EVOLUTIONARY RELATIONSHIPS IN SUPEROXIDE DISMUTASE

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1. Introduction

Superoxide dismutase, an enzyme previously shown to be ubiquitous among oxygen metabolising organisms [1], is responsible for the dismutation of superoxide radicals into peroxide and molecular oxygen:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \qquad [2]$$

Two distinct types of superoxide dismutase have been isolated from aerobic organisms. A blue-green Cu–Zn protein with a mol. wt of 32 000 and comprising two identical subunits has been obtained from a wide range of eukaryotes (e.g. [3,4]) while a pinkish-purple Mn-containing enzyme, also composed of identical subunits, has been isolated from eukaryotic mitochondria [5] and from prokaryotes [6,7]. In addition, an Fe-containing superoxide dismutase, similar to its Mn-containing counterpart, has been obtained from *E. coli* [8]. The prokaryotic Mn and Fe-containing enzymes are dimers with mol. wts of 40 000 and the mitochondrial Mn-enzyme, with a mol. wt of 80 000, is a tetramer.

The subunits of the Mn and Fe-containing dismutases are of the same chain length, comprising approx. 185 to 190 residues [6–8]. In addition they possess similar amino acid compositions and N-terminal sequences [9]. The Cu–Zn enzyme from bovine erythrocytes on the other band possesses a smaller subunit (of 150 residues [10]), with a different amino acid composition [11] and N-terminal sequence [9]. These results led Steinman and Hill [9] to suggest that the two classes of dismutase are probably composed of entirely different proteins of independent evolutionary origin. Insofar as the sequence comparisons of the respective protein chains were confined to the N-terminal segments of 25 to 30 residues, the possibility nevertheless remained that failure to detect sequence homologies between the different classes of dismutase might have been due to the differences in chain length.

In order to test this possibility we have carried out an extended sequence analysis of 60 residues from the N-terminus on a new superoxide dismutase isolated from *B. stearothermophilus.* Like its counterparts from *E. coli* [6] and *Streptococcus mutans* [7], the thermophile dismutase is a typical Mn-containing prokaryotic dismutase comprising two identical subunits each of about 185–190 amino acids (J. I. Harris and E. Kolb, unpublished results). The results are compared with sequences of the N-terminal 30 residues in the Cu–Zn enzyme from bovine erythrocytes, as well as in other Mn- and Fe-containing dismutases. The implications of these comparisons for the evolution of superoxide dismutase and for the endosymbiotic theory of mitochondrial evolution are discussed.

2. Materials and methods

The superoxide dismutase used in this work was obtained as a by-product during the course of a multi-enzyme isolation procedure designed to prepare glycolytic enzymes and tRNA synthetases from *B. stearothermophilus* (cf. [12]). The pure dismutase obtained in a yield of 1 g from 10 kg frozen cells gave a single band by gel-electrophoresis with and without 0.1% SDS, and a single N-terminal residue proline, (unpublished results of J. I. Harris and M. Runswick).

Sequence analyses by automated Edman degradation were performed in a Beckman 890B sequencer using the standard Quadrol double-cleavage programme [13]. Native protein (8 mg, 400 nmol) was dried under vacuum in the reaction cup and degradations were

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commenced at the second acid-cleavage step so as to stabilise the protein film in the cup. Sequencer chemicals were from Beckman Instruments Inc. Phenylthiohydantoins were identified by a combination of t.l.c. [13], g.l.c. [14] and amino acid analysis following hydrolysis with HI [15]. All residues were identified by at least two of these methods in each of two separate sequencer runs. Repetitive yield was calculated as 96% between leucine 4, leucine 14 and leucine 38.

3. Results and discussion

The sequence obtained from the intact protein (residues 1 to 60) was found to be:

Pro–Phe–Glu–Leu–Pro–Ala–Leu–Pro–Tyr–Pro^{10}–
Tyr–Asp–Ala–Leu–Glu–Pro–His–Ile–Asp–Lys^{20}–
Glu–Thr–Met–Asn–Ile–His–His–Thr–Lys–His^{30}–
His–Asn–Thr–Tyr–Val–Thr–Asn–Leu–Asn–
Ala^{40}–Ala–Leu–Glu–Gly–His–Pro–Asp–Leu–
Gln–Asn^{50}–Lys–Ser–Leu–Glx–Glx–Leu–Pro–
Ser–Asx–Leu^{60}–

The validity of the sequence was further confirmed by sequence analysis of the N-terminal CNBr fragment (residues 1 to 23) by automated solid-phase degradation (J. Bridgen, unpublished results). The second CNBr fragment in the sequence was also isolated and subjected to 38 cycles of sequencer analysis under the same conditions as the native protein and no discrepancies were found between any of these sequences.

Comparison of the N-terminal sequence of the thermophile superoxide dismutase with N-terminal sequences of other dismutases (fig. 1) shows that there is no significant sequence homology between the first 30 residues from the N-terminus of the Cu–Zn enzyme from bovine erythrocytes and any part of the N-terminal 60 residues of the *B. stearothermophilus* Mn enzyme. Furthermore, comparison of the complete sequence of the bovine erythrocyte dismutase [10] with the partial sequence of the *B. stearothermophilus* enzyme also fails to reveal any significant homology. This result provides additional evidence for the view [9], that the Cu–Zn and Mn-containing dismutases have in all probability evolved independently by convergent evolution from different ancestral proteins.

There is, on the other hand, a very high degree of sequence homology between the prokaryotic dismutases, and between the prokaryotic and mitochondrial enzymes, respectively. Thus, in the sequence of the *B. stearothermophilus* Mn-enzyme 24 out of 29 residues from the N-terminus are identical with at least one of the corresponding residues from the two *E. coli* dismutases. Moreover, in the sequence of the mitochondrial dismutase 20 out of 27 residues from the N-terminus are identical with residues in the same position in at least one of the three bacterial dismutases. A similarly high degree of homology between bacterial sequences has also been observed [17] in comparisons of the first 50 residues of tryptophan synthetase α-chains of *Shigella dysenteriae* and *Salmonella typhimur-*

B. stearothermophilus	Mn	Pro	Phe	Glu	Leu	Pro^{5}	Ala	Leu	Pro	Tyr	Pro^{10}	Tyr	Asp	Ala	Leu	Glu^{15}
E. coli	Mn	Ser	Tyr	Thr		Ser				Ala						
E. coli	Fe	Ser								Ala	Lys					Ala
Chicken liver mitochondria	Mn	Lys	His	Thr		Asp				Asp		Gly				
Bovine erythrocyte	Cu/Zn	Ac–Ala	Thr	Lys	Ala	Val	Cys	Val	Leu	Lys	Gly	Asp	Gly	Pro	Val	Gln

B. stearothermophilus	Mn	Pro	His	Ile	Asp	Lys^{20}	Glu	Thr	Met	Asn	Ile^{25}	His	His	Thr	Lys
E. coli	Mn		Phe			Gln			Glu	Leu	?		?		
E. coli	Fe			Ser	Ala		?	Ile	Glu	Tyr		Tyr	Gly		
Chicken liver mitochondria	Mn			Ser	Ala		Ile		Gln	Leu		?	?		
Bovine erythrocyte	Cu/Zn	Gly	Thr	Ile	His	Phe	Glu	Ala	Lys	Gly	Asp	Thr	Val	Val	Val	

Fig. 1. Comparison of the N-terminal region of superoxide dismutase from *B. stearothermophilus* with the N-terminal regions of two other bacterial and one mitochondrial dismutase. Residues identical to those in the *B. stearothermophilus* sequence have not been included. The corresponding region of the bovine erythrocyte enzyme is also shown.

ium with the corresponding N-terminal region of the *E. coli* enzyme. On the other hand, the complete sequence of cytochrome *c*-551 has been found to vary by as much as 25 to 35% in four species of *Pseudomonas* [18], and also shows considerable variation within one strain of a given species [19]. Clearly one cannot draw firm conclusions from sequence comparisons involving only 15 to 20% of a protein chain, and in the case of superoxide dismutase it is entirely possible that the N-terminal sequence is highly conserved because it is a functionally important part of the molecule. In any event it seems to be clearly established that the mitochondrial and bacterial dismutases are structurally related and that they have evolved from a common ancestral protein, an observation that provides further direct evidence for the endosymbiotic theory of mitochondrial evolution [cf. 20–22].

The question remains as to why an organism finds it necessary to produce more than one type of superoxide dismutase. In the case of *E. coli* it has been suggested [23] that the Fe enzyme, which is located primarily in the periplasmic space, may function against external superoxide radicals whereas the Mn enzyme, which is found mainly in the cell matrix, shields against intracellular O_2^-. However, since the potentially harmful sources of O_2^- *in vivo* are unknown in both prokaryotic and eukaryotic cells, it is not yet possible to correlate structural differences with mechanisms of dismutase action. Moreover, it is unlikely that the functional similarity between mitochondrial and prokaryotic dismutases could have arisen by a process of convergent evolution as this would have implied that mitochondria and bacteria had been subjected to similar selective pressures for a considerable period of time. Convergent evolution may, however, explain how the eukaryotic and prokaryotic (and in particular the bovine erythrocyte and liver mitochondrial) proteins appear quite different in primary structure but possess similar enzymatic functions. In this respect superoxide dismutase appears to resemble aldolase [24,25] and similar relationships have also been observed previously for serine proteinases [26]. However, the extent to which the two classes of dismutase may possess similar three-dimensional structures remains to be established from the results of *X*-ray crystallographic work which is already in progress [27].

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References

[1] McCord, J. M., Keele, B. B. and Fridovich, I. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1024–1027.
[2] Fridovich, I. (1972) Accounts Chem. Res. 5, 321–326.
[3] McCord, J. M. and Fridovich, I. (1969) J. Biol. Chem. 244, 6049–6055.
[4] Bannister, J., Bannister, W. and Wood, E. (1971) Eur. J. Biochem. 18, 178–186.
[5] Weisiger, R. A. and Fridovich, I. (1973) J. Biol. Chem. 248, 3582–3592.
[6] Keele, B. B., McCord, J. M. and Fridovich, I. (1970) J. Biol. Chem. 245, 6176–6181.
[7] Vance, P. G., Keele, B. B. and Rajagopalan, K. V. (1972) J. Biol. Chem. 247, 4782–4786.
[8] Yost, F. J. and Fridovich, I. (1973) J. Biol. Chem. 248, 4905–4908.
[9] Steinman, H. M. and Hill, R. L. (1973) Proc. Natl. Acad. Sci. U.S. 70, 3725–3729.
[10] Steinman, H. M., Naik, V. R., Abernethy, J. L. and Hill, R. L. (1974) J. Biol. Chem. 249, in press.
[11] Keele, B. B., McCord, J. M. and Fridovich, I. (1971) J. Biol. Chem. 246, 2875–2880.
[12] Atkinson, A., Phillips, B. W., Callow, D. S., Stones, W. R. and Bradford, P. A. (1972) Biochem. J. 127, 63–64P.
[13] Edman, P. and Begg, G. (1967) Eur. J. Biochem. 1, 80–91.
[14] Laursen, R. A. (1971) Eur. J. Biochem. 20, 89–102.
[15] Pisano, J. J., Bronzert, T. J. and Brewer, H. B. (1969) J. Biol. Chem. 244, 5597–5607.
[16] Bridgen, J. and Secher, D. S. (1972) FEBS Lett. 29, 55–57.
[17] Li, S.-L. and Yanofsky, C. (1972) J. Biol. Chem. 247, 1031–1037.
[18] Ambler, R. P. and Wynn, M. (1973) Biochem. J. 131, 485–498.
[19] Ambler, R. P. (1974) Biochem. J. 137, 3–14.
[20] Sangan, L. (1967) J. Theor. Biol. 14, 225–274.
[21] Margulis, L. (1968) Science 161, 1020–1022.
[22] Raven, P. H. (1970) Science 169, 641–646.
[23] Gregory, E. M., Yost, F. J. and Fridovich, I. (1973) J. Bacteriol. 115, 987–991.
[24] Jack, R. S. and Harris, J. I. (1971) Biochem. J. 124, 68–69P; Jack, R. S. (1973) Ph.D. dissertation, University of Cambridge.

[25] Stribling, D. and Perham, R. N. (1973) Biochem. J. 131, 833–841.
[26] Smith, E. L. (1970) in: The Enzymes (Boyer, P. D., ed.) Vol. 1, pp. 267–339, Academic Press, New York and London.
[27] Thomas, K. A., Rubin, B. H., Bier, C. J., Richardson, J. S. and Richardson, D. C. (1974) J. Biol. Chem. 249, 5677–5683.