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# The effect of exogenous zinc ions on the pattern of oxygen consumption of the hepatic mitochondria of albino rats

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**Summary**

The effect of incubation of coupled liver mitochondria on varying concentration of zinc ion was determined. A low concentration of 6 *u*M zinc ion was found to inhibit the rate of oxygen consumption of the liver mitochondria significantly [P<0.01]. There was uncoupling of the liver mitochondria when subjected to varying incubation periods. There was no change observed in the control experiment. Zinc-citrate inhibited the rate of oxygen consumption significantly [P<0.01] when compare with the control. The changes observed in the Zn-aspartate were insignificant. Zn-EDTA had no inhibitory or stimulatory effect on the rate of liver mitochondrial oxygen consumption.

**Keywords**: *Zinc, ions, liver mitochonolria, omol oxygen consumption*

**Résumé**

L'effect d'incubation des mitochondries couplés du foi sur les concentrations variées de zinc était évalué. A la faible concentration de 6uM, l'ion zinc blocquait significativement le taux de consommation d 'oxygéne aux mitochondries du foi. Il n'avait pas de cuplement des mitochondries du foi quand soumis à different période d'incubation , ni de changement observé au groupe de controle. Le citrate de zinc blocquait le taux de consommation d'oxygene significamment (P<0.001) lorsque comparé avec leur controle. Les changements observés dans l'acide aspartique de zinc n'étaient pas significative. L'EDTA de zinc n'avait pas d'effect sur le taux de consommation d'oxygéne par les mitochondries du foi.

## Introduction

Zinc is an essential component of all cells mediating a variety of cellular activities such as metalloenzyme activity [1]. Mitochondrial zinc constitutes a significant fraction of total cellular zinc. Estimates place the total cellular zinc content of mammalian cells at approximately 0.2 mM. Liver mitochondria *in situ* contain only about 0.6 nmol Zn mg[1] proteins which is less than that in the cytosol [2].

Zinc is known to be active at the lowest concentrations; although its concentration in tissues is relatively high compare with other heavy metals and only iron is present in a higher quantity. Zinc has been reported to accumulate in the liver of animals [3] and most recently Guan et al, [4] reported that prostate and liver accumulate high levels of zinc which results in alter mitochondrial function such as inhibition of m-aconitase and citrate oxidation and induction of mitochondrial apoptogenesis.

The role of zinc as an effective inhibitor of the liver mitochondrial oxygen consumption is being highlighted in this study.

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## Materials and methods

Young adult male Wistar rats weighing 275-350 grams were employed as the source of tissues for these studies. The handling of the animals was in conformity with the National Institute of Health [NIH] and University guidelines for the care and use of animals for research. The preparation of liver mitochondria has been described previously [5,6]. All procedures were carried out at 2° – 4° C on ice. Generally, rat liver tissue were chopped into 1 mm pieces in isolation buffer [250 mM sucrose, 10 mM HEPES and 1 mM EDTA, pH 7.30], homogenized in a motor-driven glass homogenizer, and centrifuge at 500 x g for 5 minutes in a refrigerated Beckman preparative ultracentrifuge. The supernatant fluid was centrifuge for 7 minutes at 12000 xg and the resulting pellet was washed twice in isolation buffer containing 0.25% fatty acid free BSA, and washed once in reaction buffer [250 mM sucrose, 10 mM HEPES and 5 mM $KH_2PO_4$]. The final mitochondrial pellets were suspended in reaction buffer and adjusted to provide a mitochondrial concentration around 20 mg protein $ml^{-1}$. Protein assay was performed by a method of Bradford [7]. The condition of the mitochondrial preparations was checked by determination of oxygen consumption and respiratory control with the aid of a fiber optic oxygen monitoring system. Preparations that did not meet the criteria of no detectable endogenous respiration and succinate-stimulated respiratory control ratio > 2.5 were generally excluded from the studies. All zinc solutions

were dissolved in medium containing 250 mM sucrose and 10 mM HEPES, pH 7.30. Generally 75 *u*l mitochondrial suspension containing 250 *u*g mitochondrial protein was added to 75 *u*l reaction buffer containing O *u*M (control), 2*u*M, 4 *u*M, and 6 *u*M of zinc chloride respectively. Each of the reacting system was incubated for a period of 15 minutes at 37°C. After incubation period, the reaction was initiated by the addition of 50 mM succinate. The rate of oxygen consumption was monitored, and 2 *u*M of ADP was added to complete the reaction.

Experiment was further carried out on varying the time of incubation of the liver mitochondria with 6*u*M $ZnCl_2$, 0 [control], 5, 10, and 15 minutes and the rate of oxygen consumption at each incubation period was monitored.

Zinc-ligand solutions were prepared to provide a Zn/ligand molar ratio of 1:3. These served as a source of free $Zn^{2+}$ ions; zinc citrate (Zn-cit), zinc-aspartate (Zn-asp), zinc-EDTA, and zinc-chloride. Effect of each of these zinc substrates incubation with liver mitochondria on the rate of oxygen consumption was monitored. A control experiment without zinc incubation was carried out in each case.

## Results

Zinc inhibition on the rate of liver mitochondrial oxygen consumption was repeated two or three times to ensure the reproducibility of the results. The data and the plot were analyzed by Sigma Plot 8.0.

In these studies, Figure 2 shows oxygen monitor tracing of sucinate-stimulated rate of oxygen consumption by the rat liver mitochondria. The rate of inhibition of oxygen consumption by the liver mitochondria was determined with $ZnCl_2$ as a source of free $Zn^{2+}$ The result on the effect of different concentration of $ZnCl_2$ on the rate of oxygen consumption reveals significant inhibition at the level of 6 *u*M (Table 1).

**Table 1:** Effect of time of incubation of 6 *u*m zinc chloride on the rate of oxygen consumption by the rat liver mitochondria

| Time [Minutes] | Succinate [*u*l oxygen/min/mg protein] | ADP [*u*l oxygen/min/mg protein] |
|---|---|---|
| Control [No zinc incubation] | 0.193±0.006 | 0.385±0.001 |
| 5 | 0.189±0.007 | 0.218±0.007 |
| 10 | 0.155±0.007 | 0.189±0.010 |
| 15 | 0.160±0.018 | 0.193±0.006 |

*Units of the rate of oxygen consumption are in microliter of Oxygen utilized per minute per milligram of protein Each value is the mean ± S.E of five observations*

Effect of incubation time of $ZnCl_2$ on the rate of oxygen consumption by the liver mitochondria was evident at 15 minutes in a succinate initiated reaction (Table 2).

When different ligands of zinc were incubated with the liver mitochondria there was significant inhibition on the rate at which oxygen was consumed by zinc-citrate when compared with zinc chloride, zinc-aspartate or zinc-

**Table 2:** Effect of different concentration of zinc chloride on the rate of oxygen consumption in the rat liver mitochondria

| Zinc Concentration [uM] | Succinate *u*l oxygen/min/mg protein | Adenosine di-phosphate [ADP] *U*l oxygen/ min/mg protein |
|---|---|---|
| Control [No zinc] | 0.023±0.018 | 0.387±0.005 |
| 2 | 0.166±0.007 | 0.24±0.033 |
| 4 | 0.129±0.007 | 0.29±0.033 |
| 6 | 0.116±0.016* | 0.23±0.007* |

*Units of the rate of oxygen consumption are in microliter of Oxygen utilized per minute per milligram of protein.*
*Each value represents the mean ± S.E for five observations.*
** Significant difference from the control*

EDTA. Zinc chloride has some inhibitory effect on the oxygen consumption but less significant. In contrast, no demonstrable inhibition of oxygen consumption by Zn-EDTA was evident (Figure 1). This indicates that undissociated Zn-EDTA is not permeable across the mitochondrial inner membrane; that zinc is not released from EDTA for availability to inhibit oxygen consumption.

## Discussion

These studies reveal the relevance of zinc to the inhibition of liver mitochondrial oxygen consumption, and presumably ATP synthesis, which are dependent upon

**Fig.1:** Liver mitochondria incubated with 6 uM of different types of zinc for 15 minutes

succinate [substrate] oxidation. The inhibition at the level of 6 *u*M was reported (Table 2). Estimates place the total cellular zinc content of mammalian cells at approximately 0.2 mM. For this discussion we would define three pools of zinc that comprises of the total cellular zinc; (a) tightly bound zinc (mainly metalloenzyme, metalloproteins, nucleoproteins) that is immobile unreactive pool; (b) loosely bound zinc ( such as amino acid, and citrate bound) which constitute a mobile reactive pool; (c) free $Zn^{2+}$ ion which would be a reactive pool. The mobile unreactive pool comprises >95% of the total cellular zinc. The free $Zn^{+2}$ ion concentration is negligible; estimated to be in the nanomolar to picomolar range, and even as low as femtomolar concentration [8]. Therefore, zinc bound to mainly low molecular weight ligands (Zn-ligands) comprises the major mobile reactive pool of zinc.

Figure 1 indicates that zinc-citrate has a significant inhibitory effect on the rate of oxygen consumption while Zn-EDTA has none due to the fact that it is not able to dissociate to release $Zn^{+2}$ ions across the mitochondria to effect the inhibition of oxygen consumption. Our recent findings indicated that zinc chelated with citrate (Zn-cit) is a major form of zinc which represents an important potential cytosolic source of transportable zinc into mitochondria and uniquely responsible for the inhibition of the oxygen consumption [3]

Table 1 show that the longer the incubation period of the mitochondria with $ZnCl_2$( free $Zn^{2+}$) the more there is transportation of more zinc ions into the mitochondria to inhibit oxygen consumption.

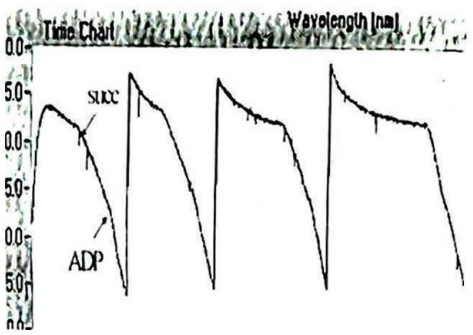


**Fig. 2:** Oxygen monitor tracing of succinate-stimulated rate of oxygen consumption by the rate liver mitochondria

**Abbreviations**

BSA bovine serum albumin
EDTA ethylenediaminetetraacetic acid
HEPES N-2-hydroxyethylpiperazine-N'-2-ethansulfonic acid

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