

Example of Short Report

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X-RAY CRYSTAL STRUCTURE OF HUMAN MYELOPEROXIDASE AT 1.8 Å RESOLUTION

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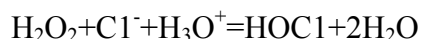
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INTRODUCTION. Myeloperoxidase (MPO, EC 1.11.1.7) is a heme-containing enzyme found in mammalian neutrophils, where it catalyzes the hydrogen peroxide-mediated peroxidation of halide ions and the pseudohalide thiocyanate according to the following reaction (1,2):



We have extended the x-ray crystal structure of the human enzyme to 1.8 Å resolution and characterized the halide-binding sites.

METHOD. Crystals of human MPO isoform C were grown by a method similar to those reported previously. X-ray diffraction data were recorded using a 30-cm MAR image plate detector (MAR Research, Hamburg, Germany) mounted on a Rigaku Ru300 x-ray generator equipped with long focusing mirrors (Area Detector Systems Corp., Poway, CA). A native low temperature (-180°C) diffraction data set to 1.8 Å resolution was recorded from a single crystal, flash-frozen in a stream of cold nitrogen from a crystal cooling device (Area Detector Systems Corp.).

RESULTS. The final model consists of 10,307 nonhydrogen atoms, including 1140 amino acids, 2 hemes, 16 sugars, 2 calcium ions, 2 chloride ions, 4 sulfates, 6 acetates, and 838 water molecules. The crystallographic *R*-factor for reflections in the 30 to 1.8 Å range is 19.7%, and the corresponding free *R*-factor is 23.9%. The root mean square deviations from ideality for bond lengths and angles are 0.013 Å and 1.23°. Results confirm that the heme is covalently attached to the protein via 2 ester linkages as well as a sulfonium ion linkage between the β-carbon of the pyrrole ring A vinyl group and the sulfur atom of Met²⁴³. Bromide binds in the distal heme cavity, in close proximity to the distal His⁹⁵, where it replaces the water molecule hydrogen bonded to Gln91 (see Fig. 1).

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Fig.1. Entire MPO dimer, viewed along the molecular dyad axis.

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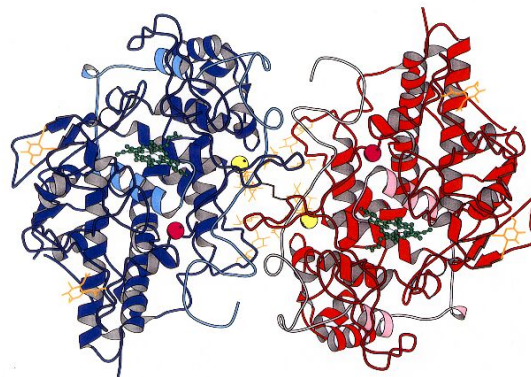
DISCUSSION. The mode of heme binding revealed by our studies may be unique to MPO. Although other members of this gene family all contain the conserved Asp^{P94} and Glu²⁴², they lack methionine at position 243 (3). It is therefore likely that other mammalian peroxidases also have heme-protein ester linkages analogous to those of MPO,

but that the sulfonium ion linkage is a feature unique to MPO. We propose that the reported spectral changes that accompany halide binding to MPO result from halide binding to the site that we observe in the distal cavity. We propose that the resting MPO the distal cavity bromide-binding site corresponds to the site for inhibition of MPO by halides. Catalysis of halide peroxidation by MPO does not necessitate a halide substrate-binding site on the resting enzyme, because it is only necessary for halides to interact with compound I for peroxidation to occur.

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