CYTOCHROME C AND THE ROLE OF ZINC IONS IN ELECTRON TRANSPORT IN RAT LIVER MITOCHONDRIA.

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Summary: The inhibition of electron transfer by zinc ions in the electron transport system of the rat liver mitochondria was investigated. There was an increase in the rate at which oxygen was consumed in rat liver mitochondria pre-incubated with cytochrome c. However, the rate of inhibition of oxygen consumption by zinc ions was significantly reduced [P<0.01] in the liver mitochondria that was pre-incubated with cytochrome c. Zinc ions inhibited the electron transfer at the complex II, which is at the level of succinate electron transfer and not at the level of NADH [Complex I] of the rat liver mitochondria.

***Key Words**: Zinc, electron transport system, Succinate and NADH*

Introduction

Zinc is one of the metal ions that influence mitochondrial functions. It is active at the lowest concentrations, and has been reported to be an uncoupler and inhibitor of rat liver mitochondrial oxygen consumption at a concentration of 6 uM [Kukoyi et al, 2002]. Kleiner [1974] reported that zinc ions caused a respiratory stimulation in coupled mitochondria isolated from rat liver at a concentration lower than 4 uM.

Mitochondrial electron carriers function in serially ordered complexes vis-à-vis electrons moving from NADH, succinate, or some other primary electron donors through flavoproteins, ubiquinone, iron-sulfur proteins and cytochromes [nearly all of which are embedded in the inner membrane] and finally to oxygen [Voet and Voet, 1995] Complexes II and I catalyze electron transfer to ubiquinone from two different electron donors: NADH [Complex I] and succinate [Complex II]. Complex III carries electrons from ubiquinone to cytochrome c, and complex IV completes the sequence by transferring electrons from cytochrome c to oxygen [Walker, 1992]. If cytochrome c oxidase is blocked by any agents that inhibit the flow of electrons to oxygen, electron acceptors that function before the inhibited step will be expected to become fully reduced. This investigation was undertaken to detect the site of zinc inhibition in the electron transport chain in liver mitochondria.

Materials and Methods

Male Wistar rats weighing 275-350 grams were used. The handling of the animals was in conformity with the National Institute of Health [NIH] and University of Maryland guidelines for the care and use of animals for research. The rats were sacrificed and the liver were excised and placed in isolation buffer solution containing 250 mM sucrose, 10 mM HEPES and 1 mM ethylenediamine tetraacetic acid [EDTA], pH 7.30.

The mitochondria were isolated from the liver according to the methods described by Kukoyi et al [2002]. The protein content of the suspended mitochondria was determined by the method of Bradford [1976]. The rates of oxygen consumption of the liver mitochondria incubated with cytochrome c were determined using Fiber Optic Oxygen Monitor Model 210 with 50 mM succinate as substrate. The control experiment lacked cytochrome c. The inhibition of oxygen consumption by 20 uM zinc chloride was measured.

The cytochrome c, succinate and NADH were used as substrates to determine their viability on the rate of oxygen consumption of the liver mitochondria.

The concentration of cytochrome c from bovine heart mitochondria was determined from the absorbancy at 550 nm of the reduced solution using the Beer's Law. DW-2000 UV-VIS Spectrophotometer was used to measure nmole of cytochrome c reduced per milligram mitochondrial protein per minute.

The reaction system contained prepared 250 microgram of mitochondria, cytochrome c, cytochrome c oxidase inhibitor potassium cyanide in 250 mM sucrose, 10 mM HEPES, pH 7.3. This system was incubated with 20 uM of different variation of zinc ions complexes [Zinc-EDTA, Zinc Chloride, Zinc-Aspartate, and Zinc-Citrate] for 5 minutes at 37°C. The nanomole of cytochrome c reduced per milligram protein per minute was determined spectrophotometrically after the addition of reducing agents, succinate and NADH.

Control experiments were not incubated with zinc ions.

Results

We previously demonstrated that the addition of 6 uM of zinc ions markedly inhibited the rate of hepatic mitochondrial oxygen consumption [Kukoyi et al., 2002]. In the present report concentration of zinc used was increased to 20 uM to complement the liver mitochondrial zinc. Our present study shows the site of inhibition of electron transport by zinc ion in the electron transport chain. As shown in Table 1, inhibition of the rate of oxygen consumption of the liver mitochondria pre-incubated with cytochrome c was observed when compared with control. However, table 2 shows the rate of oxygen consumption with cytochrome c, succinate and NADH as substrates. There was an increase in the inhibition of the rate of the oxygen consumption of liver mitochondria, and NADH increased the rate of oxygen consumption 3- to 4 folds compared to cytochrome c and succinate respectively.

The result shows zinc-citrate, zinc-aspartate and zinc-chloride inhibited the rate of cytochrome c reduction while zinc-EDTA had no effect on the reduction compared to the control that lacked zinc ions.

Discussion

Zinc is an essential trace element and plays important role as a component of many co-factors and enzymes [Vallee and Galdes, 1984]. Cytochrome c is a peripheral membrane protein, which is loosely bound to the outer surface of the inner mitochondrial membrane [Moore and Pettigrew, 1990]. It alternately binds to cytochrome c of complex III and to cytochrome c oxidase [Complex IV] and thereby functions to shuttle electrons between them [Smith et al., 1981]. Potassium cyanide is an inhibitor of cytochrome c oxidase. The rate at which cytochrome c receives electrons and becomes reduced is an indication of a functional electron transport chain. Figure 1 shows zinc inhibition of the transfer of electrons from succinate [complex II] to cytochrome c. Cytochrome c was eventually not reduced while in figure 2, electrons were transferred from NADH [complex I] to cytochrome c and was reduced. These observations suggest that zinc complexes inhibit the electron transfer at the complex II and not at the level of NADH [complex I].

TABLE 1:Effect of zinc on the rate of oxygen consumption in rat liver mitochondria pre-incubated with cytochrome C.

	Succinate	Zinc chloride
Control Experiment [No cytochrome c incubation	0.378±0.032	0.116±0.008
Mitochondria with cytochrome c incubation	0.756±0.015*	0.151±0.012*

Our preliminary results [Table 1] on the effect of zinc ions on the rate of oxygen consumption of the rat liver mitochondria pre-incubated with cytochrome c shows an increase in the inhibition rate compared to the control that lacked cytochrome c. The rate of oxygen consumption increased in mitochondria that were pre-incubated with cytochrome c. Table II shows that cytochrome c has little effect on the rate of oxygen consumption compared to both the succinate and NADH.

Table 2: Effect of cytochrome c, succinate and NADH on the rate of the liver mitochondria oxygen consumption.

Cytochrome c	0.068
Succinate	0.089
NADH	0.232

Units of the rate of oxygen consumption are in microliter of oxygen consumed per minute per milligram of protein

Our previous findings [Kukoyi et al, 2002] indicated that 6 uM of zinc ions was the minimum concentration of zinc ions required to inhibit the rate of oxygen consumption of the rat liver mitochondria significantly.

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Received: December 8, 2002
Accepted: January 27, 2003