Zinc Inhibition of Mitochondrial Aconitase and Its Importance in Citrate Metabolism of Prostate Epithelial Cells

(Received for publication, May 1, 1997, and in revised form, August 27, 1997)

Leslie C. Costello, Yiyian Liu, Renty B. Franklin, Mary Claire Kennedy

From the Cellular and Molecular Biology Section /Oral Craniofacial and Biological Sciences, Dental School, University of Maryland, Baltimore, Maryland 21201 and Department of Biochemistry, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Prostate epithelial cells possess a uniquely limiting mitochondrial (m-) aconitase activity that minimizes their ability to oxidize citrate. These cells also possess uniquely high cellular and mitochondrial zinc levels. Correlations among zinc, citrate, and m-aconitase in prostate indicated that zinc might be an inhibitor of prostate m-aconitase activity and citrate oxidation. The present studies reveal that zinc at near physiological levels inhibited m-aconitase activity of mitochondrial sonicate preparations obtained from rat ventral prostate epithelial cells. Corresponding studies conducted with mitochondrial sonicates of rat kidney cells revealed that zinc also inhibited the kidney m-aconitase activity. However the inhibitory effect of zinc was more sensitive with the prostate m-aconitase activity. Zinc inhibition fit the competitive inhibitor model. The inhibitory effect of zinc occurred only with citrate as substrate and was specific for the citrate $\rightarrow$ cis-aconitate reaction. Other cations (Ca$^{2+}$, Mn$^{2+}$, Cd$^{2+}$) did not result in the inhibitory effects obtained with zinc. The presence of endogenous zinc inhibited the m-aconitase activity of the prostate mitochondrial preparations. Kidney preparations that contain lower endogenous zinc levels exhibited no endogenous inhibition of m-aconitase activity. Studies with pig prostate and seminal vesicle mitochondrial preparations also revealed that zinc was a competitive inhibitor against citrate of m-aconitase activity. The effects of zinc on purified beef heart m-aconitase verified the competitive inhibitor action of zinc. In contrast, zinc had no inhibitory effect on purified cytosolic aconitase. These studies reveal for the first time that zinc is a specific inhibitor of m-aconitase of mammalian cells. In prostate epithelial cells, in situ mitochondrial zinc levels inhibit m-aconitase activity, which provides a mechanism by which citrate oxidation is limited.

Prostate secretory epithelial cells have the specialized function and capability of accumulating and secreting extraordinarily high levels of citrate. This is achieved by the existence of a uniquely limiting m-aconitase activity that minimizes the oxidation of citrate via the Krebs cycle. Consequently, citrate synthesized by these cells is accumulated and secreted (which we refer to as "net citrate production"), thereby accounting for the extremely high (20–150 mM) citrate content of human prostatic fluid. In typical mammalian cell metabolism, m-aconitase is not a regulatory, rate-limiting enzyme. Consequently, the steady-state citrate/isocitrate ratio of most cells is generally maintained at about 11/1, which is established by the aconitase equilibrium reaction, 88 citrate $\rightarrow$ 4 cis-aconitate $\rightarrow$ 8 isocitrate. In contrast, the citrate/isocitrate ratio in prostate is generally about 30/1. Also, the intracellular citrate concentration of prostate cells is estimated to be about 1.2 mM as compared with about 0.1–0.4 mM for typical mammalian cells. These and other relationships of prostate citrate metabolism and regulation are detailed in our recent review articles (1–4).

This paper addresses the mechanism associated with this uniquely limiting m-aconitase activity of prostate cells. The level of prostate m-aconitase enzyme appears to be similar to that associated with other cells, although the levels of m-aconitase activity and citrate oxidation are significantly lower in prostate cells (5–7). Thus the limiting m-aconitase activity is due to unique properties of the enzyme and/or unique mitochondrial conditions that inhibit the enzyme activity. Based on the close correlations between zinc and citrate levels in prostate and the fact that prostate cells accumulate the highest level of zinc in the body, we reasoned that zinc might be involved in the m-aconitase/citrate relationship (1–4). Moreover, earlier studies provided evidence that zinc could be an inhibitor of prostate m-aconitase activity (8). However, to our knowledge no other studies have been reported concerning zinc as an inhibitor of m-aconitase activity. This report 1) demonstrates that zinc at low concentrations is a specific inhibitor of mammalian cell m-aconitase activity, 2) presents preliminary kinetic information concerning the mechanism of zinc inhibition, 3) establishes that physiological levels of zinc inhibit the m-aconitase activity of prostate epithelial cells, and 4) provides the basis for establishing an important and unique role of zinc in prostate citrate metabolism.

MATERIALS AND METHODS

Young adult male Wistar rats weighing between 300 and 350 g were employed as the source of tissues for these studies. The handling and treatment of animals were in accordance with the regulations and guidelines of the National Institutes of Health and the University of Maryland. The preparation of prostate epithelial cells and kidney cells from male rats as well as the procedure for obtaining mitochondrial preparations has been described previously (5–7). Generally, the mitochondria were isolated in 250 mM sucrose containing 50 mM HEPES buffer, pH 7.2. The mitochondrial preparations were generally sonicated in 50 mM HEPES buffer. The sonicates were centrifuged at 20,000 $\times$ g for 30 min, and the supernatant fluid was the source for m-aconitase activity. In some experiments, the supernatant fluid was passed through a Bio-Rad 10DG desalting column to remove endogenous substrates. All steps were conducted at 2–4 °C. The protein concentration of the sonicate preparations was determined by the method

* This research was supported by National Institutes of Health Grants DK-28015 (to L. C. C. and R. B. F.), CA-71207 (to L. C. C. and R. B. F.), and GM-51831 (to M. C. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This advertisement is in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence should be addressed: OCB/Dental School, 666 W. Baltimore St., Baltimore, MD 21201. Tel.: 410-706-7618; Fax: 410-706-0193; E-mail: lcostell@umbabnet.ab.umd.edu.

2 The abbreviations used are: m- and c-, mitochondrial and cytosolic aconitase, respectively.
of Bradford (9). Before use, the sonicates were incubated with activating solution (5 mM cysteine, 0.5 mM ferrous ammonium sulfate) to activate the m-aconitase. Specific modifications of these procedures are described in the presentation of the results.

Purified m-aconitase was prepared in the laboratory of Dr. Kennedy from beef heart mitochondria and was activated anaerobically with Fe$^{2+}$, dithiothreitol, and dithionite as described previously (10). To remove excess low molecular activating agents, the protein solution was rapidly desalted anaerobically on G-50 Sephadex columns equilibrated with 0.1 M HEPES/K$^+$, pH 7.5, using the method of Penefsky (11). The active enzyme was frozen in 25-$\mu$l pellets in liquid nitrogen and stored at −77 °C until used.

Mitochondrial aconitase activity was determined fluoroenzymatically by coupling the m-aconitase reaction with isocitric dehydrogenase as described previously (7). Fluorometer tubes contained 1.0 ml of 50 mM HEPES with 0.1 mM Mg$^{2+}$ (pH 7.2). Generally 10 $\mu$l of excess isocitric dehydrogenase (Sigma), 10 $\mu$l of excess NADP, and 5–20 $\mu$l of mitochondrial extract (to provide ~50 $\mu$g of mitochondrial protein) were added to establish the base-line fluorescence. The aconitase reaction was run at 25 °C and initiated by the addition of substrate (usually from 0.01–1.0 mM final concentration citrate or cis-aconitate), and the reduction of NADP was recorded. The m-aconitase activity is reported as $\mu$mol of NADPH formed (i.e. substrate utilized)/mg of protein/min. This assay measures m-aconitase activity in the direction citrate $\rightarrow$ cis-aconitate $\rightarrow$ isocitrate. For this assay to work properly, isocitric dehydrogenase must not be limiting, and we check these conditions with each assay. In some experiments, aconitase activity was determined by dual-wavelength spectrophotometry by tracking either the utilization or formation of cis-aconitate at 240/280 $\mu$m.

The zinc content of mitochondrial preparations was determined by atomic absorption as described previously (12). Enzyme-inhibitor kinetics were analyzed with the enzyme-PC computer program developed by Lutz and Rodbard (13). This program provides analysis for pure, partial, and mixed types of competitive, uncompetitive, and noncompetitive inhibition. The results presented are representative of the data obtained from experiments that were repeated two or more times to ensure the consistency and reproducibility of the results.

RESULTS

We previously demonstrated that the addition of zinc in the range of 1–10 mM markedly inhibited the m-aconitase activity of prostate mitochondrial preparations (8). Recently the in situ concentration of mitochondrial zinc was determined to be in the range of 50–1100 ng/mg of mitochondrial protein (equivalent to approximately 0.15–3.0 $\mu$g zinc), depending upon the cell type (12). Within this range, the zinc levels of prostate cells is 2–20 times greater than the level in nonprostate cells. The present studies were initiated to establish the effect of more physiological concentrations of zinc on prostate m-aconitase activity. The results (Fig. 1) demonstrate that zinc in the range of 0.004–0.015 mM (equivalent to approximately 3–11 $\mu$g of zinc/mg of mitochondrial protein) inhibited the m-aconitase reaction, citrate $\rightarrow$ isocitrate, in the presence of 0.05 mM citrate as substrate. The inhibition exhibited a linear dose response effect over this range. In parallel assays, zinc also exhibited an inhibitory effect on kidney m-aconitase activity (Fig. 1). For the prostate preparation, the $I_{50}$ (50% inhibition) = 0.01 $\mu$g zinc, and $I_{25}$ = 0.004 $\mu$g zinc. The $I_{50}$ concentration was equivalent to approximately 3 $\mu$g of zinc/mg of mitochondrial protein. For kidney, $I_{50}$ = 0.011 mM (9 $\mu$g of zinc/mg of protein), and $I_{25}$ = 0.02 mM. Consequently, the m-aconitase activity of the prostate preparation was 2–3 times more sensitive to the inhibitory effect of zinc than the kidney preparation. This difference between prostate and kidney was consistently observed in all the experiments and with varying concentrations of citrate as substrate. It is also important to note that the effective inhibition concentration (e.g. $I_{50}$ = 0.004 $\mu$g zinc) occurs when the zinc concentration is less than 10% that of the citrate (substrate) concentration. Under the conditions of this assay (0.05 mM citrate, 0.004 $\mu$g zinc, pH 7.2), about 94% of the citrate (0.047

FIG. 1. The effect of zinc on the m-aconitase activity (Vel) of mitochondrial preparations from rat ventral prostate (VP) epithelial cells and kidney (K) cells. The activity was determined by fluoroenzymatic assay of the reaction citrate $\rightarrow$ isocitrate. The reaction system contained 0.05 mM citrate and 30 $\mu$g of mitochondrial protein. %I, % inhibition.

$2$ J. Glusker, personal communication.
zinc within the concentrations employed is specific for and limited to citrate as substrate and essentially inhibits the m-aconitase reaction in the direction citrate → isocitrate. This is extremely important since, within the context of the Krebs cycle function, citrate is the natural substrate of m-aconitase, and the effect of zinc would be to inhibit citrate oxidation.

We then attempted to obtain some preliminary information concerning the kinetic properties of the enzyme activity and the inhibitor characteristics of zinc on prostate and kidney m-aconitase. The m-aconitase activity for the reaction citrate → isocitrate of kidney mitochondrial preparations ($V_m = 80–110$ nmol/mg of protein/min) was always consistently and significantly higher than prostate ($V_m = 30–40$ nmol/mg of protein/min). This verifies the earlier reports that m-aconitase activity of prostate epithelial cells is lower than other cells (1–4). However the $K_m$ (0.05–0.08 mM) for citrate was essentially the same for kidney and prostate preparations. A typical representation of the enzyme-inhibitor characteristics obtained in these studies is presented in Fig. 2. The Lineweaver-Burk plots and computer analysis of inhibitor models revealed that at the low concentrations (up to about 0.0125 mM zinc in the presence of 0.05 mM citrate as substrate), zinc acted as a competitive inhibitor of the m-aconitase reaction citrate → isocitrate in prostate and kidney preparations. The $K_i$ values were slightly but consistently higher for prostate ($K_i = 0.007$ mM) than for kidney (0.004 mM). At zinc concentrations greater than 0.0125 mM (for example 0.025 mM) and approaching stoichiometry with the citrate concentration, the inhibitory effect of zinc did not conform to any of the typical classes of the inhibitor model. At the higher zinc concentrations, chelation with citrate significantly decreased the concentration of free citrate as substrate. Therefore the decrease in m-aconitase activity with high zinc concentrations was probably a reflection of decreased substrate availability rather than a true inhibitory effect of zinc on m-aconitase.

We then attempted to determine if this inhibitory effect was specific for zinc. Consequently the possible effects of Ca$^{2+}$, Mn$^{2+}$, and Cd$^{2+}$ were determined. Ca$^{2+}$ or Mn$^{2+}$ concentrations up to $10 \times$ greater than the zinc range employed exhibited no inhibitory effect on the m-aconitase reaction. At concentrations equivalent to or higher than the citrate concentration, some inhibition resulted, but such effects would be of no physiological importance. Cd$^{2+}$ in the range of 0.01–0.1 mM did exhibit an inhibitory effect on prostate and kidney m-aconitase. However the inhibition fit either an uncompetitive or noncompetitive type of inhibition but definitely did not fit the competitive inhibition model. This is consistent in part with an effect of Cd$^{2+}$ on the sulfhydryl groups of m-aconitase since Cd$^{2+}$ exhibits a higher affinity for thiols than zinc. Consequently, the inhibition of m-aconitase by low zinc concentrations is not representative of a generalized divalent cationic or chelating effect that is shared by other cations.

In all the studies described above, the mitochondrial preparations were obtained by isolation with sucrose medium in the absence of EDTA or other chelating agents. Therefore, there was no attempt to extract any of the endogenous zinc that was present in the mitochondria. Consequently, the possibility existed that the m-aconitase activity of the mitochondrial preparations might be inhibited by the presence of endogenous zinc. This was an extremely important factor to consider, since pros-
The mitochondria reportedly contain much higher zinc levels than nonprostate mitochondria (12). This relationship was investigated by comparing the m-aconitase activities of mitochondria prepared with medium containing EDTA and with medium lacking EDTA for both prostate and kidney preparations. In these studies, each isolated mitochondrial preparation was divided into two aliquots before sonication. Each aliquot was washed three times in either sucrose medium or sucrose medium containing 1.0 mM EDTA. This was followed by two washings in sucrose medium without EDTA to remove any traces of EDTA from the final preparation. Sonication and final preparation proceeded in the regular manner. Fig. 3 presents the enzyme kinetic results of this study. Treatment of the mitochondria with EDTA had no effect on kidney m-aconitase activity. In contrast, EDTA treatment resulted in a consistent increase in prostate m-aconitase activity. The results also demonstrated that the minus-EDTA preparation exhibited the properties of a competitive inhibitor, which is consistent with the probability that this effect was due to endogenous zinc. These results are consistent with earlier studies (8), which demonstrated that EDTA treatment of prostate mitochondria increased citrate oxidation while having no effect on kidney mitochondrial citrate oxidation.

To obtain additional evidence that endogenous zinc was responsible for these results, the effects of the addition of zinc or EDTA to the aconitase reaction assay medium for EDTA and minus-EDTA-treated prostate preparations were determined (Fig. 4). The isolation of prostate mitochondria in sucrose/EDTA medium resulted in an increased m-aconitase activity, with no such effect observed with the kidney mitochondrial preparation, which is consistent with the results presented in Fig. 3. For prostate, the addition of 0.006 mM zinc to the reaction mixture for the EDTA preparation resulted in the inhibition of m-aconitase activity, which approached the activity of the minus-EDTA preparation in the absence of added zinc. The addition of 0.0125 mM zinc to the assay reaction containing 0.1 mM citrate decreased the m-aconitase activity below the activity of the minus-EDTA-treated preparation. Under these conditions about 92–96% of the citrate remained unchelated and available as substrate. This reaffirms and extends the zinc inhibition effects presented in Figs. 1 and 2. With kidney, EDTA treatment had no effect on the m-aconitase activity, and the addition of zinc to the assay medium inhibited the activity.

We also determined the effects of the addition of EDTA to the reaction medium on the m-aconitase activity (Fig. 4). When 0.1 mM EDTA was added to the reaction of the minus-EDTA preparation of prostate, the m-aconitase activity was increased and approached the activity of the EDTA-treated preparation, but the addition of 0.1 mM EDTA to the reaction assay had no effect on the activity of the EDTA preparation. This demonstrated the endogenous presence of an m-aconitase inhibitor in the minus-EDTA mitochondrial preparation, which was most likely zinc. In contrast, the addition of EDTA to the assay medium had no significant effect on the m-aconitase activity of either EDTA or minus-EDTA-treated kidney preparations.

To relate these EDTA effects to zinc, the zinc content of the mitochondrial preparations was determined. EDTA treatment reduced the prostate zinc concentration from 344 to 122 ng/mg of protein and reduced the kidney zinc concentration from 55 to 22 ng/mg of protein. These results confirm the reported (12) significantly higher mitochondrial level of zinc in prostate epithelial cells as compared with kidney or other cells. It must be noted that although EDTA treatment decreased the zinc content of both prostate and kidney preparations, the levels of zinc in the prostate preparation remained proportionately higher than the kidney levels. This correlates with the observed differences in the effects on the m-aconitase activity of the preparations and on citrate oxidation as reported earlier (8). The endogenous levels of zinc in the kidney mitochondria are apparently too low to exhibit an inhibitory effect on m-aconitase activity so that EDTA treatment has no effect on the activity. The much higher endogenous level of zinc does inhibit the m-aconitase activity of prostate mitochondria; therefore, the reduction of zinc concentrations by EDTA treatment results in increased m-aconitase activity. Even after EDTA treatment the zinc level (122 ng/mg of protein) of the prostate mitochondria remained significantly higher than the level (22 ng/mg protein) of the kidney preparation, which indicates that prostate mitochondria contains a much higher nondiffusible (i.e., not extracted from the intact mitochondria during isolation) zinc component than kidney mitochondria. The relationship of this component to m-aconitase will require future studies involving the purification of the prostate enzyme. As in our previous report (12), the zinc level of the mitochondrial preparations represents the remaining zinc upon completion of the isolation and harvesting of the mitochondrial pellet. It is most probable that the in situ mitochondrial level of zinc is somewhat higher than the remaining level. Nevertheless, collectively these results demonstrate that under in situ or physiological conditions, zinc is an inhibitor of m-aconitase activity in the direction of citrate → isocitrate in prostate epithelial cells.

We also had the opportunity to obtain some fresh pig prostate and seminal vesicle glands that are also citrate-producing glands. Mitochondrial preparations were obtained from the glandular epithelial cells in the same manner described for rat ventral prostate, and the effects of zinc on pig prostate and seminal vesicle m-aconitase activity were determined. The results (Fig. 5) demonstrate that zinc significantly inhibited m-aconitase activity of both preparations and that zinc inhibition fit the competitive inhibition model. The kinetic values for the prostate and seminal vesicle activities, respectively, were $V_m = 12$ and $20 \text{ nmol/mg of protein/min}$, $K_m = 0.009$ and 0.08 mM, and $K_i = 0.001$ and 0.01 mM. Despite some specific cell and species variations in the values of the kinetic parameters, these studies with a variety of sources of mitochondria from rat and
pig tissues consistently demonstrate that low levels of zinc inhibit the m-aconitase activity of the mitochondrial preparations. Considering the diversity of the somewhat crude preparations, the consistency of the zinc inhibition is rather remarkable.

It became essential to determine if the inhibitory effect was due to a direct effect of zinc on the substrate-aconitase complex or an interaction of zinc with other components of the mitochondrial preparations, which in turn inhibited the m-aconitase activity. Therefore we determined the effects of zinc on purified m-aconitase isolated from bovine heart (10). The results (Fig. 6) demonstrate that zinc was a competitive inhibitor of the m-aconitase reaction citrate $\rightarrow$ isocitrate. The purified enzyme exhibited a $K_m = 0.08$ mM and $K_i = 0.002$ mM. Zinc inhibition was obtained with as little as 0.001 mM zinc in the presence of 0.1 mM citrate. This adds support to the likelihood that zinc at levels approximating the in situ level of mitochondrial zinc inhibits the m-aconitase activity of prostate cells. With 0.1 mM cis-aconitate as substrate, zinc concentrations lower than 0.025 mM had no effect on m-aconitase activity, and as much as 0.05 mM zinc exhibited only 25% inhibition (Fig. 6). Consequently the competitive inhibition effect of low zinc levels was specific for the citrate $\rightarrow$ cis-aconitate reaction. Moreover, the inhibitory effect of zinc was independent of the enzyme concentration over the range of 0.15–3.0 $\mu$g of enzyme (results not shown). These results with purified m-aconitase essentially corroborate the results obtained with the mitochondrial preparations and demonstrate that the in situ effect of zinc is due to a direct inhibition of m-aconitase enzyme. The use of the purified active enzyme allowed us to compare the zinc effects on m-aconitase activity in the presence and absence of the cysteine/Fe$^{2+}$ activating solution used in the mitochondrial sonicate studies. The inhibition by zinc was essentially the same in either case so that the presence of activating solution was not involved in this effect. The effects of zinc on m-aconitase and purified cytosolic (c-) aconitase were compared. Under conditions (0.2 mM citrate, 0.01 mM zinc), which resulted in 45% inhibition of m-aconitase, zinc had no effect on c-aconitase activity. Thus this inhibitory effect is specific for the m-aconitase isozyme. This is an important distinction since both isozymes contain identical active site structures.

DISCUSSION

These studies conclusively demonstrate that zinc at physiological or near physiological levels is a specific inhibitor of mammalian cell m-aconitase. The kinetic results demonstrate that zinc acts as a competitive inhibitor of m-aconitase and that this effect occurs only with citrate as substrate. Whether free
zinc or a zinc-citrate complex competes with citrate for the enzyme active site is unknown at this time. Zinc inhibition was achieved under conditions in which the molar ratio of zinc to citrate was as low as 0.01 (for the purified enzyme). Such conditions would result in free citrate 99.3% and zinc-citrate chelate 0.7%. For a zinc-citrate chelate to be the competitive inhibitor form would require that the chelate has an extremely high affinity binding to the enzyme. Moreover, if a zinc-citrate complex competed with free citrate for the active site of the enzyme, one might expect that c-aconitase as well as m-aconitase might be inhibited, which is not the case. From knowledge of the structure of m-aconitase as determined by x-ray crystallography, binding of a zinc-citrate chelate to the active site might not be expected because of steric constraints (16). It is of particular significance to note that Lauble et al. (16, 17), in their crystallographic studies, located an apparent zinc binding site in a loop at the top of domain 4.3 Refinement of the structures in which either trans-aconitate or 4-hydroxy-trans-aconitate is bound to the enzyme (16, 17) has revealed a region of electron density too high to be accounted for by a water molecule.3 In addition, there are four short contacts to His-692, Asp-695, and His-717 at an average distance of 2.52 Å, which provide typical ligands for the binding of zinc to proteins. Cytosolic aconitase lacks this putative zinc binding site (18), which might provide an explanation for the absence of zinc inhibition and would indicate a possible site for the inhibitory effect of zinc on m-aconitase. It is apparent that the mechanism of zinc inhibition of m-aconitase will require additional studies with the purified enzyme.

In regard to the uniquely limiting m-aconitase activity associated with citrate-producing prostate cells, the present report establishes that the higher levels of mitochondrial zinc that characterize these cells inhibit m-aconitase activity and subsequent citrate oxidation. In contrast, the endogenous zinc concentration of kidney (nonprostate cells) mitochondrial preparations appears to be too low to exhibit an inhibitory effect on m-aconitase activity. The existence in prostate cells of a limiting m-aconitase activity is a unique relationship, since m-
aconitase generally is not considered to be a limiting and regulating enzyme in the pathway of citrate metabolism of mammalian cells. However prostate cells have the unique function of producing and secreting high levels of citrate. The inhibition of the citrate → cis-aconitate reaction minimizes citrate oxidation and provides the most efficient metabolic step to optimize net citrate production. This relationship also provides an explanation for the high citrate/isocitrate ratio (30–40 to 1), which characterizes the prostate. Moreover, testosterone and prolactin regulate citrate oxidation of prostate epithelial cells by altering the level of mitochondrial zinc, which in turn altered the m-aconitase activity (5–7, 12). Consequently it is evident that zinc plays a key role in regulating m-aconitase activity and subsequent citrate oxidation of prostate epithelial cells.

These relationships are of particular significance in prostate cancer. In contrast to the high citrate and zinc content of normal human prostate, prostate cancer tissue contains very low citrate and zinc levels that are similar to the levels typically associated with most normal soft tissue (1–4). Whereas normal human prostate epithelial cells are citrate-producing cells, malignant prostate epithelial cells are citrate-oxidizing cells. These relationships coupled with the current results of this study provide compelling evidence 1) that m-aconitase activity is not limited in malignant prostate cells, thereby permitting the typical oxidation of citrate via the Krebs cycle, and 2) that the malignant cells contain a low level of mitochondrial zinc, which alleviates the inhibition of the m-aconitase activity. This concept warrants further investigation and will be important for understanding the metabolic implications in the pathogenesis and treatment of prostate cancer.

Acknowledgments—The authors express their sincere appreciation to Dr. Paul Srere (VA Medical Center, Dallas, TX) and Dr. Jenny Glusker (Fox Chase Cancer Center, Philadelphia, PA) for their valuable comments during the course of this investigation and in the preparation of this paper. We thank Dr. Vernon Pursel (Reproduction Laboratory, Beltsville Agricultural Research Center) for providing the pig tissues employed in this study.

REFERENCES
1. Costello, L. C., and Franklin, R. B. (1991) Prostate 18, 25–46
2. Costello, L. C., and Franklin, R. B. (1991) Prostate 19, 181–205
3. Costello, L. C., and Franklin, R. B. (1997) Urology 50, 3–12
4. Franklin, R. B., and Costello, L. C. (1997) The Prostate: Basic and Clinical Concepts (Nax, R. K., ed) pp. 115–150, CRC Press, Inc., New York
5. Costello, L. C., Liu, Y., and Franklin, R. B. (1995) Mol. Cell. Endocrinol. 112, 45–51
6. Liu, Y., Costello, L. C., and Franklin, R. B. (1996) Metabolism 45, 442–449
7. Costello, L. C., Liu, Y., and Franklin, R. B. (1996) Urology 48, 654–659
8. Costello, L. C., and Franklin, R. B. (1981) Enzyme 26, 281–287
9. Bradford, M. (1976) Anal. Biochem. 72, 248–254
10. Kennedy, M. C., Emptage, M. E., Dreyer, J.-L., and Beinert, H. (1983) J. Biol. Chem. 258, 11098–11105
11. Penefsky, H. S. (1977) J. Biol. Chem. 252, 2891–2899
12. Liu, Y., Costello, L. C., and Franklin, R. B. (1996) Prostate 30, 26–32
13. Lutz, R. A., and Rodbard, D. (1986) Enzyme 36, 197–206
14. Westergaard, N., Banke, T., Wahl, P., Sonnewald, U., and Schouseboe, A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3367–3370
15. Sillen, L., and Martell, A. E. (1964) Stability Constants of Metal-Ion Complexes, 2nd Ed., Chemical Society, London
16. Lauble, H., Kennedy, M. C., Beinert, H., and Stout, C. D. (1994) J. Mol. Biol. 237, 437–451
17. Lauble, H., Kennedy, M. C., Emptage, M. H., Beinert, H., and Stout, C. D. (1996) Proc. Nat. Acad. Sci. U. S. A. 93, 13699–13703
18. Frishman, D., and Heinz, M. W. (1996) Eur. J. Biochem. 239, 197–200