Volume 221, number 1, 87-90 FEB 05054 August 1987

The primary structure of iron superoxide dismutase from *Escherichia coli*

M. Eugenia Schininà, Letizia Maffey, Donatella Barra, Francesco Bossa, Krystyna Puget* and Adolf M. Michelson*

Department of Biochemical Sciences and CNR Centre for Molecular Biology, University of Rome La Sapienza, 00185 Rome, Italy and **Fondation Edmond de Rothschild, Institut de Biologie Physico-Chimique, Paris, France*

Received 8 July 1987

The complete amino acid sequence of iron superoxide from *Escherichia coli* has been determined. The sequence was deduced from analysis of peptides obtained after cleavage of the carboxymethylated apoenzyme with trypsin, *Stapholococcus aureus* protease or CNBr. The polypeptide chain is made up of 192 residues and is easily aligned with the other known amino acid sequences of iron and manganese superoxide dismutases from various sources. The iron superoxide dismutase from *E. coli* shows a significantly higher homology with the iron enzyme from a different organism than with the manganese isoenzyme from *E. coli*.

Iron superoxide dismutase; Primary structure; Sequence homology

1. INTRODUCTION

The superoxide dismutases (SOD, EC 1.15.1.1) are a class of metallo-proteins containing different metals, namely iron (FeSOD), manganese (MnSOD), or both copper and zinc (Cu,ZnSOD). The superoxide dismutases containing either manganese or iron have been grouped in a class distinct from the copper/zinc-containing isoenzymes on the basis of structural evidence and biological distribution. Structure-function relationships on the copper/zinc SODs have been the subject of various reviews [1-5], whereas information on the manganese- or iron-superoxide dismutases is less available.

The MnSOD is present in mitochondria and in bacteria, whilst the FeSOD is essentially a prokaryotic protein. In some cases MnSOD and FeSOD can be present in the same bacterial species. This is the case in *Escherichia coli*. The iron superoxide dismutase initially thought to be present in the periplasmic space of this species [6] was eventually confirmed to be present in the cytoplasm [7]. Although the iron and manganese SODs have similar biological activity, their biosynthesis in *E. coli* has been found to be influenced by either the superoxide radical or the culture conditions [8-11]. The available crystallographic data for the *Thermus thermophilus* MnSOD [12] have clarified structural characteristics, such as the identity of the metal ligands and the hydrophobicity of the active site, for this class of isoenzymes. In the case of the FeSOD, X-ray diffraction studies on both *Pseudomonas ovalis* and *E. coli* proteins [13,14] have provided only evidence for the tertiary structure of the two enzymes. This is because no amino acid sequence is available for either protein.

We therefore have undertaken the determination of the amino acid sequence of FeSOD isolated from *E. coli*. The results obtained provide the basis for (i) a more detailed interpretation of the available crystallographic data, and (ii) a better understanding of structural and evolutionary

Correspondence address: F. Bossa, Dipartimento di Scienze Biochimiche, Università La Sapienza. Piazzale Aldo Moro, 5, 00185 Roma, Italy

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

relationships between two isoenzymes from the same organism.

2. MATERIALS AND METHODS

Iron superoxide dismutase from *E. coli* was purified according to Yamakura [15]. Apoprotein prepared by dialysis against 8 M urea containing 10 mM EDTA adjusted to pH 3.8 with acetic acid, was carboxymethylated with iodo[^{14}C]acetate as in [16]; two samples (about 6 mg each) were digested with trypsin or *Staphylococcus aureus* V-8 protease, respectively [17]. Another 2 mg aliquot of apoprotein was cleaved at the level of tryptophan residues with CNBr according to [18]. Isolation of tryptic and *S. aureus* protease peptides was carried out by high-performance liquid chromatography on a macroporous reverse-phase column (Aquapore RP-300, 4.6 × 250 mm, 10 μm, Brownlee Labs) with gradients of 0–70% acetonitrile in 0.2% trifluoroacetic acid. Elution of peptides was monitored on a Beckman 165 spectrophotometer at both 220 and 280 nm. Analytical techniques for the determination of the N-terminal residue and amino acid composition of the peptides were carried out as described in [17]. Sequence analysis was performed on an Applied Biosystems model 470A gas phase protein sequencer equipped with an Applied Biosystems model 120A PTH analyzer for the on-line detection of phenylthiohydantoin amino acids. Samples were loaded onto trifluoroacetic acid-treated glass fiber filters, coated with polybrene and prewashed according to the manufacturer's instructions. The CNBr digest of the protein was directly analyzed on the automatic sequencer, by a modification of the express technique described by Shlyapnikov et al. [19].

3. RESULTS AND DISCUSSION

The complete amino acid sequence of the *E. coli* iron superoxide dismutase is given in fig.1. The sequence is made up of 192 residues and was reconstructed by isolation and analysis of a complete set of tryptic peptides which were aligned by overlapping peptides obtained following *S. aureus* protease and chemical cleavages.

In fig.1 the *E. coli* iron superoxide dismutase sequence is compared with that of the mangano isoenzyme from the same organism [20] and with the known amino acid sequence of *P. leiognathi* iron protein [17]. Gaps are inserted to maximize the homologies and to compensate for differences in length.

The amino acid sequences of mangano superoxide dismutases from human liver [16], mouse [21], *Saccharomyces cerevisiae* mitochondria [22], *Bacillus stearothermophilus* [23] and *T. thermophilus* [24] are not reported in fig.1 for the sake of clarity, however residues conserved in all the primary structures of the two FeSODs and six MnSODs are underlined. This indicates that 41 positions (18.8%) are conserved in the eight SODs. In fig.1 the positions of the identified ligands to the metal in the *T. thermophilus* MnSOD are also shown. These residues appear to be conserved in all the known primary structures both for manganese- and iron-containing proteins suggesting that His-26, -73 and -160 and Asp-156 are the candidates for metal binding also in the iron superoxide dismutase from *E. coli*. This is in fair agreement with the tentative assignment from the crystallographic data [14].

The percentage values of the extent of sequence homology between iron- and manganese-superoxide dismutases are reported in table 1. It appears that the iron protein from *E. coli* exhibits a higher homology with the FeSOD from *P. leiognathi* (75.1%) than with the manganese isoenzyme from the same species (41.4%). The homology between the *E. coli* iron and manganese isoenzymes is of the same order as that observed between the different manganese superoxide dismutases.

A more detailed analysis of the possible alignments between the two FeSODs and the six MnSODs (not reported) indicates the presence of certain amino acids in various critical structural positions in the amino acid sequence reported in fig.1. Thus the residues in positions 76, 77, 84, 92, 100, 150, 151, 179, 194 and 195 are conserved in all the MnSODs whereas the FeSODs have a different conserved amino acid. Five of these positions are in the 69–100 region, around ligand His-81, where the homology values are 97% for FeSODs and 29% for MnSODs. Furthermore, the residues in position 25, 72, 125 and 180 appear to be discriminated on the basis of whether the enzyme was isolated from mitochondria or from prokaryotes. The most critical differences between the two *E. coli* isoenzymes appear in positions 18, 90, 95, 96

Table 1

Sequence homologies between iron- and manganese-superoxide dismutases

	Mouse (Mn)	*S. cerevisiae* (Mn)	*B. stearoth.* (Mn)	*T. thermoph.* (Mn)	*E. coli* (Mn)	*P. leiognathi* (Fe)	*E. coli* (Fe)
(Mn) Human liver	93.9	45.5	48.8	49.3	43.3	37.7	40.2
(Mn) Mouse cDNA		43.3	49.0	48.1	42.9	38.7	40.2
(Mn) *S. cerevisiae*			39.2	41.6	39.0	35.7	34.5
(Mn) *B. stearoth.*				58.7	60.5	50.7	51.0
(Mn) *T. thermoph.*					51.2	38.0	40.2
(Mn) *E. coli*						39.5	41.4
(Fe) *P. leiognathi*							75.1

Values are given as percentage of sequence homologies

and 181 which in the mangano form are occupied by amino acids peculiar only for this organism.

These data confirm that the manganese- and iron-containing superoxide dismutases belong to the same family and suggest a very early occurrence of the possible gene duplication giving rise to the divergence of two distinct metallo-proteins without changing their function. The high extent of conservation of the iron SOD sequences would suggest, if confirmed by more sequence data, extra constraints on the structure of this sub-class of SOD, possibly imposed by some specific functional or topological features.

Further structural information would therefore be useful to confirm the discriminant characteristic of some positions in the primary structure of these isoenzymes.

ACKNOWLEDGEMENTS

We thank Dr J.V. Bannister for advice and helpful comments. This work was supported in

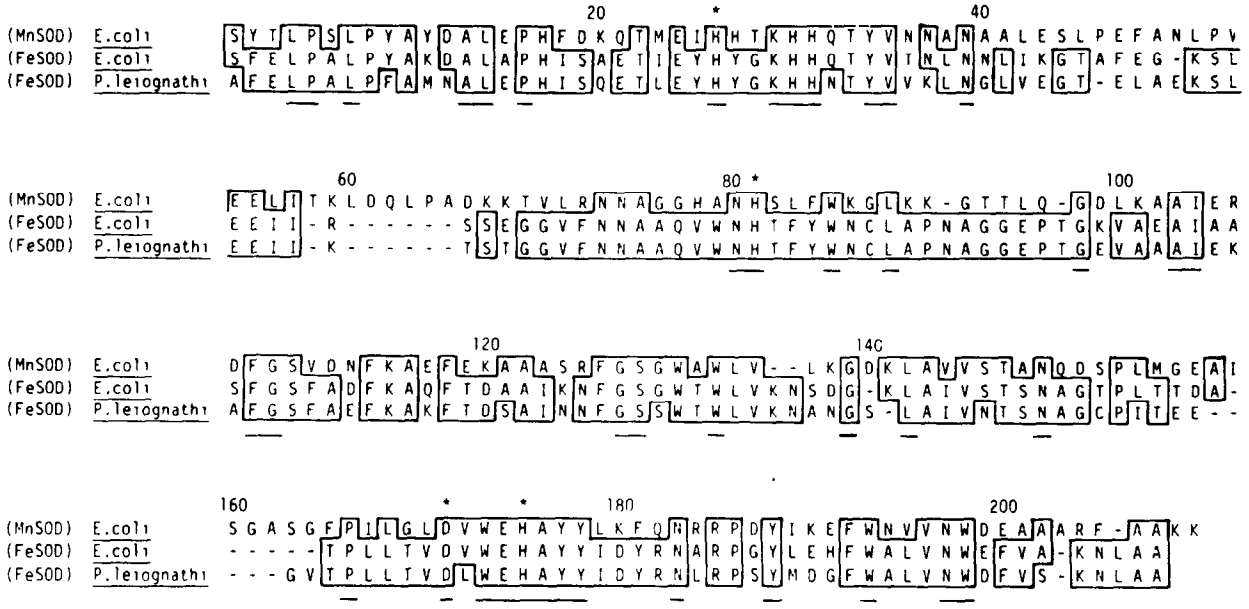

Fig.1. Comparison of the amino acid sequence of *E. coli* FeSOD with those of the mangano isoenzyme from the same organism and of FeSOD from *P. leiognathi*. Gaps were introduced to compensate the difference in length and to maximize the homologies. Metal ligands are indicated with an asterisk. Boxes indicate positions at which residues are identical. Underlined residues are conserved in all the manganese- and iron-superoxide dismutases so far known.

part by a grant from the Italian Ministero della Pubblica Istruzione.

REFERENCES

[1] Steinman, H.M. (1983) in: Superoxide Dismutase (Oberley, L. ed.) vol. 1, pp. 11-68, CRC Press, Boca Raton, FL.
[2] Bannister, J.V. and Rotilio, G. (1984) Dev. Biochem. 26, 146-189.
[3] Parker, M.W., Schininà, M.E., Bossa, F. and Bannister, J.V. (1984) Inorg. Chim. Acta 91, 307-317.
[4] Bannister, J.V. and Calabrese, L. (1986) Methods Biochem. Anal. 32, 279-312.
[5] Parker, M.W., Bossa, F., Barra, D., Bannister, W.H. and Bannister, J.V. (1986) in: Superoxide and Superoxide Dismutase in Biology, Chemistry and Medicine (Rotilio, G. ed.) pp. 237-245, Elsevier, Amsterdam, New York.
[6] Gregory, E.M., Yost, F.J. and Fridovich, I. (1973) J. Bacteriol. 115, 987-991.
[7] Britton, L. and Fridovich, I. (1977) J. Bacteriol. 131, 815-820.
[8] Hassan, H.M. and Fridovich, I. (1977) J. Biol. Chem. 52, 7667-7672.
[9] Hassan, H.M. and Fridovich, I. (1977) J. Bacteriol. 129, 1574-1583.
[10] Moody, C.S. and Hassan, H.M. (1984) J. Biol. Chem. 259, 12821-12825.
[11] Pugh, S.Y.R. and Fridovich, I. (1985) J. Bacteriol. 162, 196-201.
[12] Stalling, W.C., Pattridge, K.A., Strong, R.K. and Ludwig, M.L. (1984) J. Biol. Chem. 259, 10695-10699.
[13] Ringe, D., Petsko, G.A., Yamakura, F., Suzuki, K. and Ohmori, D. (1983) Proc. Natl. Acad. Sci. USA 80, 3879-3883.
[14] Stalling, W.C., Powers, T.B., Pattridge, K.A., Fee, J.A. and Ludwig, M.L. (1983) Proc. Natl. Acad. Sci. USA 80, 3884-3888.
[15] Yamakura, F. (1976) Biochim. Biophys. Acta 422, 280-294.
[16] Barra, D., Schininà, M.E., Simmaco, M., Bannister, J.V., Bannister, W.H., Rotilio, G. and Bossa, F. (1984) J. Biol. Chem. 259, 12595-12601.
[17] Barra, D., Schininà, M.E., Bannister, W.H., Bannister, J.V. and Bossa, F. (1987) J. Biol. Chem. 262, 1001-1009.
[18] Ozols, J. and Gerard, C. (1977) J. Biol. Chem. 252, 5986-5989.
[19] Shlyapnikov, S.V., Bezborodova, S.I., Kulikov, V.A. and Yakovlev, G.I. (1986) FEBS Lett. 196, 29-33.
[20] Steinman, H.M. (1978) J. Biol. Chem. 253, 8708-8720.
[21] Hallewell, R.A., Mullenbach, G.T., Stempien, M.M. and Bell, G.I. (1986) Nucleic Acid Res. 14, 9539.
[22] Ditlow, C., Johansen, J.T., Martin, B.M. and Svendsen, I.B. (1982) Carlsberg Res. Commun. 47, 81-91.
[23] Brock, C.J. and Walker, J.E. (1980) Biochemistry 19, 2873-2882.
[24] Sato, S., Nakada, Y. and Nakazada-Tomizawa, K. (1987) Biochim. Biophys. Acta 912, 178-184.