Identity of the metal ligands in the manganese- and iron-containing superoxide dismutases

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Alignment of the amino acid sequence of peptides obtained following digestion of *Photobacterium leiognathi* iron superoxide dismutase with the known sequence of *Bacillus stearothermophilus* manganese superoxide dismutase shows that the residues found to form ligands to the manganese are conserved in the iron enzyme. This indicates that the metal ligands in both proteins are identical.

Superoxide dismutase *Manganese* *Iron* *Amino acid sequence* *Metal ligand*

1. INTRODUCTION

The superoxide dismutases (EC 1.15.1.1) are a class of metalloproteins containing either copper and zinc or manganese or iron. The copper/zinc superoxide dismutase is the most extensively studied form of the enzyme [1–3]. The N-terminal sequences of manganese- and iron-containing superoxide dismutases indicated the proteins to be related [2] although their mechanisms of superoxide dismutation were found to be different [4,5]. The identity of the two superoxide dismutases has recently been confirmed by metal exchange experiments. The iron-containing superoxide dismutase isolated from *Bacteriodes fragilis* could be reconstituted with either iron or manganese without loss of activity [6] whilst the anaerobic *Propionibacterium shermanii* synthesised the manganese or iron enzyme depending on the metal supply [7]. The two enzymes had similar amino acid composition. The X-ray structure around 3 Å resolution has been reported for two iron-containing superoxide dismutases, however, in the absence of a primary structure for an iron superoxide dismutase, the amino acid ligands to the metal could not be identified [8,9]. Recently, the manganese and iron superoxide dismutases have been demonstrated to possess structural homology and the ligands to the manganese superoxide dismutase have been identified [10]. We have recently started the determination of the primary structure of the iron superoxide dismutase from *Photobacterium leiognathi*. The data so far obtained show that the residues indicated to be ligands to the manganese are also present in the iron protein.

2. MATERIALS AND METHODS

Iron superoxide dismutase was isolated from *P. leiognathi* by the method of Yamakura [11]. Apoprotein was prepared by incubation with 70% formic acid for 2 h at 40°C followed by dialysis against 50% acetic acid and lyophilization. Apoprotein was carboxymethylated with iodo-[^{14}C]acetate and then digested with trypsin or chymotrypsin. After digestion peptides were separated by high-performance liquid chromatography [12]. Analytical techniques for determination of amino acid compositions of peptides, N-terminal residues, mammal sequences, carboxypeptidase digestions and state of amidations of acid residues were carried out as described in [12].

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3. RESULTS AND DISCUSSION

The amino acid sequence deduced from analysis of tryptic and chymotryptic peptides obtained from *P. leiognathi* iron-containing superoxide dismutase is reported in fig.1 and aligned with the known sequence for the *Bacillus stearothermophilus* manganese superoxide dismutase [13]. The alignment has been carried out in such a way as to maximise the homology between the two proteins. The peptides sequenced represent over 80% of the total sequence of the iron superoxide dismutase. A total of 79 residues were found to align correctly with the manganese superoxide dismutase sequence. This represents about 47% identity of the partial iron superoxide dismutase primary structure with the primary structure of the manganese superoxide dismutase. Sequence homologies between the various manganese superoxide dismutases have been found to be between 40 and 60% [2].

The predicted ligands for the iron were determined from the crystal structure to be residues 26, 69, 148 and 152 in the amino acid sequence [8,9]. However, the metal ligands in manganese superoxide dismutase were found to correspond to positions 26, 81, 163 and 167 which correspond to His, His, Asp and His in the sequence of the *B. stearothermophilus* enzyme. In the absence of a complete primary structure for an iron superoxide dismutase it is not possible to determine the definite numbering of the amino acid ligands to the iron with the exception of His 26. However, the

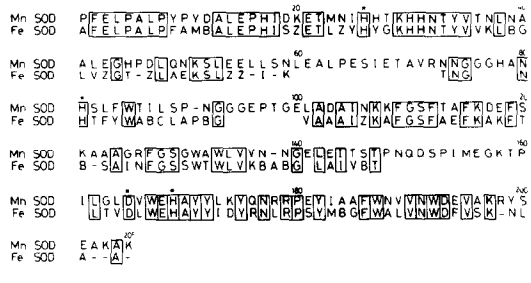

Fig.1. Amino acids sequence of *Bacillus stearothermophilus* manganese superoxide dismutase (Mn SOD) and *Photobacterium leiognathi* iron superoxide dismutase (Fe SOD). Identical residues are boxed. Metal ligands are indicated with an asterisk. Gaps in the sequence of the Fe SOD relate to undetermined sequences.

alignment reported in fig.1 indicates that the ligands reported from the manganese are conserved in the iron enzyme, indicating that the iron and the manganese may have similar ligands. This is plausible because it has been shown to be possible for the iron to be exchanged for manganese [6]. A similar situation may exist between the iron- and manganese-containing acid phosphatases [14]. Also conserved in the manganese and iron enzyme sequences are most of the glycine residues which may be connected with the three-dimensional packing of the molecule. In fact, the spatial arrangement of the principal secondary structural features present in the iron superoxide dismutases was found to be conserved in the manganese superoxide dismutase [10].

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REFERENCES

[1] Bannister, J.V. and Rotilio, G. (1984) Developments in Biochemistry, vol.26, pp.146–189, Elsevier, New York.

[2] Parker, M.W., Schenina, M.E., Bossa, F. and Bannister, J.V. (1984) Inorg. Chim. Acta 91, 307–317.

[3] Fielden, E.M. and Rotilio, G. (1984) in: The Copper Proteins (Lontie, R. ed.) vol.2, pp.27–61, CRC Press, FL.

[4] McAdam, M.E., Fox, R.A., Lavelle, F. and Fielden, E.M. (1973) Biochem. J. 165, 71–79.

[5] Lavelle, F., McAdam, M.E., Fielden, E.M., Roberts, P.B., Puget, K. and Michelson, A.M. (1977) Biochem. J. 161, 3–11.

[6] Gregory, E.M. and Dapper, C.H. (1983) Arch. Biochem. Biophys. 220, 293–300.

[7] Meier, B., Barra, D., Bossa, F., Calabrese, L. and Rotilio, G. (1982) J. Biol. Chem. 257, 13977–13980.

[8] Ringe, D., Petsko, G.A., Yamakura, F., Suzuki, K. and Ohmori, D. (1983) Proc. Natl. Acad. Sci. USA 80, 3879–3883.

[9] Stallings, W.C., Powers, T.B., Partridge, K.A., Fee, J.A. and Ludwig, M. (1983) Proc. Natl. Acad. Sci. USA 80, 3884–3888.

[10] Stallings, W.C., Partridge, K.A., Strong, K.K. and Ludwig, M. (1984) J. Biol. Chem. 259, 10695–10699.
[11] Yamakura, F. (1976) Biochim. Biophys. Acta 422, 280–294.
[12] Barra, D., Schinina, M.E., Simmaco, M., Bannister, J.V., Bannister, W.H., Rotilio, G. and Bossa, F. (1984) J. Biol. Chem. 259, 12595–12601.
[13] Brock, C.J. and Walker, J.E. (1980) Biochemistry 19, 2873–2882.
[14] Kawabe, H., Sugiwa, Y., Terauchi, M. and Tanaka, H. (1984) Biochim. Biophys. Acta 784, 81–89.