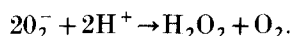


Characterization of Crystals of Genetically Engineered Human Manganese Superoxide Dismutase

The genetically engineered human manganese superoxide dismutase crystallizes in space group $P2_12_12$ with $a = 75.51 \text{ \AA}$, $b = 79.00 \text{ \AA}$, $c = 67.95 \text{ \AA}$. At room temperature the crystals are not stable against radiation, so we cooled them to 90 K and collected a data set to 3 Å resolution at this temperature.

Before any compound, and in particular a macromolecule, is used as a drug for humans it would be advantageous to establish its three-dimensional structure. Detailed knowledge of the crystal structure and mechanism of action of such drug-enzymes could be especially useful in the intelligent design of second generation molecules with improved properties. These premises led us to undertake the study of the three-dimensional structure of the recently cloned human manganese superoxide dismutase (MnSOD†) (Beck *et al.*, 1987), a member of a family of enzymes, the SODs, which is believed to be of great therapeutic potential in various situations, originating from oxidative damage (Oberley, 1985). The three-dimensional structure of a related MnSOD from *Thermus thermophilus* HB38 has been determined at 2.4 Å resolution (Stallings *et al.*, 1985; Ludwig *et al.*, 1986) (1 Å = 0.1 nm).

SODs are metalloproteins which were found to catalyse the dismutation of superoxide radicals (McCord & Fridovich, 1969):



O_2^- radicals are generated in many biological oxidations, e.g. in the autooxidation of flavins (Ballou *et al.*, 1969), hemoproteins (Misra & Fridovich, 1972b), reduced ferredoxin (Misra & Fridovich, 1971) and hydroquinones, as well as in the final stages of the purine metabolism, i.e. in the oxidation of xanthine to uric acid catalysed by xanthine oxidase (Misra & Fridovich, 1972a). Subcellular organelles, such as mitochondria (Loschen *et al.*, 1974) and chloroplasts (Halliwell, 1975) also produce superoxide radicals. Three different types of SOD have been characterized in terms of the metals bound in their active sites: the Mn or Fe-containing forms, which are similar in sequence and structure (Stallings *et al.*, 1984) versus the Cu/Zn SOD, which appears to bear no obvious sequence or structural relationship to the two other enzymes. The Cu/Zn enzyme is primarily found in the cytoplasm of eukaryotic cells, whereas the iron form is essentially a prokaryotic enzyme. The Mn form appears to have originated in prokaryotes, but is also found in mitochondria of eukaryotes.

Human MnSOD is a multimeric enzyme made up

of 198 amino acids per subunit with a molecular weight of 22,000, whose amino acid sequence has been determined (Barra *et al.*, 1984), and exists as a tetramer (McCord *et al.*, 1977). The enzyme has been cloned and expressed in *Escherichia coli* and the cDNA sequence was found, with minor corrections, to agree with the above (Beck *et al.*, 1987). The recombinant MnSOD was purified from the bacterial extract, essentially according to previous procedures (McCord *et al.*, 1977; Asayama & Burr, 1984; Beck *et al.*, 1988). The protein used for the study described here was at least 98% pure (as determined by SDS/polyacrylamide gel electrophoresis), possessed a specific activity of 3600 units/mg, as determined by the xanthine/xanthine oxidase-cytochrome *c* assay (McCord & Fridovich, 1969), and had appropriate absorption spectra (in the visible and ultraviolet regions) and metal content (McCord *et al.*, 1977).

Crystals were obtained by the hanging drop technique at room temperature by equilibrating a solution containing 3.4 mg of enzyme/ml in ammonium phosphate buffer (pH 5.9), 10% 2-methyl-2,4-pentanediol (MPD) against 32% MPD. After two to three days, crystals grew with dimensions of 0.5 mm × 0.12 mm × 0.12 mm. The space group and cell dimensions were determined from them *via* precession photographs and confirmed on a Rigaku AFC5-R rotating anode diffractometer to be $P2_12_12$, with $a = 75.51 \text{ \AA}$, $b = 79.00 \text{ \AA}$, $c = 67.95 \text{ \AA}$.

On the basis of the volume of the unit cell, approximately $4.1 \times 10^5 \text{ \AA}^3$, and the measured density (floating method; bromobenzene/benzene) of 1.26 mg/ml, there are two subunit molecules per asymmetric unit. The calculated V_m value, assuming two molecules per asymmetric unit, is 2.3 Å/dalton, which fits very well in the range of values for proteins with a molecular weight between 20,000 and 40,000 (Matthews, 1968). At room temperature the crystals were found to last only half a day in the X-ray beam without radiation damage. We therefore decided to cool the crystal with liquid nitrogen to about 90 K following the shock cooling technique described by Hope (1988). Under these conditions the MnSOD crystals were found to be stable, and a complete set of X-ray data to 3 Å resolution was collected.

A search for heavy-atom derivatives has been initiated. We plan to determine the structure *via*

† Abbreviation used: SOD, superoxide dismutase.

multiple isomorphous replacement or the molecular replacement method based on the atomic coordinates of the related thermophilic bacterial MnSOD (Stallings *et al.*, 1985).

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