<14) hydrogen bonding to the oxydianion in the wild-type SBP (*8*).

Although the active site Ser in proteinases has been successfully mutated to Cys, the ultimate goal or desired result has not been achieved; engineered thiol trypsin exhibits essentially no enzymatic activity with normal amide and ester substrates (*3*). The cause of the failure to achieve the desired result is not clearly understood (*15*). Small geometrical changes resulting from the mutation could have an effect far more profound than that described here. Although little publicized, the catalytic Ser or Cys residues of proteolytic enzymes are, like Ser^{130} of SBP, engaged in cooperative hydrogen bonding; they simultaneously donate to a His residue that is part of the charge relay system and accept an NH group or a solvent molecule.

The results presented here demonstrate the sensitivity of a Ser to Cys mutation. If the effect of the mutation is as deleterious as the one reported here, one wonders what the extent of the effect might be if the residue is directly involved in enzyme catalysis. In any case the use of site-directed mutagenesis to probe or quantitate structure-function relations is fraught with uncertainty. To minimize the uncertainty, it is important that many mutational changes be made. For amino acid replacements to be rationally selected and generated and for interpretation of the results to be properly made, a well-refined high-resolution structure is a prerequisite.

Finally, there seems to be a propensity of hydroxyl-containing residues, as well as peptide units, to interact with charged groups and ligands (*9, 16, 17*). An excellent example has been recently shown in the well-refined 1.7 Å resolution structure of the phosphate-binding protein in complex with phosphate; the completely dehydrated and sequestered phosphate is held in place by a total of 12 hydrogen bonds, 5 with peptide unit NH groups and 4 with hydroxyl side chains (*17*). We have further noted that ligand-gated ion channels are known to contain regions rich in hydroxyl side chains and that, like in the binding protein-phosphate complex, these hydroxyls may have properties that are particularly important in achieving the requisite specificity and speed of ion movements (*17*). The sensitivity and differential effects of mutations of Ser to Cys, Ala, and Gly at a highly specific ion-binding site as demonstrated here could be used to probe similar residues residing in these channels or in other ligand-binding sites.

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11. The expression vectors and methods used in the mutagenesis in *E. coli* have been described (*6*). Mutations of $Ser^{130} \rightarrow$ Gly, $Ser^{130} \rightarrow$ Ala, and $Ser^{130} \rightarrow$ Cys and were directed by oligonucleotides 5′-CGCCACCACCGCTTTTC-3′, 5′-CGCCACCAGCGCTTTTC-3′, and 5′-CGCCACCACAGCTTTTC-3′, respectively, where the bold underlined base indicates mismatches. In each mutagenesis, the entire SBP gene was sequenced to ensure that the desired mutation was attained.
12. Proteins were purified from a 12-liter growth of appropriate mutant *E. coli* cells with the use of the method previously described for the wild-type SBP (*5, 6*). Purification of SBP with this method is achieved mainly by the use of DEAE-53 ion exchange chromatography. The Ala^{130} and Gly^{130} SBP mutants were further purified by high-performance liquid chromatography (HPLC) with a preparative Synchrom Q300 anion exchange column. In the purification of the Ser^{130}Cys mutant, β-mercaptoethanol was added to all solutions to a final concentration of 1 mM. Moreover, after chromatography on a DEAE column, the mutant protein was further purified by isoelectric focusing (ampholyte pH 6 to 8) with a Bio-Rad Rotofor Preparative IEF Cell. Protein purity was determined by SDS-polyacrylamide gel electrophoresis and isoelectric focusing with Pharmacia's PhastGel. Protein concentration was determined spectrophotometrically by using an extinction coefficient of 1.2 ml mg^{-1} cm^{-1} (*7*).
13. Note that the binding activity of Gly^{130} and Ala^{130} SBP mutants was measured at pH 7.5, the pH at which the wild-type protein activity is normally measured (*7, 8*). The Cys^{130} SBP mutant has maximum activity at about pH 6.0 (see Table 1).
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Crystal Structure of Defensin HNP-3, an Amphiphilic Dimer: Mechanisms of Membrane Permeabilization

CHRISTOPHER P. HILL, JEFF YEE,* MICHAEL E. SELSTED,† DAVID EISENBERG

Defensins (molecular weight 3500 to 4000) act in the mammalian immune response by permeabilizing the plasma membranes of a broad spectrum of target organisms, including bacteria, fungi, and enveloped viruses. The high-resolution crystal structure of defensin HNP-3 (1.9 angstrom resolution, *R* factor 0.19) reveals a dimeric β sheet that has an architecture very different from other lytic peptides. The dimeric assembly suggests mechanisms by which defensins might bind to and permeabilize the lipid bilayer.

NEUTROPHILS CONSTITUTE 50 TO 70% of the total white blood cells in humans. They play a vital role in the immune response by ingesting invading microorganisms, which are then destroyed by one of two general mechanisms. The "oxygen-dependent" mechanism results from the production of superoxide, which is converted to potent oxidants termed "reactive oxygen intermediates" (*1*). The other, "oxygen-independent," defense mechanism occurs when the microbicidal-cytotoxic proteins of cytoplasmic granules are discharged into the phagocytic vacuole (*2*).

Defensins account for ~30% of the total protein in human azurophil granules (*3*). They are small (molecular weight of 3500 to 4000), cationic, disulfide cross-linked proteins that show in vitro activity against Gram-negative and Gram-positive bacteria (*3, 4*), fungi (*5*), mammalian cells (*6*), and enveloped viruses (*7*). The work of Lehrer and colleagues shows that defensins permeabilize both the inner and outer membranes of *Escherichia coli*, and that inner-membrane permeabilization is coincident with cell death (*8*). A membrane potential is apparently required for defensin action, since cells are killed only when metabolically active and they are protected by membrane-depolarizing agents such as carbonylcyanide M-chlo-

C. P. Hill, J. Yee, D. Eisenberg, Molecular Biology Institute and Departments of Chemistry and Biochemistry, University of California, Los Angeles, CA 90024–1570.
M. E. Selsted, Departments of Medicine and Pathology, University of California, Los Angeles, CA 90024–1732.

*Present address: University of California College of Medicine, Irvine, CA 92717.
†Present address: Department of Pathology, University of California College of Medicine, Irvine, CA 92717.