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Terminal oxidation and the effects of zinc in prostate versus liver mitochondria

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Abstract

Although the total zinc content of cells generally approximates 0.2 mM, the cytosolic free zinc ion concentration is negligible (subnanomolar concentrations). However, all reported studies of effects of zinc on cellular respiration and terminal oxidation involved μM – mM levels of free zinc ions. Prostate cells and their mitochondria accumulate 3–10 fold more zinc than other mammalian cells. We considered that a cytosolic pool of mobile reactive low molecular weight zinc ligands could inhibit respiration and terminal oxidation. The effects of ZnLigands, especially ZnCitrate, versus free Zn^{++} ions on respiration and terminal oxidation were studied with prostate and liver mitochondria. ZnLigands were equally as effective as free Zn^{++} ions in the inhibition of respiration and terminal oxidation of both prostate and liver mitochondria, which supports our concept that zinc can be transferred from cytosolic donor ZnLigands directly to zinc-binding sites of terminal oxidation components. Also, the respiration and specific activities of terminal oxidation components of prostate mitochondria are 20–50% of liver mitochondria. Zinc inhibition and inherently low levels of electron transport components are likely major factors responsible for the low respiration that characterizes prostate cells.

Keywords

Zinc; Prostate and liver; Mitochondrial respiration; Terminal oxidation; Citrate; Succinate and NADH oxidation

1. Introduction

‘In biological systems, very little, if any, zinc is free in solution’ (Vallee and Falchuk, 1993). Estimates place the total cellular zinc content of mammalian cells at approximately 0.2 mM (Outten and O’Halloran, 2001; Vallee and Falchuk, 1993). However the cytosolic concentration of free Zn^{++} ions is estimated to be in the nM–fM range; i.e. a negligible pool of zinc (Outten and O’Halloran, 2001; Williams and Frausto da Silva, 2000; Peck and Ray, 1971). Of the total cellular zinc, about 50% is cytoplasmic (cytosol and organelles). The cytosol provides the available pool of zinc for effects on mitochondrial function. In the

virtual absence of free Zn^{++} ions, essentially all the cytosolic zinc exists bound to either immobile macromolecules (~ 90%) or to mobile low molecular weight ligands (~ 10%). The former would not provide an available source of zinc to mitochondria, but the latter could provide the available zinc pool for mitochondrial interaction. The outer mitochondrial membrane pore structure permits the passage of molecules up to ~ 10 kDa; so that molecules of this size and smaller would constitute the low molecular weight zinc ligands for the delivery of zinc into the intermembrane space. This potential pool of zinc donors for interaction within the mitochondria would approximate a low μM range. Therefore, for physiological relevancy, any studies relating to the intracellular effects of zinc on mitochondrial function must involve zinc forms and concentrations that would reflect the cellular composition of zinc.

A focus of this study involves the effects of zinc on mitochondrial respiration and terminal oxidation, with special emphasis on prostate cells. Several studies with mammalian cells have reported that zinc ions inhibit mitochondrial respiration and terminal oxidation (Hunter and Ford, 1955; Skulachev et al., 1967; Nicholls and Malviya, 1968; Kleiner and von Jagow, 1972; Lorusso et al., 1991; Link and von Jagow, 1995). However all these studies employed μM – mM concentrations of free Zn^{++} ions; i.e. conditions that are non-existent in normal or pathological cellular environments. Therefore, the role and effects of zinc in mammalian cells that reflect intracellular conditions had not been established. This important physiological and metabolic relationship is addressed in this report.

Prostate secretory epithelial cells and their mitochondria accumulate uniquely high levels of zinc; generally 3–10 fold higher than other mammalian cells (Liu et al., 1997; Costello and Franklin, 1998). It is notable that prostate cells are characterized as exhibiting a high aerobic glycolysis and low respiration (Barron and Huggins, 1944; Nyden and Williams-Ashman, 1953; Harkonen, 1981). However the cause of these unique metabolic relationships of prostate cells has not been established. Unlike other mammalian cells, prostate cells contain a high concentration of citrate, which is a major ligand that comprises about 30% of the cellular zinc (Kavanagh, 1983; Larue and Morfin, 1984). These relationships caused us to consider that zinc might have an inhibitory effect on terminal oxidation in these cells, which could result in their low respiration. With these conditions in mind, the effects of representative cellular levels and forms of zinc on respiration and terminal oxidation of prostate mitochondria were determined; and for comparison, the effects on liver mitochondria.

2. Experimental

2.1. Rat tissue preparation

Young adult male Wistar rats weighing between 300 and 350 g were employed as the source of tissues and mitochondria preparations. The handling, treatment, and euthanasia of animals were in accordance with the regulations and guidelines of the National Institutes of Health and the University of Maryland. Rat ventral prostate lobes and liver tissue were resected immediately upon the sacrifice of animals. The tissues were chopped into 1 mm³ pieces in cold 2–4 °C mitochondrial isolation buffer containing 250 mM sucrose, 10 mM HEPES and 1 mM EDTA, pH 7.35; homogenized in a motorized glass homogenizer, and centrifuged at

500 × g for 5 min. The supernatant fluid was centrifuged for 7 min at 12,000 × g and the resulting pellet was washed twice in the isolation buffer containing 0.25% dialyzed fatty acid-free bovine serum albumin, and washed once in zinc uptake reaction buffer (250 mM sucrose, 10 mM HEPES and 5 mM KH₂PO₄). The final mitochondrial pellets were suspended in reaction buffer and adjusted to provide a mitochondrial concentration around 20 mg protein/ml. Protein assay was performed by the method of Bradford (1976). The condition of the mitochondrial preparations was checked by determination of oxygen consumption and respiratory control.

2.2. Measurement of mitochondrial oxygen consumption

Mitochondrial oxygen consumption was measured at 26 °C with a fiber optic oxygen monitoring system. Aliquots of 250–500 µg mitochondrial protein were added to a small chamber containing 250 µl reaction buffer (250 mM Sucrose, 10 mM HEPES, 5 mM KH₂PO₄, and 1 mM MgCl₂, pH 7.35) with magnetic stirring. Generally, the mitochondrial suspensions were preincubated with or without ZnLigands for 5 min at room temperature. Respiration was initiated by the addition of succinate at a final concentration of 4 mM, which was followed by adding ADP to a final concentration of 0.4 mM to obtain state 3 respiration. The final reaction volume was 300 µl. The respiration rates were calculated as nmol oxygen consumed per minute per mg mitochondrial protein.

2.3. Measurement of enzyme activities in the electron transport chain

Because of the combination of low inherent terminal oxidation activity and high turbidity of prostate mitochondrial preparations, the specific activities of the electron transport chain reactions were determined by dual wavelength spectrophotometry with a DW-2000A spectrophotometer. For these studies the mitochondrial preparations were mildly sonicated. Depending upon enzyme activity, aliquots of 50–200 µg mitochondrial protein were loaded into the quartz cuvette containing ETC reaction buffer (250 mM Sucrose, 10 mM HEPES, 1 mM MgCl₂, pH 7.35). Substrates and other reagents were added and mixed thoroughly to a final reaction volume of 2 ml. NADH or succinate was used as a substrate. Either decylubiquinone or oxidized cytochrome *c* was employed as the electron acceptors for NADH or succinate oxidation; and reduced cytochrome *c* was the substrate for cytochrome oxidase activity. For NADH oxidation the wavelength pair was 340/378 nm; for cytochrome *c* reduction or oxidation, the wavelength pair was 550/540 nm. Time-based reactions were tracked for 30 s to 4 min depending upon the enzyme activity. Specific activities in the presence and absence of zinc were calculated as nmol per minute per mg mitochondrial protein.

2.4. Preparation of zinc solutions

For these studies we employed zinc solutions prepared in either mitochondrial reaction buffer or ETC buffer in the form of ZnCl₂ to represent the concentration of free Zn⁺⁺ ions; ZnCitrate as a major mobile reactive form of zinc in prostate; ZnCysteine as a mobile reactive form in which the free Zn⁺⁺ ion concentration is negligible; ZnEDTA as a tightly bound unreactive form in which the free Zn⁺⁺ ion concentration is negligible. The ZnLigand solutions contained 20 µM ZnCl₂ and 60 µM ligand. The basis for these

concentrations and characterizations of zinc ligand forms and their reactivity is presented in Guan et al. (2003). The log K_f values (formation constants) are ZnCitrate ~ 5 , ZnCysteine ~ 10 , ZnEDTA ~ 16 . At a ligand/ ZnCl_2 concentration ratio of 60 μM ligand and 20 μM ZnCl_2 , the estimated free Zn^{++} ion concentration for ZnCitrate is $\sim 3 \mu\text{M}$; for ZnCysteine is $\sim 1 \text{ nM}$; and for ZnEDTA is $< 1 \text{ pM}$.

All the experiments were repeated a minimum of three times to establish the consistency of the effects. The representative results are presented.

3. Results

3.1. Respiration studies

We first determined the effects of free Zn^{++} ions and ZnLigands on succinate-stimulated respiration of mitochondria from rat ventral prostate cells. Fig. 1 shows that respiration was similarly inhibited by ZnCl_2 , ZnCitrate, and ZnCysteine. The concentration of total zinc in all solutions was 20 μM , but the free Zn^{++} ions in each solution was 20 μM , $\sim 3 \mu\text{M}$, and $\sim 1 \text{ nM}$, respectively. Therefore the inhibition was dependent upon the total zinc concentration and independent of the concentration or availability of free Zn^{++} ions. ZnEDTA, which contains negligible free Zn^{++} ions, had no effect on respiration; which demonstrates that zinc inhibition is dependent upon the concentration of the total zinc that exists in a reactive form. This is dependent upon the binding affinity of the ZnLigand. ZnLigands with log $K_f \sim 10$ (ZnCysteine) or lower will effectively inhibit respiration. As the log K_f increases above ~ 10 toward that of ZnEDTA (~ 16), the tightly bound zinc becomes unavailable for inhibition; i.e. a tightly bound un-reactive form of zinc. Also the rate of respiration of ZnEDTA-treated mitochondria in this experiment and in experiments described below was identical to the initial mitochondrial respiration, which demonstrates that the mitochondrial preparations maintained functionality throughout the duration of the experiment.

Fig. 1 also reveals that zinc inhibition of succinate-stimulated respiration of coupled and uncoupled mitochondria was $\sim 50\text{--}60\%$; and state 3 respiration was inhibited by $\sim 70\text{--}80\%$. These results indicate that the inhibitory effect of zinc is due to an effect on some electron transport component. This expectation is confirmed by the studies on terminal oxidation presented below.

To test whether or not the inhibitory effect of zinc is specific to the prostate mitochondria, similar experiments were conducted in uncoupled and coupled liver mitochondria (Fig. 2). The effects were virtually identical to the results obtained with prostate mitochondria in that zinc resulted in marked inhibition ($\sim 50\text{--}60\%$) of succinate-stimulated respiration, and $\sim 70\text{--}80\%$ of state 3 respiration. Also, the dependency of zinc inhibition on the total concentration of zinc as loosely bound ZnLigands and not free Zn^{++} ions is evident with liver as well as prostate mitochondria.

In the studies described above the mitochondria were pre-incubated with zinc for 5 min prior to the determination of respiration. Mitochondrial respiration was also determined prior to exposure to zinc and immediately following the addition of zinc. Fig. 3 shows that the inhibition of succinate-stimulated respiration occurs immediately upon the addition of zinc,

which is indicative of a rapid exchange and interaction of zinc with the affected respiratory component.

The comparative studies of prostate and liver mitochondria revealed that the rate of succinate-stimulated oxygen consumption of prostate mitochondria is markedly lower than that of liver mitochondria. Table 1 summarizes the rates obtained from several different prostate and liver mitochondrial preparations. It is apparent that succinate-stimulated prostate respiration is only 20–25% of liver mitochondrial respiration. This same relationship and the inhibitory effects of zinc also apply to NADH-stimulated respiration (not shown). The implications and extension of these comparative differences are further described below.

3.2. Terminal oxidation studies

The results of the respiration studies raised two immediate issues that we addressed. What is the cause of the zinc inhibition of respiration? Why is the respiration of prostate mitochondria dramatically lower than liver mitochondria? To address these questions, we determined the effects of zinc on the terminal oxidation of succinate and NADH. Succinate and NADH oxidation by prostate mitochondria leading to cytochrome *c* reduction was similarly inhibited (~ 40–60% inhibition) by ZnCl₂, ZnCitrate, and ZnCysteine, with no inhibitory effect of ZnEDTA (Fig. 4). The results show that the inhibition is not dependent upon the availability of free Zn⁺⁺ ions, but is dependent upon the total concentration of reactive ZnLigands. This replicates the zinc effects on respiration described above.

With liver mitochondria (Fig. 5), the inhibitory effects (~ 50–60%) of zinc on succinate oxidation leading to cytochrome *c* reduction were virtually identical to the effects obtained with prostate mitochondria. The inhibitory effect (~ 15–20% inhibition) of zinc on NADH oxidation leading to cytochrome *c* reduction was consistently lower than the ~ 40–60 inhibition obtained with prostate mitochondria. To corroborate this low inhibitory effect of zinc, we determined NADH oxidation more directly by the change in absorbance at 340 nM in the presence of ubiquinone. NADH oxidation was decreased by ~ 20–40%, which confirms that zinc inhibited the oxidation of NADH. As shown in Table 1, the specific activities for NADH oxidation measured by cytochrome *c* reduction or by direct NADH oxidation with ubiquinone were virtually the same.

The possibility also existed that zinc inhibition of mitochondrial respiration was due to an inhibitory effect on cytochrome *c* oxidase. Fig. 6 reveals that zinc has no effect on cytochrome *c* oxidase activity.

Table 1 contains the comparative specific activities of the terminal oxidation reactions conducted in the studies described above. It is clear that all the reactions of prostate mitochondria from substrate oxidation through cytochrome oxidase are markedly lower (~ 50–80% lower) than liver mitochondria. This correlates well with the significantly lower respiration of prostate mitochondria.

4. Discussion

These studies reveal that zinc, under conditions that mimic its cytosolic concentration and form, inhibits the respiration and terminal oxidation of prostate and liver mitochondria. The inhibition does not require the presence of a free Zn^{++} ion pool; which is a negligible form of reactive zinc in cells. ZnLigands that have relatively low binding affinities ($\log K_f \sim 10$ or lower) are equally as effective as equimolar concentrations of free Zn^{++} ions in the inhibition of respiration and terminal oxidation. This reveals that the inhibitory effect involves a direct intermolecular transfer of zinc from the donor ZnLigand to the recipient electron transport component. A similar mechanism is involved in the import of zinc into mitochondria by a putative inner membrane zinc uptake transporter that has an apparent $\log K_f \sim 11$ (Guan et al., 2003). Consistent with this mechanism, Halhouth et al. (2001) reported that the direct intermolecular transfer of zinc from donor ligand to recipient peptide does not involve an intermediary free Zn^{++} ion step, and is dependent upon the relative zinc-binding affinities of the interacting components.

Hunter and Ford (1955) first reported that micro-molar levels of free zinc ions dramatically inhibited coupled liver mitochondrial oxygen consumption stimulated by β -hydroxybutyrate. Similar inhibitory effects of zinc were obtained by Ye et al. (2001) with uncoupled liver mitochondria. Skulachev et al. (1967) found that μM free zinc ions inhibited electron transfer between cytochrome *b* and c_1 of complex III and had no effect on the cytochrome *c* oxidase; and our results are in general agreement with those observations. Similar observations were reported by studies in bovine heart mitochondria (Nicholls and Malviya, 1968; Kleiner and von Jagow, 1972; Lorusso et al., 1991; Link and von Jagow, 1995). Link and von Jagow (1995) proposed that zinc inhibits complex III by interfering with proton transfer reactions at the Q_p center of the bc_1 complex. However, all these studies involved effects of free Zn^{++} ions, which do not exist in the intracellular environment. Prior to the results presented herein, no information was available concerning the effects of zinc under physiological cellular conditions; nor on prostate mitochondrial electron transport and respiration. It is now evident that μM concentrations of zinc that is loosely-bound to ligands is equally as effective as free Zn^{++} ions in the inhibition of respiration and terminal oxidation.

Our studies were not designed to establish the specific component(s) that might be directly involved in this inhibitory effect. The inhibition of both succinate and NADH oxidation by prostate and liver mitochondria leading to cytochrome *c* reduction strongly supports the likely inhibition of complex III by zinc. However, Link and von Jagow (1995) determined that the bc_1 zinc-binding sites have binding affinities equivalent to $\log K_f \sim 6-7$. Seemingly, this would be inconsistent with the effective inhibition obtained with ZnCysteine ($\log K_f \sim 10$). One might suggest that cysteine could have been oxidized to release Zn^{++} ions. However, the rapidity and the extent (equivalent to free Zn^{++} ion and to ZnCitrate) of the zinc inhibition by ZnCysteine argue against such a mechanism. Conceivably, the effects observed in this report are due to a direct transfer of zinc from donor ZnLigands to zinc-binding site(s) in complex III and possibly complexes I and/or II. Follow-up studies are now essential to establish the interaction of ZnLigands rather than free Zn^{++} ions with the respiratory complexes.

Prostate cells contain several-fold higher citrate levels than other mammalian cells. Consequently citrate is an important ligand for zinc in these cells. Although the exact cytosolic concentration of Zn-citrate (mainly Zn_3Cit_2) is unknown, a reasonable estimate is in the range of 30–100 μM . Prostate epithelial cells can contain ~ 3.0 mM citrate or more, which accounts for an estimated 30% of the total cellular zinc. Therefore, the observed equal inhibitory effects of 20 μM zinc in the form of Zn-citrate compared to free Zn^{++} ions demonstrates that the former is a likely physiological zinc inhibitor of terminal oxidation. However these conditions do not exist in liver cells or other mammalian cells, in which the cellular level of citrate is only about 0.1 mM and mobile reactive zinc approximates 20 μM . However, in liver cells other potential ligands including metallothionein ($\log K_f \sim 11\text{--}12$) can likely serve as zinc donors that could inhibit respiration and terminal oxidation. We have reported that metallothionein is an effective chaperone for the delivery of zinc to the mitochondrial inner membrane for direct exchange with zinc uptake transporter, so it is likely that this relationship is applicable to zinc inhibition in liver cells. This was suggested by Ye et al. (2001) who found that zinc-metallothionein entered the mitochondrial intermembrane space where it inhibited the respiration of liver mitochondria. They showed that, in the absence of a significant free Zn^{++} pool, zinc could be transferred from metallothionein to zinc-acceptor site; and that localized oxidation of zinc-metallothionein would enhance the effect by facilitating its release of zinc.

Because of the specialized function of high citrate production, these prostate cells possess a unique intermediary metabolism (for reviews see Costello and Franklin, 1998, 2000; Costello et al., 2004). The Krebs cycle is truncated at the first step of citrate oxidation due to the inhibition of m-aconitase by zinc (Costello et al., 1997). Prostate cells have a high rate of aerobic glycolysis and low respiration (Barron and Huggins, 1944; Nyden and Williams-Ashman, 1953; Harkonen, 1981). However, the cause of the low respiration had been unknown. The present studies reveal that the respiration and specific activities of terminal oxidation of the prostate mitochondria are dramatically lower than liver mitochondria. Presumably, the levels or copies of the electron transport complexes are inherently low in these cells, and we are now exploring this possibility. In addition, the high levels of mobile reactive zinc that characterize the prostate cells will inhibit terminal oxidation. This limiting terminal oxidation and the truncated Krebs cycle due to zinc-inhibited m-aconitase activity are likely important factors that account for the low respiration that is characteristic of these cells. It is important to recognize the metabolic-energetic implications associated with these cells. The accumulation of citrate as an end product of metabolism results in an energy production of 14 ATP/glucose as compared to 38 ATP/glucose. The accelerated aerobic glycolysis is an essential compensation to increase energy production essential for the survival and functioning of these highly specialized cells. Other normal mammalian cells cannot survive with an aconitase-inhibited citrate oxidation as is evident from the lethal inhibitory effects of fluorocitrate on m-aconitase. Thus the low cellular respiration, high aerobic glycolysis, low terminal oxidation and mitochondrial respiration, and the inhibitory effects of zinc are integrated metabolic adaptations required for the specialized function of net citrate production and survival of the prostate secretory epithelial cells.

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References

- Barron ESG, Huggins C. The metabolism of isolated prostatic tissue. *J Urol*. 1944; 51:630–634.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976; 72:248–254. [PubMed: 942051]
- Costello LC, Franklin RB. Novel role of zinc in the regulation of prostate citrate metabolism and its implications in prostate cancer. *Prostate*. 1998; 35:285–296. [PubMed: 9609552]
- Costello LC, Franklin RB. The intermediary metabolism of the prostate: A key to understanding the pathogenesis and progression of prostate malignancy. *Oncology*. 2000; 59:269–282. [PubMed: 11096338]
- Costello LC, Liu Y, Franklin RB, Kennedy MC. Zinc inhibition of mitochondrial aconitase and its importance in citrate metabolism of prostate epithelial cells. *J Biol Chem*. 1997; 272:28875–28881. [PubMed: 9360955]
- Costello LC, Feng P, Milon B, Tan M, Franklin RB. The role of zinc in the pathogenesis and treatment of prostate cancer: Critical issues to resolve. *Prostate Cancer Prostatic Diseases*. 2004; 7:111–117. [PubMed: 15175662]
- Guan ZX, Kukoyi B, Feng P, Kennedy MC, Franklin RB, Costello LC. Kinetic identification of a mitochondrial zinc uptake transporter in prostate cells. *J Inorg Biochem*. 2003; 97:199–206. [PubMed: 14512198]
- Halthout Y, Fabrisa D, Fenselau C. Stoichiometry in zinc ion transfer from metallothionein to zinc finger peptides. *Internat J Mass Spectrom*. 2001; 204:1–6.
- Harkonen P. Androgenic control of glycolysis, the pentose cycle and pyruvate dehydrogenase in the rat ventral prostate. *J Steroid Biochem*. 1981; 14:1075–1084. [PubMed: 7300327]
- Hunter FE, Ford L. Inactivation of oxidative and phosphorylative systems in mitochondria by preincubation with phosphate and other ions. *J Biol Chem*. 1955; 216:357–369. [PubMed: 13252035]
- Kavanagh JP. Zinc binding properties of human prostatic tissue, prostatic secretion and seminal fluid. *J Reprod Fertil*. 1983; 68:359–363. [PubMed: 6864650]
- Kleiner D, von Jagow G. On the inhibition of mitochondrial electron transport by Zn(2+) ions. *Fed Eur Biochem Soc Lett*. 1972; 20:229–232.
- Larue JP, Morfin RF. Identification of a low molecular weight ligand for zinc in the human hyperplastic prostate. *Endocr Res*. 1984; 10:171–181. [PubMed: 6389106]
- Link TA, von Jagow G. Zinc ions inhibit the QP center of bovine heart mitochondrial bc1 complex by blocking a protonatable group. *J Biol Chem*. 1995; 270:25001–25006. [PubMed: 7559629]
- Liu Y, Franklin RB, Costello LC. Prolactin and testosterone regulation of mitochondrial zinc in prostate epithelial cells. *Prostate*. 1997; 30:26–32. [PubMed: 9018332]
- Lorusso M, Cocco T, Sardanelli AM, Minuto M, Bonomi F, Papa S. Interaction of Zn²⁺ with the bovine-heart mitochondrial bc1 complex. *Eur J Biochem*. 1991; 197:555–561. [PubMed: 1851092]
- Nicholls P, Malviya AN. Inhibition of nonphosphorylating electron transfer by zinc. The problem of delineating interaction sites. *Biochemistry*. 1968; 7:305–310. [PubMed: 4991481]
- Nyden SJ, Williams-Ashman HG. Influence of androgens on synthetic reactions in ventral prostate tissue. *Am J Physiol*. 1953; 172:588–600. [PubMed: 13030791]
- Outten CE, O'Halloran TV. Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. *Science*. 2001; 292:2488–2492. [PubMed: 11397910]
- Peck EJ Jr, Ray WJ Jr. Metal complexes of phosphoglucomutase in vivo. *J Biol Chem*. 1971; 246:1160–1167. [PubMed: 5543683]
- Skulachev VP, Chistyakov VV, Jasaitis AA, Smirnova EG. Inhibition of the respiratory chain by zinc ions. *Biochem Biophys Res Commun*. 1967; 26:1–6. [PubMed: 4291553]

- Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. *Physiol Rev.* 1993; 73:79–118. [PubMed: 8419966]
- Williams RJP, Frausto da Silva JJR. The distribution of elements in cells. *Coordinat Chem Rev.* 2000; 247:200–202.
- Ye B, Maret W, Vallee BL. Zinc metallothionein import into liver mitochondria modulates respiration. *Proc Natl Acad Sci.* 2001; 98:2317–2322. [PubMed: 11226237]

Fig. 1.

Effects of zinc on ventral prostate mitochondria oxygen consumption. Respiration was initiated by the addition of 4 mM succinate. State III respiration rate was determined by adding ADP at a final concentration of 0.4 mM. ZnLigands contained 20 μ M ZnCl₂ and 60 μ M ligand. Values are nmol oxygen consumed/min per mg protein.

Fig. 2.
Effects of zinc on liver mitochondrial oxygen consumption. Conditions were the same as in Fig. 1.

Fig. 3. Rapid effect of zinc on mitochondrial oxygen consumption. (A) Uncoupled mitochondrial preparations. Values in percentage are inhibition of respiration by zinc. (B) Coupled liver mitochondrial preparation. Values are nmol oxygen/min per mg protein. Conditions were the same as in Fig. 1.

Fig. 4.

Effect of zinc on NADH and succinate oxidation in VP mitochondria. Ventral prostate mitochondria were preincubated with ZnLigands for 5 min in a cuvette containing 2 ml reaction buffer (250 mM sucrose, 10 mM HEPES, 1 mM MgCl₂, pH 7.35) supplemented with 5 mM cyanide and 3 μ M oxidized cytochrome *c*.

Fig. 5. Zinc effect on NADH and succinate oxidation in liver mitochondria. Conditions were the same as Fig. 4. Ubiquinone was 5 μ M.

Fig. 6.
Effects of zinc on cytochrome *c* oxidase activity in liver and ventral prostate mitochondria. Mitochondria were preincubated with zinc for 5 min prior to the addition of reduced cytochrome *c*. Conditions were the same as in Fig. 4, except cyanide was excluded.

Table 1

Terminal oxidation activities of liver and prostate mitochondria

Reaction		Liver mitochondria	Prostate mitochondria
Oxygen consumption	State III	55.09 ± 7.53	10.15 ± 0.21
	State IV	20.96 ± 3.02	5.6 ± 0.84
NADH → UQ		255.52 ± 3.73	114.75 ± 0.41
NADH → cytochrome <i>c</i>		285 ± 12.81	90.78 ± 8.28
Succinate → cytochrome <i>c</i>		51.56 ± 1.56	12.4 ± 0.05
Cytochrome <i>c</i> oxidase		223.75 ^a	113.75 ± 3.75

Unit: nmol/min per mg protein.

^aFrom one experiment. Others results were from at least three separate experiments.