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Iron- and manganese-containing superoxide dismutases can be distinguished by analysis of their primary structures

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The iron- and manganese-containing superoxide dismutases have very similar three-dimensional structures but can be distinguished by various biochemical means. The primary structures of six manganese-containing and three iron-containing superoxide dismutases are known. Analysis of the aligned amino acid sequences of these enzymes taken together with structural data from X-ray diffraction studies demonstrates that the two classes of enzyme can be distinguished on the basis of a small number of single-site substitutions that are positioned in and close to the active site of the enzyme.

Iron; Manganese; Superoxide dismutase; Amino acid sequence; Sequence homology

1. INTRODUCTION

The superoxide dismutases (EC 1.15.1.1, SOD) are a group of metalloproteins which have been classified according to their metal cofactor. The most primitive class has been considered to be the iron enzyme because of its presence in anaerobic bacteria and other prokaryotes. The manganese enzyme is present in prokaryotes and mitochondria whilst the copper/zinc enzyme is found almost exclusively in eukaryotic species. The SOD enzyme catalyses the disproportionation of superoxide radicals to hydrogen peroxide and oxygen [1].

Structural studies have indicated that the iron and manganese enzymes have similar primary, secondary and tertiary structures but bear no resemblance to the copper/zinc enzyme [2–8]. Atomic models derived from X-ray diffraction studies exist for the manganese enzymes from *Thermus thermophilus* and *Bacillus stearothermophilus* [6,7] and the iron enzymes from *Pseudomonas ovalis* and *Escherichia coli* [3,4]. These studies demonstrate that the iron and manganese proteins have very similar tertiary folds and that the ligands to the metal cofactor are identical. However, with a few exceptions [9–12], the metal requirement of the manganese and iron enzymes is specific. The iron enzyme can be inhibited with millimolar amounts of azide and inactivated by hydrogen peroxide whereas the manganese enzyme requires much higher levels of azide for inhibition and appears to be reasonably stable towards hydrogen peroxide [13].

The primary structures of three iron SODs have been published within the last year [14–16]. This report describes a comparison of these structures with those of six known manganese SOD primary structures and uses a knowledge of the three dimensional structure of the manganese enzyme from *B. stearothermophilus* to pinpoint residues that may be responsible for conferring the distinguishing properties of the iron and manganese SODs.

2. METHODS

Complete amino acid sequences of six manganese SODs from

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human liver [8,26], mouse liver [17], *Saccharomyces cerevisiae* [18], *B. stearothermophilus* [19], *T. thermophilus* [20] and *E. coli* [21] and three iron SODs from *E. coli* [15], *Ps. ovalis* [16] and *Photobacterium leiognathi* [14] have been published. The alignment of the sequences was carried out initially using the computer program ALIGN (in which the mutation data matrix was used and a gap penalty of 12 assigned) but small adjustments were required when the pairs of aligned sequences were brought together. The program ALIGN was made available by the National Biomedical Research Foundation (Georgetown University Medical Center, Washington, USA).

Details of the crystal structure determination of the manganese SOD from *B. stearothermophilus* are given elsewhere [7]. The current model of the structure was examined using an Evans and Sutherland interactive computer graphics system.

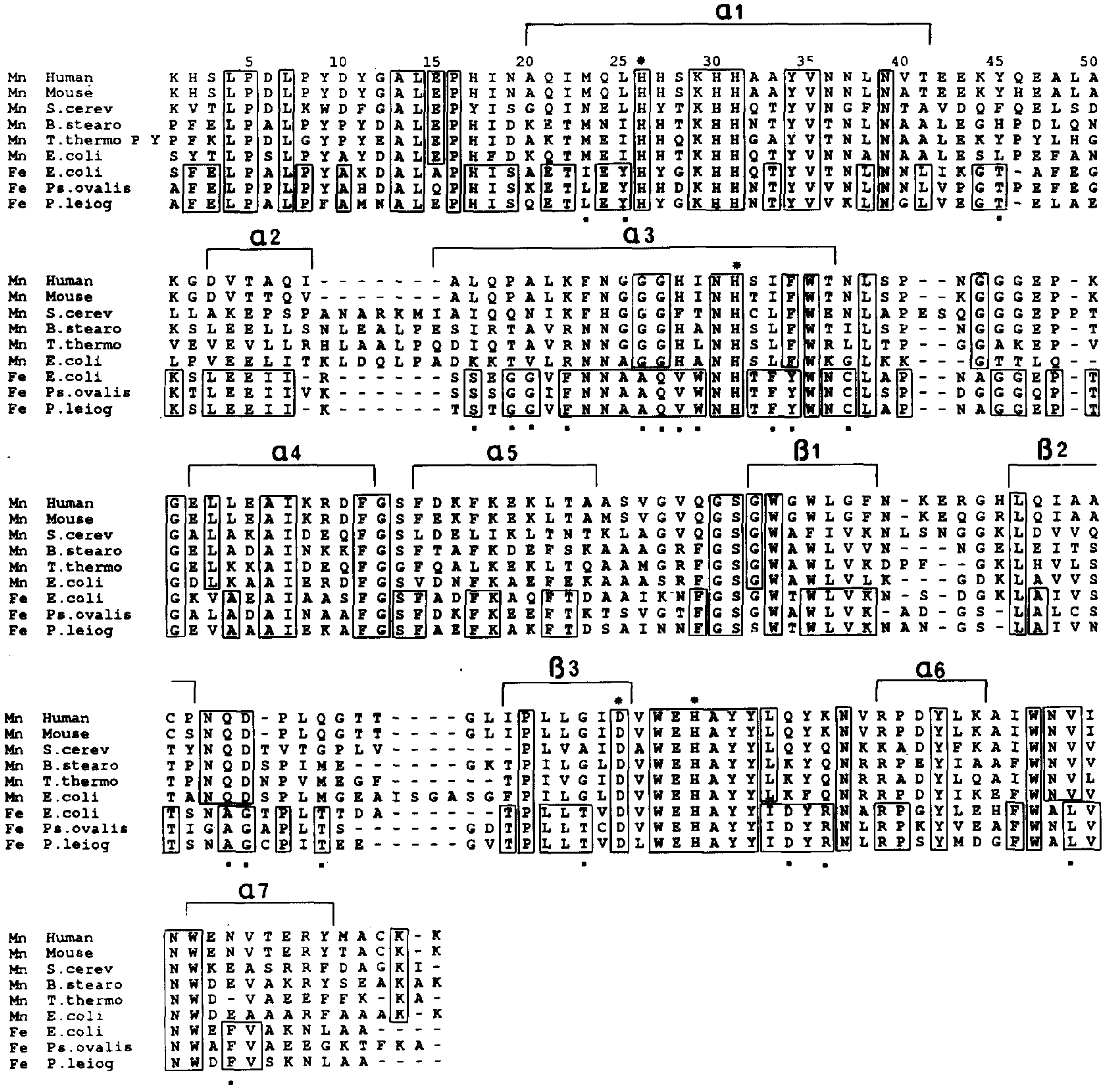

Fig.1. Aligned amino acid sequences of manganese and iron superoxide dismutases. Residues marked with an asterisk are ligands to the metal cofactor. Invariant residues are boxed. The locations of the α-helix and β-sheet secondary structures in the *B. stearothermophilus* enzyme are indicated. Residues designated with a blackened square have been pinpointed as potential discriminators between the iron and manganese proteins.

3. RESULTS AND DISCUSSION

The sequence alignment of the SODs is shown in fig.1. The degree of sequence identity between the SODs was calculated based on the alignments shown in fig.1 with common gaps excluded from the calculations. The results of these calculations have been compiled in table 1. The iron SOD sequences show a relatively high identity of between 65 and 74% compared to typical values found for bacterial proteins [22]. The iron SOD sequences are more closely related to each other than to any prokaryotic manganese SOD sequence. It is puzzling to find that the *B. stearothermophilus* manganese SOD is more closely related to the iron SODs than is the related thermophilic manganese SOD from *T. thermophilus*. The low degree of identity between the yeast (*S. cerevisiae*) sequence and all other sequences appears anomalous.

There are 39 residues distributed throughout the entire length of the sequences that are invariant (fig.1). It has been possible to postulate a role for most of these residues (table 2) based on an examination of the crystal structure of the manganese SOD from *B. stearothermophilus* (fig.2) and other data [7]. Presumably, the positioning of prolines and glycines as well as hydrophobic residues of the correct size and shape are critical in determining the fold of the protein. It has been postulated that the relatively high conservation of the N-terminal arm (residues 1–19) is necessary because it could play a role as a recognition site such as may be required for protein transport in the cell [7]. The active sites of manganese SODs are characterised by a hydrophobic box of aromatic residues [6,7] and many of these residues are invariant (table 2). It has been postulated that Lys 29 may be responsible for electrostatic steering of the substrate and that Tyr 34 plays a catalytic role [6,7]. The reason for the invariance of four asparagine residues is unclear.

The aligned sequences were examined for situations where a residue was invariant or conservatively substituted in one class of enzyme but not found in the other class. The results of this investigation are shown in figs 1,2 and table 3. It is particularly striking that many of the potential discriminating residues are positioned on helix 3 and many occur within a sphere of 10 Å radius from the metal cofactor. (This sphere is used to define the active site in table 3.) Residues in table 3 have been classified as being either primary or secondary discriminating candidates. Residues considered as primary candidates are found to be totally invariant within each enzyme class whereas secondary candidates have residues that are not invariant in the manganese class although in many cases the residue has been conservatively substituted [23]. An exception to the classification is the substitution of a valine for leucine at position 179 which has been considered as a secondary candidate because it is a conservative substitution and is unlikely to play any functional role. Comparison of the first reported iron SOD sequence with the known structures of the manganese SODs suggested that residues at positions 77 and 154 were prime candidates for discriminating residues [8].

Table 1

Degree of amino acid sequence identity between SODs

	Mn human	Mn mouse	Mn *S. cerev*	Mn *B. stearo*	Mn *T. thermo*	Mn *E. coli*	Fe *E. coli*	Fe *Ps. ovalis*	Fe *P. leiog*
Mn human	x								
Mn mouse	94	x							
Mn *S. cerev*	44	43	x						
Mn *B. stearo*	50	48	39	x					
Mn *T. thermo*	49	48	42	62	x				
Mn *E. coli*	43	42	39	60	52	x			
Fe *E. coli*	40	40	34	49	40	42	x		
Fe *Ps. ovalis*	42	42	34	52	41	43	65	x	
Fe *P. leiog*	37	38	34	49	37	40	74	65	x

The degree of sequence identity is expressed as a percentage

Table 2

Invariant residues in SODs

Residue	Role
Leu 4	st/recog?
Pro 5	st/recog?
Leu 7	st/recog?
Ala 13	st/recog?
Leu 14	st/recog?
Pro 16	st/recog?
His 26	ligand
Lys 29	guidance?
His 30	sc/act
His 31	sc/act
Tyr 34	act/catalytic?
Val 35	st
Asn 39	?
Asn 80	?
His 81	ligand
Trp 85	act
Leu 88	st
Gly 101	st
Ala 106	st
Ile 107	st
Phe 111	st
Gly 112	st
Gly 130	st
Ser 131	sc
Trp 133	act
Leu 146	st
Pro 170	st
Asp 175	ligand
Trp 177	st
Glu 178	sc
His 179	ligand
Ala 180	st
Tyr 181	act
Tyr 182	sc
Asn 187	?
Tyr 192	st
Trp 197	st
Asn 201	?
Trp 202	st

st, structural; recog, recognition; ligand, ligand to metal cofactor; guidance, electrostatic guidance for substrate; sc, subunit contact; act, maintains aromatic nature of active site; ?, unknown

Gln 154 interacts with Tyr 34 in the crystal structure of *B. stearothermophilus* manganese SOD and Tyr 34 is suspected of being involved in the catalytic mechanism [7]. It was proposed that the space vacated by the substitution of a glutamine for an alanine at residue 154 in iron SOD could be filled by the substitution of glycine for glutamine at residue 77 [8]. The precise positioning of these residues in the active site might be modulated by the substitutions of glycine for alanine at position 76 and aspartic acid for glycine at position 155. It is clear from table 3 that significant changes in the pattern of residue interactions may occur in going from one class to the other because of a significant clustering of potential discriminating residues about both these regions as well as other regions in the active site. It should be pointed out that the model coordinates of the active site of *T. thermophilus* manganese SOD produce a good fit to the electron density map of the *E. coli* iron SOD and that structural changes between the classes are thus expected to be subtle [24]. Biochemical evidence suggests that some of the residues listed as secondary candidates do play important distinguishing roles. The inactivation of the iron SODs by hydrogen peroxide is associated with the reaction of two tryptophan, one cysteine and one histidine residue per dimer molecule [25]. There is a tryptophan and a cysteine residue at positions 79

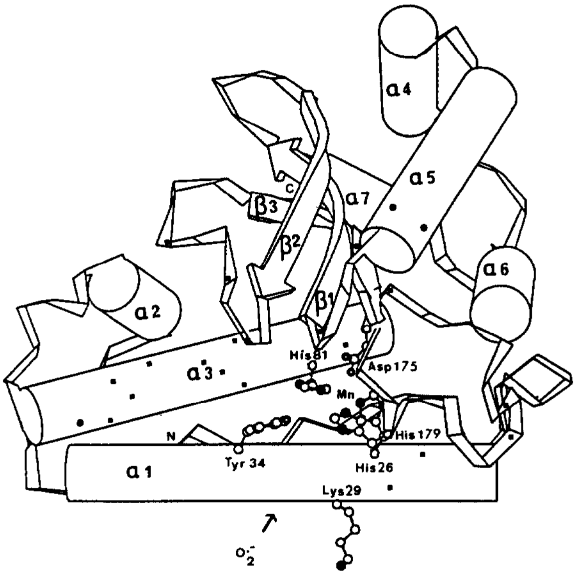

Fig.2. Chain fold of *B. stearothermophilus* manganese SOD. α-Helices are labelled α1–α7 and β-strands β1–β3. The location of the metal and its ligands are shown, as are Tyr 34 and Lys 29 in relation to the probable pathway of approach of the superoxide ion ($O_2^{\cdot -}$). Positions of residues that may discriminate between iron and manganese SODs are shown as darkened squares (residues in foreground) and circles (residues hidden from view). Picture produced by a computer program written by Lesk and Hardman (1982).

Table 3

Residues that may distinguish between manganese and iron SODs

Residue			Comment
No.	MnSOD	FeSOD	
Primary candidates			
76	Gly	Ala	active-site residue
77	Gly	Gln	active-site residue, interacts with Tyr 34 in FeSOD?
84	Phe	Tyr	active-site residue, conservative substitution interacts with Tyr 192 in FeSOD?
154	Gln	Ala	active-site residue, interacts with Tyr 34 in MnSOD
155	Asp	Gly	active-site residue, interacts with Arg 72 in MnSOD
Secondary candidates			
23	Met	Alp	active-site residue, conservative substitution
25	Alp	Tyr	active-site residue, interacts with Asn 187 in FeSOD?
45	Arom	Thr	
67	Alp	Ser	
69	Mix	Gly	
70	Alp	Gly	
72	Bas	Phe	interacts with Asp 155 in MnSOD
78	His	Val	active-site residue
79	Alp	Trp	active-site residue
83	Alp	Phe	active-site residue
87	Mix	Cys	appears buried
159	Mix	Thr	
173	Gly	Thr	active-site residue, interacts with carbonyl of residue 134 in FeSOD?
184	Mix	Asp	conservative substitution
186	Mix	Arg	conservative substitution
199	Val	Leu	conservative substitution
204	Mix	Phe	

Alp, aliphatic side chain; Bas, basic side chain; Mix, more than one residue type; Arom, aromatic side chain. NB: A residue was judged to be of a certain type if it occurred more than four times in the manganese class or all the time in the iron class

and 87, respectively, which are unique to iron SODs (fig.1 and table 3) and furthermore Trp 79 is positioned close to the active site of the enzyme. It can be concluded that residues 76, 77, 79, 84, 87, 154 and 155 are the most likely residues responsible for conferring the distinguishing properties of the iron and manganese classes of SOD.

The identification of residues responsible for conferring particular properties to the SODs will aid the design of protein engineering experiments currently being performed in a number of laboratories. The degree of specificity of metal binding might be related to the degree of sequence similarity between the two classes and in particular to the identity of residues that are found at the key positions listed above; the primary structure of an enzyme that is active with either metal might clarify this issue. Higher resolution X-ray diffraction studies of both classes are required for more detailed explanations of the observed differences between them.

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