Superoxide Dismutases in Mitochondria from *Helianthus tuberosus* and *Neurospora crassa*

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Superoxide dismutase has been studied in a wide range of organisms (Fridovich, 1975). Early observations suggested that superoxide dismutases isolated from eukaryotes (McCord & Fridovich, 1969; Misra & Fridovich, 1972; Beauchamp & Fridovich, 1973) showed little diversity in their properties, having mol.wts. near 32000 and containing two copper and two zinc atoms per molecule. The activity of these enzymes was inhibited by cyanide. In contrast, two different cyanide-insensitive enzymes could be isolated from prokaryotes, e.g. *Escherichia coli*, which contained either two manganese atoms or one iron atom per molecule (Keele et al., 1970; Yost & Fridovich, 1973). From these original observations it would appear that there was a distinction between the superoxide dismutases in eukaryotes and prokaryotes. However, more recent observations suggest that this may not be correct (Puget & Michelson, 1974; Lumsden & Hall, 1975).

Weisiger & Fridovich (1973b) have reported that mitochondria isolated from chicken liver contained both manganese and cupro-zinc superoxide dismutases. The manganese enzyme was located in the matrix space and the cupro-zinc enzyme in the intermembrane space. A similar spatial distribution of these two enzymes has been reported in mitochondria isolated from rat liver by Peeters-Joris et al. (1975). Misra & Fridovich (1972) found that soluble extracts of *Neurospora crassa* contained only the cupro-zinc enzyme; however, the isolation procedure used by these workers involved extraction with an ethanol/chloroform mixture, which is known to inactivate the manganese enzyme (Weisiger & Fridovich, 1973a). The distribution of superoxide dismutase within the mitochondrial is of interest with respect to the endosymbiotic theory of mitochondrial evolution (Fridovich, 1975; Lumsden & Hall, 1975). Tyler (1975) reported that superoxide ions, formed during respiratory-chain activity, inhibited NADH oxidation in heart muscle submitochondrial particles and concluded that mitochondrial superoxide dismutase protected the NADH dehydrogenase against the harmful effects of superoxide ions. Superoxide dismutase may have a similar role in plant and fungal mitochondria, although there have been no reports of superoxide dismutase distribution and cyanide sensitivity in plant mitochondria. Misra & Fridovich (1972) reported a cupro-zinc superoxide dismutase in soluble extracts of *N. crassa* hyphae, but did not isolate mitochondria from this organism.

In the present work we have studied the localization of superoxide dismutase in mitochondria isolated from Jerusalem artichoke (*Helianthus tuberosus*) and *N. crassa*. We provide evidence that in both these types of mitochondria the matrix contains a cyanide-insensitive enzyme (probably manganese dismutase) and the intermembrane space contains a cyanide-sensitive enzyme (cupro-zinc superoxide dismutase). Mitochondria were isolated from tubers of Jerusalem artichoke by the method of Palmer & Arron (1976) and from hyphae of *N. crassa*, which had been grown in submerged culture for 24 h, by a modification of the method of Hall & Baltscheffsky (1968). The inner and outer membranes of the mitochondria were separated by osmotic swelling and contraction and then differential centrifugation as described by Moreau & Lance (1972). The different types of superoxide dismutase were separated and assayed on polyacrylamide gels by the method of Beauchamp & Fridovich (1971).

The mitochondria from Jerusalem artichoke gave gels with three bands of superoxide dismutase activity (Fig. 1), one of which was insensitive to cyanide and the remainder were sensitive. The cyanide-insensitive enzyme activity appeared to be confined to the matrix, whereas the cyanide-sensitive enzyme activities appeared to be
Fig. 1. Activity patterns on polyacrylamide-gel electrophoretograms stained for superoxide dismutase activity of extracts from Jerusalem artichoke (H. tuberosus) in the presence or absence of $1 \text{mM}$-cyanide

The dark areas represent enzyme activity. (1) Mitochondria (no cyanide); (2) mitochondria (cyanide added); (3) mitochondrial matrix (no cyanide); (4) mitochondrial matrix (cyanide added); (5) mitochondrial intermembrane space (no cyanide); (6) mitochondrial intermembrane space (cyanide added); (7) cytosol (no cyanide); (8) cytosol (cyanide added). Cytosol constitutes supernatant after the initial homogenate has been centrifuged at $48000 \text{g}$ for 2 min.

Fig. 2. Activity patterns on polyacrylamide-gel electrophoretograms stained for superoxide dismutase activity of extracts from N. crassa in the presence or absence of $1 \text{mM}$-cyanide

(1) Mitochondria (no cyanide); (2) mitochondria (cyanide added); (3) mitochondrial matrix (no cyanide); (4) mitochondrial matrix (cyanide added); (5) mitochondrial intermembrane space (no cyanide); (6) mitochondrial intermembrane space (cyanide added); (7) cytosol (no cyanide); (8) cytosol (cyanide added).

Located in the intermembrane space. The enzyme in the matrix probably contains manganese, and the enzymes in the intermembrane space probably contain copper and zinc because they are sensitive to cyanide. Another cyanide-sensitive enzyme (thus probably a cupro-zinc enzyme) was found in the cytosol after the removal of the mitochondria, and since the cytosol preparation was contaminated with broken mitochondria, all the enzymes found in the mitochondria were also found in this soluble preparation.

A similar distribution of superoxide dismutase was found in hyphae of N. crassa (see Fig. 2). The mitochondrial matrix contained a cyanide-insensitive enzyme (probably...
a manganese enzyme) and the intermembrane space a cyanide-sensitive enzyme (cupro-zinc enzyme). The cytosol fraction contained the same two enzymes as found in the mitochondria and thus these may have originated from broken mitochondria. No distinct cytosolic superoxide dismutase was apparent, although Misra & Fridovich (1972) have suggested that one exists. Such an enzyme might have similar electrophoretic properties as the intermembrane-space enzyme, and thus would not be separated on our gels. It is clear that there is a difference between the soluble superoxide dismutases in extracts from Jerusalem artichoke and \textit{N. crassa}.

The outer membranes of plant mitochondria are difficult to remove because of the presence of polyuronic acid, which appears to confer a high degree of osmotic stability (Mannella & Bonner, 1975). When Jerusalem-artichoke mitochondria were exposed to a range of decreasing sucrose concentrations the cyanide-sensitive superoxide dismutase was more readily released than adenylate kinase, which is considered to be located in the intermembrane space (Ernster & Kuylenstierna, 1970). This suggests that the cupro-zinc superoxide dismutase can be lost through the damaged outer membrane more readily than can adenylate kinase.

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Evidence that the Monomers of Dimeric Triose Phosphate Isomerase are Active

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We have previously presented evidence (Fell & Branford White, 1975) that the monomers of dimeric triose phosphate isomerase (EC 5.3.1.1) from chicken muscle are active. In those experiments, the enzyme was attached to Sepharose 4B, and the immobilized dimeric enzyme denatured with guanidinium chloride to yield bound monomeric triose phosphate isomerase; the regain of activity on removal of the denaturant demonstrated that these monomers were active. Under appropriate conditions, we were also able to rehybridize the immobilized monomers to re-form native dimers. However, we were unable to measure the very low concentrations of protein immobilized on the gel, but we can now report the use of \textsuperscript{14}C-labelled triose phosphate isomerase to make these measurements.

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