation of pathogens to their environment. Some of the better examples of bacterial mimicry relate to mechanisms involved in colonization of the host. Pathogens such as Bordetella pertussis, Salmonella typhimurium, enteropathogenic Escherichia coli, and Yersinia species subvert different host systems to aid in their colonization (1–3).

Staphylococcus aureus is an important pathogen possessing the potential to express a variety of different virulence determinants. These include exotoxins, exoenzymes, and a family of specific protein adhesins (MSCRAMMs) that mediate the adherence of the organism to host tissues and extracellular matrix components (4–6). Some of these adhesins have been characterized in molecular detail, including the fibronectin adhesins (FnBPA and FnBPB), the collagen adhesin (Cna), and the fibrinogen adhesin (clumping factor, Cifa) (reviewed in Refs. 5 and 6). Site-specific mutants have been isolated and compared with parental strains in both in vitro and in vivo models of adherence and infection, and there is significant evidence that each of these adhesins is an important determinant for colonization and virulence (5, 6). These proteins have similar features, including a signal peptide at the NH\(_2\) terminus (which is cleaved during secretion across the plasma membrane) and at the COOH terminus, an LPXTG motif preceding a hydrophobic membrane spanning region, and a positively charged tail, which are involved in anchoring the proteins in the cell wall.

S. aureus also expresses several proteins which interact with the immune system. Protein A is cell wall-associated protein that comprises five (in some isolates four) repeated units of 58 amino acids, each of which can bind to the Fc region of IgG (7). Protein A interferes with opsonophagocytosis (8) and is an important virulence factor (9, 10). Some of the expressed toxins (enterotoxins A–E, epidermolytic toxin A, and TSST-1) act as superantigens (11) by binding to human and mouse class II major histocompatibility complex (MHC) proteins and stimulating T cells to proliferate nonspecifically (12, 13). These superantigens can act as important mediators of toxic shock and other acute reactions.

Recently, we described a novel 72-kDa surface protein of S. aureus strain FDA 574 that is capable of binding to several extracellular matrix proteins, including fibronectin, fibrinogen, vitronectin, bone sialoprotein, and thrombospondin (14). In this paper, we report on the molecular cloning and the complete nucleotide sequence of the gene encoding this protein. The deduced protein contains repeated subdomains that share striking sequence homology with a segment of the peptide binding groove of the \( \beta \) chain of MHC class II mammalian proteins. We have designated the protein as Map (MHC class II analogous protein) and demonstrated that the purified recombinant bacterial protein specifically recognizes a 15-amino acid residue synthetic peptide derived from vitronectin.

**EXPERIMENTAL PROCEDURES**

Materials—E. coli strain J1110 was used as the bacterial host for plasmids and pBluescript SK (+) (Stratagene, La Jolla, CA) was used as the cloning vector. S. aureus strain FDA 574 was obtained from the United States Food and Drug Administration. Luria agar and broth (LA and LB; Difco) were used for growth of E. coli and S. aureus strains. Ampicillin (100 \( \mu \)g/ml) (Sigma) was added when appropriate. Restriction and modification enzymes were purchased from U. S. Biochemical Corp. or Life Technologies, Inc. Isopropyl-\( \beta \)-D-thiogalactopyranoside was purchased from Life Technologies, Inc. Purified digoxigenin was obtained from Advanced DNA Technologies Laboratory, Texas A & M University, College Station, TX. All other chemicals were molecular biology grade from Sigma or U. S. Biochemical Corp.

DNA Manipulation and Sequencing—DNA manipulations were performed using standard procedures (15, 16). DNA was sequenced with

---

* This study was supported by National Institutes of Health Grants HL47313 and AI20624 and by the Arthritis Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**To whom correspondence and reprint requests should be addressed:** Institute of Biosciences and Technology, 2121 West Holcombe Blvd., Houston, TX 77030-3303. Tel.: 713-677-7551; Fax: 713-677-7576; E-mail: mhook@ibt.tamu.edu.

The abbreviations used are: MHC, major histocompatibility complex; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; CAPS, 3-(cyclohexylamino)propanesulfonic acid.

**The Journal of Biological Chemistry**

Vol. 270, No. 37, Issue of September 15, pp. 21457–21460, 1995
© 1995 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.
S. aureus expresses a MHC Class II Analog

the Sequenase 2.0 DNA sequencing Kit (U. S. Biochemical Corp.) and with the thermocycling sequencing method using the Circum Vent Thermal Cycle Dideoxy DNA Sequencing Kit (New England Biolabs). Oligonucleotides corresponding to sequences from the Bluescript clones were used as DNA sequencing primers. The final sequence was determined from both strands.

Cloning of the map Gene from S. aureus FDA 574—Polyclonal antibody raised against the purified native Map (14) were used to screen a λgt11 library constructed from genomic DNA of S. aureus FDA 574. One clone with a 5.7-kilobase pair insert was detected and called pMAP4. The NH2-terminal amino acid sequences of trypsin generated in SDS-polyacrylamide gel electrophoresis. Some His6-tagged proteins were used as DNA sequencing primers. The final sequence was determined from both strands.

Expression and Purification of Native and Recombinant Map—Native Map was extracted from S. aureus FDA 574 cells with 1 M LiCl and purified as described previously (14). The map gene was amplified from chromosomal DNA of S. aureus FDA 574 by PCR, using the primers 5′-CGGCAAATCACTTCAAGT-3′ and 5′-CGGTCGACGCTACGGTAAT-3′, containing the restriction sites BamHI and SalI, respectively (underlined). The reaction mixtures contained 10 ng of target DNA, 200 pm of forward and reverse primers, 1.5 mM MgCl2, 2 μM of each dNTP, 10 mM Tris-HCl, pH 9, 50 mM KCl, 0.1% Triton X-100, and 2.5 units of Taq DNA polymerase (Life Technologies, Inc.). The reaction mixtures were overlaid with 100 μl of mineral oil and amplified for 30 cycles consisting of a 1-min denaturation period at 94 °C, a 1-min anneal temperature at 55 °C, and a 1-min extension period at 72 °C. After amplification, 10 μl was analyzed by agarose gel electrophoresis (1% agarose). The PCR product was treated with SDS and proteinase K prior to digestion and ligation (17). The product was digested with BamHI and SalI and ligated with pBluescript SK+ (Stratagene) to form pMAP1. This plasmid was transformed into E. coli strain M15 (Qiacore Inc.). A culture harboring pMAP1 was grown in LB until it reached an A600 of 0.6. Isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 0.5 mM, and cells were incubated at 37 °C for another 4 h. Cells were harvested, lysed by passage through a French press (twice at 20,000 pounds/inch²), and the lysate was centrifuged at 50,000 × g for 20 min. The Map fusion protein, which contained six histidines at the amino terminus, was localized to the insoluble pellet. This pellet was solubilized in 6 M guanidine hydrochloride, and the Map fusion protein was purified by metal chelating chromatography (18) under denaturing conditions. The recombinant Map protein migrated slightly slower than the native map in SDS-polyacrylamide gel electrophoresis. Some His6-tagged proteins have been shown to bind to 5′-10 amino acids after the putative signal sequence core. The sequence of the 10 amino acids after the putative signal sequence core is identical to the region of 19 residues followed by the repeated domains. Typical motifs, associated with cell wall-anchored proteins and found in most Gram-positive bacterial surface proteins, were present even in the COOH terminus of the Map protein. This is in agreement with our previous study (14), which demonstrated that the Map protein can be released from the bacterial cell surface by extraction with 1 M LiCl, suggesting that Map is not covalently anchored to the cell wall.

S. aureus FDA 574 Map Is Homologous to Eukaryotic MHC Class II Proteins—Within each of the six repeated 110-amino acid domains, there is a subdomain of 31 residues (Fig. 1B). These subdomains are highly homologous with the amino-terminal domain of many MHC class II proteins from different mammalian species (Fig. 2). For example, when compared with the amino-terminal domain of the β chain of HLA-DR, the respective subdomains were 48, 48, 29, 28, 39, and 10% identical. However, if conservative amino acid substitutions are included, the respective subdomains were 61, 65, 52, 59, 52, and 45% similar. The three-dimensional structure of the human MHC antigen HLA-DR1 has been determined (25). The binding site for peptides has been located to a groove composed of 8 β sheet strands flanked by two antiparallel helical walls at the amino-terminal end of the β heterodimer. It was concluded that as well as highly polymorphic amino acids participate in peptide binding within the groove (25, 26). The region in the β chain of MHC class II molecules, which is homologous to the subdomains of Map, contains residues involved in peptide binding.

Western Ligand Blotting Analysis of Purified Native and Recombinant Map—The remarkable sequence similarity between the S. aureus FDA 574 Map protein and the eukaryotic MHC class II molecules suggested (a) that the reported binding of Map to several matrix proteins may be due to the recognition of specific and possibly similar peptide sequences in these matrix proteins rather than to other common structural features such as carbohydrates (b) that Map might be capable of binding to small peptides in an analogous fashion to MHC class II molecules. To examine these hypotheses, recombinant Map was expressed in E. coli as a fusion protein with a poly-

RESULTS AND DISCUSSION
histidine tail and purified by metal-chelating chromatography (Fig. 3, lane 2). Both native and purified recombinant Map protein bound to labeled vitronectin, fibrinogen, recombinant osteopontin (Fig. 3, lanes 3–8), and fibronectin (data not shown). The recombinant osteopontin was analyzed by electrospray mass spectroscopy and shown to have a mass that corresponds to the predicted amino acid sequence. Hence, the protein has not been subjected to any post-translational modifications. Therefore, the binding of 125I-labeled recombinant osteopontin, which does not contain any additional moieties, indicates that Map binding to the matrix protein involves a specific protein-protein interaction.

Binding of Map to a Synthetic Peptide—To investigate whether Map is capable of binding to short peptides, purified recombinant Map was labeled with 125I and studied for binding to a panel of eight immobilized synthetic peptides in an enzyme-linked immunosorbent assay type assay. Radiolabeled Map bound to a 15-amino acid peptide (residues 347–361 of vitronectin) (Fig. 4). This sequence contains the heparin binding domain of vitronectin, and this peptide was previously found to partially inhibit the adherence of S. aureus to immobilized vitronectin (22). The binding of 125I-labeled Map to immobilized peptide increased when increasing amounts of peptide were used to coat the microtiter wells (Fig. 4). In a Western affinity blot assay, binding of the 125I-labeled vitronectin peptide to both native and recombinant Map could also be detected (data not shown). The Map protein failed to bind to the other seven peptides tested. Some of these peptides were of similar size and charge as the vitronectin peptide, suggesting a specific interaction between Map and the vitronectin peptide.

Previous studies have shown that S. aureus can bind a number of different host proteins (reviewed in Ref. 5).
S. aureus Expresses a MHC Class II Analog

We thank Dr. Charles Arntzen for comments on the manuscript.

REFERENCES

1. Bliska, J. B., Galan, J. E., and Falkow, S. (1993) Cell 73, 903–910
2. Finlay, B. B., and Falkow, S. (1989) Microbiol. Rev. 53, 210–230
3. Sandros, J., and Tuanonen, E. (1993) Trends Microbiol. 1, 192–196
4. Freer, J. H., and Arbuthnott, J. P. (1983) Pharmacol. Ther. 19, 55–106
5. Patti, J. M., Allen, B. L., McGavin, M. J., and Höök, M. (1994) Annu. Rev. Microbiol. 48, 585–617
6. Foster, T. J., and McDevitt, D. (1994) Infections Associated with Indwelling Medical Devices (Bisno, A. L., and Walvogel, F. A., eds) 2nd Ed., pp. 31–44, ASM Press, Washington, D.C.
7. Uhlen, M., Guss, B., Nilsson, B., Gatenbeck, S., Philipson, C., and Lindberg, M. (1984) J. Biol. Chem. 259, 1695–1702
8. Gemmell, C. G., Tree, R., Patel, A., O’Reilly, M., and Foster, T. J. (1990) Zentralbl. Suppl. 21, 231–236
9. Patel, A. H., Nowlan, P., Weavers, E. D., and Foster, T. J. (1987) Infect. Immun. 55, 1310–1310
10. Foster, T. J., O’Reilly, M., Phoanmaeng, P., Cooney, J., Patel, A. H., and Bramley, A. J. (1990) in Molecular Biology of the Staphylococci (Novich, R. P., ed) pp. 405–417, VCH Cambridge, New York
11. Schlievert, P. M. (1993) J. Infect. Dis. 167, 997–1002
12. Kim, J., Urban, R. G., Strominger, J. L., and Wiley, D. C. (1994) Science 266, 1870–1874
13. Jardeizky, T. S., Brown, J. H., Gorga, J. C., Stern, L. J., Urban, R. G., Chi, Y., Stauffacher, C., Strominger, J. L., and Wiley, D. C. (1994) Nature 368, 711–713
14. Homonylo McGavin, M., Krajevska-Pitarasik, D., Ryden, C., and Höök, M. (1993) Infect. Immun. 61, 2479–2485
15. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
16. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Smith, J. A., Seidman, J. G., and Struhl, K. (1993) Current Protocols in Molecular Biology, Greene Publishing and Wiley-Intersciences, New York
17. Crowe, J. S., Cooper, H. J., Smith, M. A., Simms, M. J., Parker, D., and Gewert, D. (1990) Nucl. Acids Res. 19, 184
18. Schlievert, P. M., and Schumann, W. (1994) Gene (Amst.) 147, 91–94
19. McFarland, R. J., Garza, S., Butler, W. T., and Höök, M. (1995) Ann. N.Y. Acad. Sci. 760, 327–330
20. Hunter, W. M. (1978) Handbook of Experimental Immunology (Weir, D. M., ed) pp. 14.1–14.40, Blackwell Scientific Publications, Ltd., Oxford
21. McDevitt, D., Vaudaux, P., and Foster, T. J. (1992) Infect. Immun. 60, 1514–1523
22. Liu, O. D., Flock, J. I., and Wadström, T. (1994) J. Biochem. (Tokyo) 116, 457–463
23. Rothbard, B. B., and Geffer, M. L. A. (1991) Rev. Immunol. 9, 527–565
24. Hunt, D. F., Michel, H., Dickinson, T. A., Shabanowitz, J., Cox, A. L., Sakaguchi, K., Apelä, E., Grey, H. M., and Sette, A. (1992) Science 256, 1817–1820
25. Brown, J. J., Jardeizky, T. S., Gorga, J. C., Stern, L. J., Urban, R. G., Strominger, J. L., and Wiley, D. C. (1993) Nature 364, 33–39
26. Stern, L. J., Brown, J. J., Jardeizky, T. S., Gorga, J. C., Urban, R. G., Strominger, J. L., and Wiley, D. C. (1994) Nature 368, 215–221