The Structure of Human Beta-Defensin-2 Shows Evidence of Higher-Order Oligomerization

D. Hoover (NCI-FCRDC), K. Rajashankar (NCI-FCRDC and BNL-NSLS), R. Blumenthal, A. Puri, J. Oppenheim, O. Chertov, and J. Lubkowski (NCI-FCRDC)

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Introduction: Multicellular organisms share an innate defense against microorganisms that is based on small cationic peptides known as defensins. The microbicidal activity of defensins stems from the permeabilization of anionic lipid bilayers and the subsequent release of cellular contents. Interactions between defensins and bacterial membranes are governed mainly by electrostatic forces. One mechanism of permeabilization is thought to involve the formation of ion pores in bacterial membranes. A physical model of such a pore has been constructed based on the X-ray structure of the alpha-defensin human neutrophil-peptide-3 (hNP3). A second mechanism has also been proposed (sometimes called the "carpet" model) in which the peptides aggregate into positively charged patches that neutralize anionic lipid head groups of the membrane over a wide area around the peptides. This neutralization disrupts the integrity of the lipid bilayer, causing transient gaps to arise and allowing ions (and larger molecules, depending on the extent of local disruption) to permeate the membrane. Except for the X-ray structure of hNP3, no other oligomeric structures of defensins have been determined. To investigate the importance of the structure for the antimicrobial activity of human beta-defensin-2 (hBD2), we solved the X-ray structure of this defensin by using a novel method of derivatizing the protein crystals, followed by the multiwavelength anomalous diffraction (MAD) phasing protocol. Additionally, we studied the permeabilization of large unilamellar vesicles by native and reductively alkylated hBD2, as well as the oligomerization of native hBD2 in solution.

Methods and Results: Crystals of hBD2 were obtained, and two crystal forms grew together in the same hanging drops. The orthorhombic form belongs to space group $P2_12_12$ with cell constants *a* = 50.05 Å, *b* = 103.91 Å, and *c* = 28.27 Å. The monoclinic form belongs to space group $P2_1$ with cell constants *a* = 54.53 Å, *b* = 79.95 Å, *c* = 74.27 Å, and *beta* = 105.30°. Orthorhombic form crystals were soaked briefly (60 seconds) against KBr and KI (0.25 M) in the cryoprotectant solution immediately prior to freezing in the nitrogen stream and diffraction experiments. MAD data at three wavelengths near the Br edge were collected for the KBr-soaked crystal, and one data set was collected at 1.54 Å for the KI-soaked crystal. Bromide and iodide sites were located from the anomalous differences. Anomalous scattered sites were refined for each of the four data sets and initial phases were generated for data from 24 to 2.0 Å resolution. The phases were then extended to 1.4 Å and refined with a solvent content of 35%. An initial model was built automatically by placing single atoms (oxygen atoms) into electron-density peaks. The protein model was then automatically built based on the positions of the oxygen atoms, and the structure factors were improved by combining the initial experimental phases with those calculated from the model. In total, 1,334 atoms were built into the map (out of a total 1,424, or ~94% of the final model). The orthorhombic form model was completed and refined to 1.35 Å. In the final steps, anisotropic B factors for all atoms were individually refined, and 194 water molecules and one sulfate ion were added. The R-value for all reflections (20-1.35 Å) is 15.9% (R_{free} 23.5%). The solution of the monoclinic form was obtained by molecular replacement technique. The final monoclinic model contains 814 water molecules and 5 sulfate ions were added. The R-value for all reflections (25-1.7 Å) is 18.8% (R_{free} 25.5%).

Conclusions: Unlike the previously determined structures of either alpha- or beta-defensins, this X-ray structure of hBD2 displays a unique secondary-structure element at its N-terminus. The presence of this N-terminal a helix gives rise to a novel dimer topology. Light-scattering experiments indicate that dimers are the dominant oligomeric form of hBD2 in solution. This mode of dimerization is probably conserved within the beta-defensin family and has implications for the differences in activities seen between alpha- and beta-defensins. The beta-defensin mode of dimerization allows the formation of a higher-order oligomerization of the protein as an octamer. The conservation of the octamer across two crystal forms provides evidence that this octamer represents the stable, native quaternary structure of hBD2. The stability of the octamer is further supported by the burial of hydrophobic surface area and the distribution of temperature factors. It is therefore likely that the octameric form of hBD2 represents the form bound to bacterial membranes. The structural and electrostatic properties of the hBD2 octamer do not show any evidence for burial within bacterial membranes or the formation of a bilayer-spanning pore when bound to the membrane, unlike the mechanism proposed for hNP3. The uniform surface distribution of positively charged residues suggests that hBD2 disrupts the bacterial membrane via electrostatic interactions with the polar head groups of the membrane. Because of the expected crisis of bacterial antibiotic resistance, understanding the mechanism of defensins might aid the development of novel antibiotics, and the X-ray structure of hBD2 will allow for the design of more streamlined and effective antibiotics.