INTRAMITOCHONDRIAL LOCALIZATION AND RELEASE OF RAT LIVER SUPEROXIDE DISMUTASE

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1. Introduction

Mitochondrial superoxide dismutase was isolated from chicken liver mitochondria by Weisiger and Fridovich [1] in 1973. It was shown to differ markedly from the enzyme present in the cytosol of this tissue [1]. Thus, the mitochondrial enzyme is insensitive to cyanide and contains 2 Mn atoms per molecule. The mitochondrial enzyme closely resembles those previously isolated from prokaryotes [2,3], whereas the cytosol enzyme is a cupro-zinc one similar to those isolated from the cytosol of a wide variety of eukaryotes [4—8].

Recently, Weisiger and Fridovich [9] discovered that there are two types of enzyme in chicken liver mitochondria as distinguished by their cyanide sensitivities. The cyanide-sensistive enzyme was found only in the intermembrane space, whereas the cyanide-insensitive one was found primarily in the matrix space.

On the other hand, Tyler [10] observed only the mammalian-type cupro-zinc enzyme in the matrix space of rat liver mitochondria.

In the present communication we show that cyanide-sensitive superoxide dismutase of rat liver mitochondria is localized only in the intermembrane space, whereas the cyanide-insensitive one is found primarily in the matrix space. We also demonstrate some releasing properties of this enzyme.

2. Materials and methods

2.1. *Materials*

The following reagents and chemicals were obtained as indicated. L-Epinephrine: Sigma Chemical Co; xanthine: Reanal Chemical Co.; Triton X-100, cytochrome *c* (type 3): Schuchardt, München; Tris, NAD^+, α-ketoglutaric acid: Calbiochem; bovine serum albumin, EDTA: VEB Berlin-Chemie-Berlin-Adlershof; $NADH_2$: Koch-Light laboratories; digitonin: Merck Chemical Co.; DL-isocitric acid (trisodium salt): Nutritional Biochemicals Corp.; ADP: Serva Chemical Co.; D-Glucose 6-phosphate-dehydrogenase, hexokinase: Fluka Chemical Co. All other chemicals used were of reagent grade quality.

Xanthine oxidase which had been purified from unpasteurized cream by a procedure which avoided exposure to proteolytic agents was kindly provided by L.G. Nagler (Institute of chemical physics, Moscow, USSR).

2.2. *Methods*

Total superoxide dismutase was assayed in terms of its ability to inhibit the oxygen-dependent oxidation of epinephrine to adrenochrome by milk xanthine oxidase plus xanthine [5]. The standard assay was performed in 3.2 ml of 0.05 M potassium phosphate, 1×10^{-4} M EDTA, pH 7.8, in a 1.0 cm cuvette thermostated at 25°C. The reaction mixture contained 3×10^{-4} M L-epinephrine, 1×10^{-4} M xanthine and sufficient xanthine-oxidase to produce a rate of oxidation of epinephrine at 480 nm of 0.025 absorbance unit per min. Under these defined conditions, the amount of superoxide dismutase required to inhibit the rate of oxidation of epinephrine by 50% is defined as 1 unit of activity.

Cyanide-insensitive activity was measured by repeating assay in the presence of 1.0 mM cyanide.

For total superoxide dismutase assay all samples were treated with 0.1% Triton X-100 at 4°C at least 5 min prior to assay. Triton X-100 at this concentration

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did not interfere with the assay.

The activities of glutamate dehydrogenase [EC 1.4.1.3] [11], malate dehydrogenase [EC 1.1.1.37] [12], isocitrate dehydrogenase [EC 1.1.1.42] [13], adenilate kinase [EC 2.1.4.3] [13], sulphite oxidase [EC 1.8.3.1] [14], and succinate: cytochrome *c*-reductase [EC 1.3.99.1] [15] were determined in the presence of 0.1% Triton X-100. Monoaminoxidase [EC 1.4.3.4] [16] was determined in the presence of 2.5% Triton X-100 to destroy mitochondrial membranes.

Protein was determined according to Lowry et al. [17].

All spectrophotometric assays were carried out in a Unicam SP-8000 recording spectrophotometer equipped with a thermostated cell compartment.

Rat liver mitochondria were isolated by the procedure of Schneider et al. [18].

The outer membrane plus intermembrane space fraction and inner membrane plus matrix space fraction were isolated by the digitonine procedure of Schnaitman et al. [19].

Suspensions of mitochondria (15 mg protein/ml) in the isolation medium were sonicated in an MSE-150 W ultrasonic disintegrator at a frequency of 20 kcycles/sec and an amplitude of 12 μk for different periods of time.

3. Results and discussion

Superoxide dismutase of intact mitochondria is latent, and it is released after intact mitochondria are exposed to sonication or non-ionic detergents [20]. The results presented in table 1 demonstrate that rat liver mitochondria mainly contain cyanide-insensitive superoxide dismutase activity. Also we found that the total enzyme activity of rat liver mitochondrial fraction is $\frac{1}{8}$ of the total activity of cytosol.

It was found previously in our laboratory [21] that increase of ionic strength of the incubation medium caused an increase of the release of some

Table 1
Distribution between cyanide-sensitive and cyanide-insensitive superoxide dismutase activities in rat liver mitochondria.

Value	Superoxide dismutase activity		
	Total	Cyanide-sensitive	Cyanide-insensitive
Units/g tissue	266 ± 10	48 ± 12	218 ± 8
% Total activity	–	18 ± 7	82 ± 4

The mitochondrial fraction was treated with Triton X-100 (see Materials and methods) for assay.

Table 2
The effect of the high ionic strength of the incubation medium on the release of rat liver mitochondrial superoxide dismutase

	Glutamate dehydrogenase	Sulphite oxidase	Superoxide dismutase	
			Cyanide-sensitive	Cyanide-insensitive
Control	5	18	15	4
+ 0.16 M KCl	5	20	15	4
Sonication	55	90	100	46
+ 0.16 M KCl	70	100	100	68

Suspension of intact mitochondria (15 mg protein/ml) was incubated at 0°C for 30 min with and without 0.16 M KCl before (control) and after sonication for 4.5 min. Conditions of sonication described in Materials and methods. The enzyme activities of each enzyme are expressed as the percentages of total activity found.

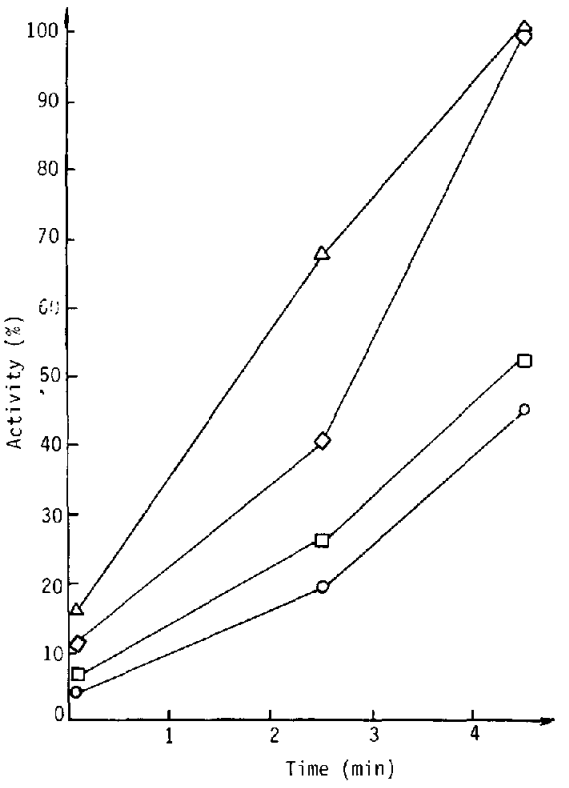

Fig. 1. The effect of sonication on release of mitochondrial superoxide dismutase. Suspension of intact rat liver mitochondria (15 mg protein/ml) was exposed to sonication for 2.5 min and 4.5 min and the indicated enzymes activities were measured in both samples. (◇—◇—◇) cyanide-sensitive superoxide dismutase (○—○—○) cyanide-insencutive superoxide dismutase (△—△—△) sulphite oxidase (□—□—□) glutamate dehydrogenase. The activities are expressed as percentages of the total units.

soluble mitochondrial enzymes under conditions of disruption of mitochondrial membranes. Table 2 shows that cyanide-sensitive mitochondrial superoxide dismutase as well as sulphite oxidase, the marker for the intermembrane space [22], are not released when intact mitochondria are exposed to high ionic strength. After sonication of intact mitochondria all the cyanide-sensitive enzyme is released, whereas a considerable part of cyanide-insensitive enzyme activity precipitates with the particulate fraction. Solubilization of cyanide-insensitive enzyme as well as glutamate dehydrogenase, the marker for the matrix space [23–25], increases at high ionic strength. This fact supports the postulated electrostatic interaction in the attachment of cyanide-insensitive superoxide dismutase to the mitochondrial membrane.

Fig. 1 shows that solubilization of cyanide-sensitive

Table 3
Compartmentation of superoxide dismutase in rat liver mitochondria

Fraction	Superoxide dismutase activity		Sulphite oxidase	Monoamine oxidase	Adenylate kinase	Glutamate dehydrogenase	Malate dehydrogenase	Isocitrate dehydrogenase	Succinate: cyt. *c*-reductase
	Cyanide-insensitive	Cyanide sensitive							
Intermembrane space plus outer membrane	9.8	99.0	95.0	92.0	98.0	10.0	15.0	20.0	–
Matrix space plus inner membrane	90.1	0.0	5.0	7.0	5.0	90.0	85.0	80.0	97.0
Percentage recovery	99.9	99.0	100.0	99.0	103.0	100.0	100.0	100.0	–

Rat liver mitochondria were prepared, fractioned, and assayed for enzyme activities as described in the text. A control mitochondrial sample was treated with 0.1% Triton X-100, but no separation was attempted. The activity of each fraction is expressed as the percentage of the total control units found in that fraction.

dismutase is similar to that of sulphite oxidase, whereas solubilization of cyanide-insensitive dismutase is similar to that of glutamate dehydrogenase. Comparison of the extent of solubilization of rat liver mitochondrial enzymes of different intramitochondrial localizations allows one to conclude that the cyanide-sensitive dismutase is localized in the intermembrane space, whereas the cyanide-insensitive one is localized in the matrix space. That this is so can be directly demonstrated by fractionating rat liver mitochondria as described in Materials and methods and assaying selected marker enzymes in the submitochondrial fractions as well as for cyanide-sensitive and cyanide-insensitive dismutases. Isocitrate, malate and glutamate dehydrogenases are markers for the matrix space [23–25]. Monoamine oxidase and succinate: cytochrome *c*-reductase are markers for outer and inner mitochondrial membranes respectively [19,26]. Adenylate kinase and sulphite oxidase are markers for the intermembrane space [14, 22]. Table 3 demonstrates that the cyanide-sensitive dismutase was found only in the intermembrane space plus outer membrane fraction, whereas the cyanide-insensitive enzyme was found primarily in the matrix space plus inner membrane fraction.

The striking similarity between the mitochondrial cyanide-insensitive superoxide dismutase and bacterial dismutases and also the gross differences between the mitochondrial cyanide-insensitive enzyme and the cytosol enzymes are considered by Fridovich [27] as evidence for the symbiotic origin of mitochondria. However, the report of Tyler [10] about the presence only of cupro-zinc superoxide dismutase in rat liver mitochondria allowed on to doubt of the correctness of this hypothesis. The present communication shows that the intramitochondrial localization of superoxide dismutase in rat liver is the same as in chicken liver.

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