THE COMPLETE AMINO ACID SEQUENCE OF HUMAN Cu/Zn SUPEROXIDE DISMUTASE

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1. Introduction

Superoxide dismutase (EC 1.15.1.1) is widely distributed in the animal and plant kingdom. Its catalytic dismutation of superoxide radicals makes it a unique enzyme in the line of defence of organisms against oxygen-centred radicals. Three different metallo-forms of the enzyme are known. These are the copper/zinc, manganese and iron forms (chemical, biochemical, biological and clinical aspects of the enzyme are reviewed in [1,2]).

The copper/zinc superoxide dismutases have been found to have similar physico–chemical properties indicating a conservation of structure and function. The properties of this class of enzyme are quite different from those of the manganese or iron enzymes. Sequence analysis has indicated a homology among the manganese and iron class of enzymes but these have no homology with the copper/zinc enzymes [3]. The copper/zinc enzyme therefore has a different ancestor from the manganese or iron enzyme which appear to have a common ancestor.

In view of the interest of superoxide dismutases not only from an evolutionary standpoint, because of their potential in the management of superoxide-related diseases and as part of our studies on the structure–function relationships of these enzymes, we report the determination of the complete amino acid sequence of the human erythrocyte (copper/zinc) superoxide dismutase [4,5]. The sequence obtained has homologies with other sequences of copper/zinc superoxide dismutase from bovine erythrocytes [6], horse liver [7] and yeast [8] but no homology with the sequenced manganese enzymes from *Bacillus stearothermophilus* [9] and *Escherichia coli* [10]. Furthermore a variable region has been found in all the copper/zinc enzymes so far investigated.

2. Materials and methods

Human erythrocyte copper/zinc superoxide dismutase was prepared according to [11]. Metal-free protein was prepared by dialysis against 10 mM EDTA at pH 3.8. Apo-protein was reduced and carboxymethylated and digested with TPCK-trypsin, chymotrypsin and *Staphylococcus aureus* protease. Details of the digestion procedures for trypsin and chymotrypsin digestion and the methods used for the subsequent isolation of the peptides have been described [4]. Digestion with *S. aureus* protease was done in 0.2 M ammonium bicarbonate overnight at 37°C at a protein/enzyme ratio of 1/80. Sequence analysis was carried out using the dansyl-Edman method as in [12] on the entire peptides or on their endopeptidase-subdigestion products after purification. Certain peptides were also sequenced on an LKB 4020 solid-phase peptide sequence using diphenylisothiocyanate to attach the peptides to the aminated CPG/100 glass support (Pierce Chemical Co., IL) as the resin. Amide groups were determined and assigned either by amino acid analysis after digestion of peptides with exopeptidases or by measurements of mobility on electrophoresis at pH 6.5 [13]. Sequencing of the N-terminal tryptic peptide and identification of the blocking group was done as in [14].

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3. Results and discussion

The complete amino acid sequence of human erythrocyte copper/zinc superoxide dismutase resulting from the sequencing of the peptides obtained from digestion with trypsin, chymotrypsin and *S. aureus* protease is given in fig.1. The amino acid composition obtained from the sequence data is in good agreement with published data [15]. The main point of contention was the presence of tyrosine. The sequence confirms the absence of this amino acid which is substituted by histidine in the human erythrocyte and horse liver enzymes and by threonine in the yeast enzyme. In contrast, the human enzyme contains tryptophan in position 32. This amino acid is not present in the other enzymes. The human erythrocyte enzyme has the N-terminal amino acid blocked by an *N*-acetyl group as the horse liver and bovine erythrocyte enzymes. A blocked N-terminal amino acid does not appear to be consistent in all the copper/zinc enzymes as both the yeast [8] and the swordfish liver [16] enzymes have a free amino-terminal valine. The human erythrocyte enzyme like the horse liver enzyme and the yeast enzyme is com-

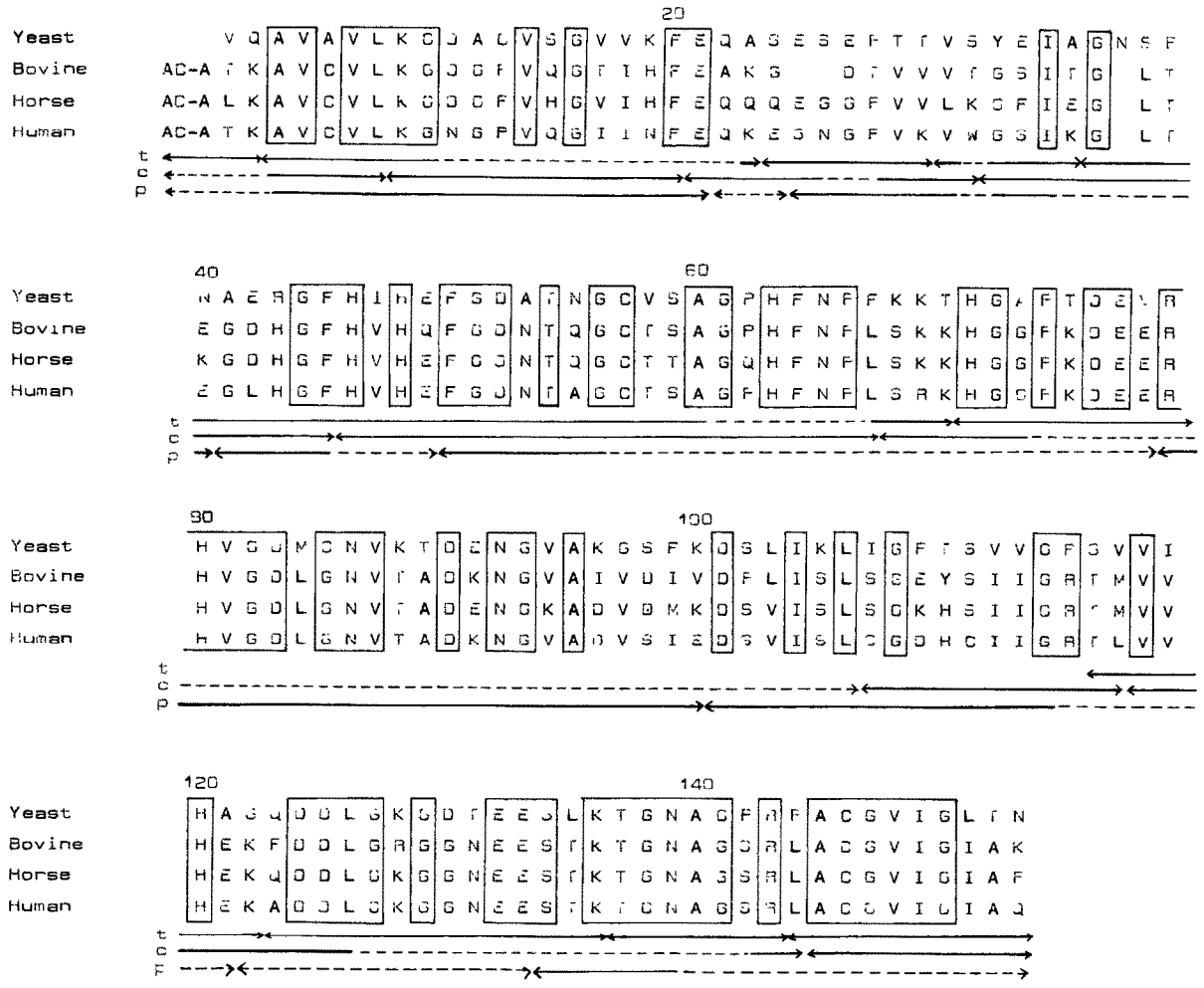

Fig.1. Comparison of the primary structures of yeast, bovine, horse and human copper/zinc superoxide dismutases. Identical residues are indicated by boxes. One letter notation is used. The N-terminus of the mammalian proteins is acetylated (AC-). Arrows under the human enzyme sequence denote peptides derived by digestion of the carboxymethylated protein with trypsin (t line), chymotrypsin (c line) and *S. aureus* protease (p line). Dashed arrows denote sequences inferred from amino acid compositions and from comparison with sequences of different peptides.

posed of 153 residues in contrast to the bovine erythrocyte which was reported to have 151 residues. The two additional residues were inserted in position 25 and 26 from considerations of the sequence homologies and from the minimum number of base changes required whilst in the case of the yeast enzyme, a further insertion between residues 37 and 38 was required because this enzyme lacks the first residue [8].

From a comparison of the sequences between human/bovine/horse enzymes the homology was found to be 74.5%; a value of 93% is found between cytochrome *c* from the same sources, suggesting a faster rate of evolutionary modification for superoxide dismutases. Including in the comparison the yeast proteins gives percentages of homology not so distant for the two classes of proteins; i.e., 49.6% for superoxide dismutases and 55.7% for cytochrome *c*, indicating for superoxide dismutases an evolutionary rate much more similar to a strictly conserved molecule such as cytochrome *c* over long evolutionary distance.

The discrepancy between the conclusions that can be derived from comparisons restricted to the mammalian superoxide dismutases and those including the yeast enzyme is related to the existence of a hypervariable region, which is clearly apparent looking at the region from residues 17–30 of the mammalian enzymes so far investigated. In fact, the discrepancy is eliminated when these residues are not taken into account for the calculations of homology. Moreover in the three-dimensional folding of the polypeptide chain, with reference to the crystallographic data obtained for the bovine enzyme [17], residues 17–30 are located on the surface of the molecule at a border of the main structural feature of this protein, i.e., a barrel formed by eight antiparallel β-strands. On the same relatively small area, the folding of the molecule concentrates most of the other substituted residues, including the residues 109–111 with the cysteine unique to the human enzyme. The existence on the copper/zinc superoxide dismutases of such a species specific hypervariable patch is possibly related to the observations on the immunochemical properties of this class of enzymes [18].

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Note added

While this manuscript was in preparation, our attention was drawn to an article [19] on the sequence of human erythrocyte superoxide dismutase. Besides the discrepancies on the assignment of amidation state for residues 11, 26, 49, 52, 53 and 92, the reported sequences have two differences now arising at position 17 (Ser in [19] versus Ile here) and 98 (Val in [19] versus Ser here). We think it is important to present briefly the more relevant experimental evidence in favour of our version for these two positions.

Residue 17: the amino acid analysis after 24 h acid hydrolysis of peptide 1–21 from *S. aureus* protease digest gave no serine (0.1 mol/mol peptide) and 0.9 mol isoleucine. After 72 h hydrolysis the latter figure became 1.4 mol/mol peptide. Carboxypeptidase Y digestion after 1 h released: Glu (1.0), Phe (1.0), Asn (1.0) and Ile (1.5) (mol/mol peptide); and after 3 h released Glu (1.0), Phe (1.0), Asn (0.9) and Ile (1.8) (mol/mol peptide). Finally, dansyl Edman degradation of a fragment from residues 10–21, produced by tryptic subdigestion, gave results fully consistent with the sequence reported in fig.1.

Residue 98: peptide 79–100 from *S. aureus* protease digest was subdigested with pepsin and the peptide mixture fractionated by paper chromatography. Amino acid analysis of the subfragment from residues 97–100 gave: Ser (0.9), Glu (1.0), Val (0.9) and Ile (1.0) (mol/mol peptide). Dansyl-Edman degradation of this fragment clearly indicated the sequence reported in fig.1.

Moreover the amino acid compositions reported in [19] for the pertinent peptides (table 2 in [19]) seem not to contradict with our version of the sequence.

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