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Effect of mercury, cadmium, nickel, chromium and zinc on kinetic properties of NADPH-cytochrome P450 reductase purified from leaping mullet (*Liza saliens*) ☆

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Abstract

Information on the mechanism of metal ion inhibition of NADPH-cytochrome P450 reductase is limited. The purpose of the present paper was to elucidate in vitro effect of Hg^{+2}, Cd^{+2}, Ni^{+2}, Cr^{+3} and Zn^{+2} ions on the purified mullet NADPH-cytochrome P450 reductase. NADPH-cytochrome P450 reductase was purified from detergent-solubilized liver microsomes from leaping mullet (*Liza saliens*). All of the metal ions caused inhibition of the enzyme activity except Zn^{+2}. At 50 μM metal concentration, Hg^{+2} inhibited the cytochrome P450 reductase activity completely (100%), while, at the same concentrations, Cd^{+2}, Cr^{+3} and Ni^{+2} caused 66%, 65% and 37% inhibition, respectively. At 50 μM metal concentration, Zn^{+2} had no apparent effect on cytochrome P450 reductase activity. The IC_{50} values of $HgCl_2$, $CrCl_3$, $CdCl_2$ and $NiCl_2$ were estimated to be 0.07 μM, 24 μM, 33 μM and 143 μM, respectively. Of the metal ions tested, Hg^{+2} exhibited much higher inhibitory effect at lower concentrations, so it was evidently a more potent inhibitor than the others. All four metal ions displayed noncompetitive type of inhibition mechanism for the purified reductase as analyzed by Dixon plot. K_i values of Hg^{+2}, Cr^{+3}, Cd^{+2}, and Ni^{+2} were calculated from Dixon plots as 0.048 μM, 18 μM, 73 μM and 329 μM, respectively.
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Keywords: Noncompetitive inhibition; Leaping mullet (*Liza saliens*); NADPH-cytochrome P450 reductase; Mercury; Cadmium; Nickel; Chromium; Zinc

1. Introduction

NADPH-Cytochrome P450 reductase (NADPH: ferrihemoprotein oxidoreductase, EC 1.6.2.4) is an integral membrane flavoprotein that catalyzes the transfer of electrons from NADPH to cytochrome P450 and is an essential component of the microsomal cytochrome P450 monooxygenase system. The monoxygenase system consists of NADPH-cytochrome P450 reductase and a family of heme proteins, cytochrome P450, and is involved in oxidative metabolism of both endogenous (steroids, fatty acids, prostaglandins, biogenic amines and retinoids) and exogenous compounds (drugs, alcohols, organic solvents, anesthetic agents, dyes, environmental pollutants and chemicals) (Lu et al., 1969; Black and Coon, 1982; Arinç, 1995; Munro et al., 2001). In addition to electron transport role in monooxygenase reactions, cytochrome P450 reductase alone can catalyze the reduction of variety of chemicals including therapeutically important antitumor drugs, antibiotics and aromatic nitro compounds (Kappus, 1986; Lu, 1991).

The important role of NADPH-cytochrome P450 reductase in the metabolism of endogenous and exogenous molecules has been recognized in many organisms from

Abbreviations: ε-ACA, ε-amino caproic acid; 2′,5′-ADP, adenosine 2′,5′-bisphosphate; DEAE, diethylaminoethyl; EROD, ethoxyresorufin O-deethylase; HEPES, *N*-2-hydroxyethylpiperazine-*N*-2,ethane sulfonic acid; NADPH, nicotinamide adenine dinucleotide phosphate reduced form; PMSF, phenyl-methyl-sulfonyl fluoride.

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plants, animals, human and fish species. NADPH-cytochrome P450 reductase has been purified from microsomes of guinea pig liver, pig liver and kidney, sheep lung and liver, rat liver, rabbit liver, human liver and beef liver (Kobayashi and Rikans, 1984; Yasukochi et al., 1980; Iscan and Arinç, 1988; Kamataki et al., 1979; Guengerich et al., 1981; Arinç and Celik, 2002). Fish cytochrome P450 reductase has been purified and some kinetic and functional properties have been characterized from scup, rainbow trout and leaping mullet (Klotz et al., 1983; Williams et al., 1983; Sen and Arinç, 1998).

The heavy metals discharged to the aquatic environment are of great concern all over the world. A number of studies have been carried out on metal accumulation in different fish species, other aquatic organisms and sediments. These studies have clearly indicated that aquatic environment is facing heavy metal pollution. All of these studies give valuable information about the contaminating concentrations of metal ions, but do not give any information about the mechanism of actions of these metals in the organisms. The metals such as selenium, iron, copper, zinc, chromium and manganese are essential metals since they play an important role in a biological system, whereas lead, cadmium and mercury are nonessential metals, as they are toxic, even in trace amounts. However, excessive accumulation of most metals, both essential and nonessential, may pose serious health risks to humans and exert adverse impacts on the ecosystem itself. For example, the accumulation of Cd^{+2} in human body (principally in kidney and liver) can cause renal disfunction and bone disease such as Itai-Itai (Nordberg, 1996; Cui et al., 2005). It is also carcinogenic in humans and laboratory animals. Nickel toxicity has been found to be associated with lung fibrosis, cardiovascular and kidney diseases and cancer (Doll et al., 1970; Miltyk and Palka, 2005). Another very toxic metal is mercury. Central nervous system is the major target organ for mercury toxicity. The other host organ systems may be gastrointestinal, respiratory, hepatic, immune, dermal, and renal (Risher et al., 2002).

Heavy metal ions exert their toxicity by multiple mechanisms including the inhibition of enzymes containing sulfhydryl groups in or near their active sites or generation of reactive oxygen species. The inhibitory effects of metal ions on purified cytochrome P450 reductase activity are quite important from essential role of this enzyme in the metabolism of xenobiotics such as, drugs, steroids, and carcinogens. As a result of improper detoxification, toxic xenobiotics may accumulate and become hazardous to the body.

However, information on the effect of heavy metals on microsomal and purified cytochrome P450 reductase activity is limited. In vivo treatment of rats with cadmium increased rat liver microsomal cytochrome P450 reductase activity after 6 hours and afterward decreased significantly (Casalino et al., 2000). Kim et al. (2002) examined the effects of Zn^{+2}, Mg^{+2}, Mn^{+2}, Ca^{+2}, Co^{+2} and Cu^{+2} on purified rat cytochrome P450 reductase and observed that only Cu^{+2} inhibited NADPH-dependent P450 reductase activity, whereas, Zn^{+2}, Mg^{+2}, Mn^{+2}, Ca^{+2} and Co^{+2} had no apparent effects on P450 reductase activity.

Leaping mullet is an economically important fish and liver microsomal CYP1A dependent ethoxyresorufin O-deethylase (EROD) activity of mullet has been used in biomonitoring studies in Izmir Bay, Turkey (Arinç et al., 2000). A recent in vitro study showed that Zn^{+2}, Ni^{+2}, Cd^{+2} and Hg^{+2} inhibited EROD activity significantly in mullet liver (Bozcaarmutlu and Arinç, 2004). The present study was undertaken to elucidate the inhibitory effects of metal ions such as Hg^{+2}, Ni^{+2}, Cd^{+2}, Cr^{+3} and Zn^{+2} on purified fish liver cytochrome P450 reductase, a component of CYP1A dependent EROD system, and to examine the mode of inhibition exerted by these metal ions on the purified mullet liver cytochrome P450 reductase.

2. Material and methods

2.1. Chemicals

All of the chemicals used in this study were of analytical grade and were purchased from Sigma Co. (St. Louis, MO, USA) and Merck Co. (Darmstadt, Germany) at the highest grade of purity available. Mercury chloride ($HgCl_2$), cadmium chloride ($CdCl_2$), nickel chloride ($NiCl_2$), chromium chloride ($CrCl_3$) and zinc chloride ($ZnCl_2$) were used to prepare the metal solutions.

2.2. Fish sampling

Leaping mullet (*L. saliens*) ($n = 80$) were collected by fish net in Izmir Bay on the Aegean coast of Turkey. The fish collected were of comparable size and weight (about 300–400 g).

2.3. Preparation of mullet liver microsomes

Leaping mullet liver microsomes were prepared as described by Arinç and Sen (1993) except that the homogenization solution contained 10 mM EDTA, 0.25 mM PMSF and 0.25 mM ε-ACA. The microsomes were resuspended in 10% glycerol containing 10 mM EDTA. Microsomal suspensions containing approximately 25–35 mg protein per milliliter were gassed with nitrogen in small polypropylene plastic bottles and stored at −70 °C until used. All the procedures involved in the preparations of microsomes and purification of enzyme were performed at 0–4 °C.

2.4. Purification of mullet NADPH-cytochrome P450 reductase

NADPH-cytochrome P450 reductase was purified from mullet liver microsomes according to the procedure described by Sen and Arinç (1998). Briefly, the purification procedure involved anion exchange chromatography of Emulgen 913-cholate solubilized microsomes on two sequential DEAE–cellulose columns. This was followed by

affinity chromatography using 2′,5′-ADP Sepharose 4B, then further purified and concentrated on hydroxylapatite column as described in detail by Sen and Arinç (1998). The purified enzyme preparation was gassed with nitrogen, aliquoted and stored at −196 °C in liquid nitrogen until use. Purified cytochrome P450 reductase was made metal-free during the preparation of microsomes by resuspending microsomes in 10 mM EDTA; metal chelating agent, containing solution. At the purification steps, cytochrome P450 was tightly bound to all chromatography columns and column content was extensively washed with EDTA containing solutions. During the purification steps, the concentration of EDTA was gradually decreased. The concentration of EDTA was negligible in the cytochrome P450 reductase activity assay mixture.

2.5. Protein determination

Protein concentration of purified cytochrome P450 reductase was determined according to the method of Lowry et al. (1951) using bovine serum albumin as a standard.

2.6. Measurements of mullet NADPH-cytochrome P450 reductase activity

NADPH-cytochrome P450 reductase activity was assayed by measuring the rate of cytochrome *c* reduction at 550 nm, spectrophotometrically, according to the method of Master et al. (1967), with some modifications. In general, NADPH-cytochrome P450 reductase activity is routinely measured in phosphate buffer. However, some of the metals such as cadmium and nickel precipitated out as phosphate salts in this buffer system, it was necessary to perform these analyses in HEPES buffer. Consequently, HEPES buffer (0.3 M, pH 7.7) was used in all the reductase studies described herein. The assay mixture contained 0.3 M HEPES, pH 7.7 containing 80 nmoles cytochrome *c*, 130 nmoles NADPH, and appropriate amounts of enzyme preparation in a final volume of 0.8 mL. Reaction was carried out at room temperature (24–25 °C). Purified enzyme preparation was diluted 1:50 with 0.3 M HEPES (pH 7.7) containing 20% glycerol solution daily. NADPH-cytochrome P450 reductase activity was found to be stable at 4 °C at least up to nine hours in the presence of glycerol. The enzyme activity was calculated using the extinction coefficient of 19.6 $mM^{-1} cm^{-1}$ for the difference in absorbance between reduced and oxidized cytochrome *c* at 550 nm (Yonetani, 1965). One unit of reductase was defined as the amount of enzyme catalyzing the reduction of 1 μmol cytochrome *c* per min under the described conditions.

2.7. Inhibition of NADPH-cytochrome P450 reductase activity by metal ions

The effects of metal ions were studied by adding various concentrations of cadmium, mercury, nickel, chromium and zinc ions on the reaction mixture containing 0.3 M HEPES, pH 7.7 buffer and appropriate amount of purified enzyme preparation. After the addition of metal ions, the reaction mixture was immediately mixed and cytochrome *c* was added. Finally reaction was initiated with the addition of NADPH and followed for 2 min at room temperature in Hitachi U-2800 double-beam spectrophotometer. The enzyme activity was calculated using the extinction coefficient of 19.6 $mM^{-1} cm^{-1}$ for the difference in absorbance between reduced and oxidized cytochrome *c* at 550 nm as described for control activity.

3. Results

NADPH-cytochrome P450 reductase was purified to electrophoretic homogenity from detergent-solubilized liver microsomes from the leaping mullet (*L. saliens*) in our laboratory. The specific activity of the enzyme was found to be 52 μmol/min/mg protein (Units/mg protein). The monomer molecular weight of the enzyme was calculated to be 77.000 Da using the SDS-PAGE which is exactly the same value obtained from Sen and Arinç (1998) (data not shown).

The effect of metal ions (Hg^{+2}, Cd^{+2}, Ni^{+2}, Cr^{+3} and Zn^{+2}) on the purified cytochrome P450 reductase activity was studied by adding various concentrations of metal ions into the assay system. Hg^{+2}, Cd^{+2}, Ni^{+2} and Cr^{+3} showed an inhibitory effect on the cytochrome P450 reductase in a concentration dependent manner (Fig. 1). At 50 μM metal concentration, Hg^{+2} inhibited the cytochrome P450 reductase activity completely (100%), while at the same concentrations Cd^{+2}, Cr^{+3} and Ni^{+2} caused 66%, 65% and 37% inhibition, respectively (Table 1). At 50 μM metal concentration, Zn^{+2} had no apparent effect on NADPH-cytochrome P450 reductase activity (Table 1). The IC_{50} values (namely, the concentration giving 50% inhibition) of $HgCl_2$, $CrCl_3$, $CdCl_2$ and $NiCl_2$ were estimated to be 0.07 μM, 24 μM, 33 μM and 143 μM, respectively. Of the metal ions tested, Hg^{+2} exhibited much higher inhibitory effect at lower concentrations and it was evidently the more potent inhibitor than the others.

In the determination of the inhibition mechanism of mercury on NADPH-cytochrome P450 reductase, the concentration of the mercury was held at two different values (0.05 μM and 0.1 μM) at the activity assay mixture, while the substrate concentration were varied (10 μM, 40 μM, 80 μM). When $1/v$ (1/μmol/min/mg protein) was plotted against 1/[S] (1/[cytochrome *c*]) (Lineweaver–Burk plot) at three different substrate concentrations, the three lines crossed apparently at a point on the *X*-axis. Lineweaver–Burk plot indicated that Michaelis constant (K_m) remained unchanged by the presence of different concentrations of mercury, while V_{max} (maximum velocity) decreased with increasing mercury concentrations (Fig. 2). $1/v$ was also plotted against inhibitor concentration [*I*] (Dixon plot) since Dixon plot is used frequently to identify the type of inhibition and to determine the K_i value (dissociation con-

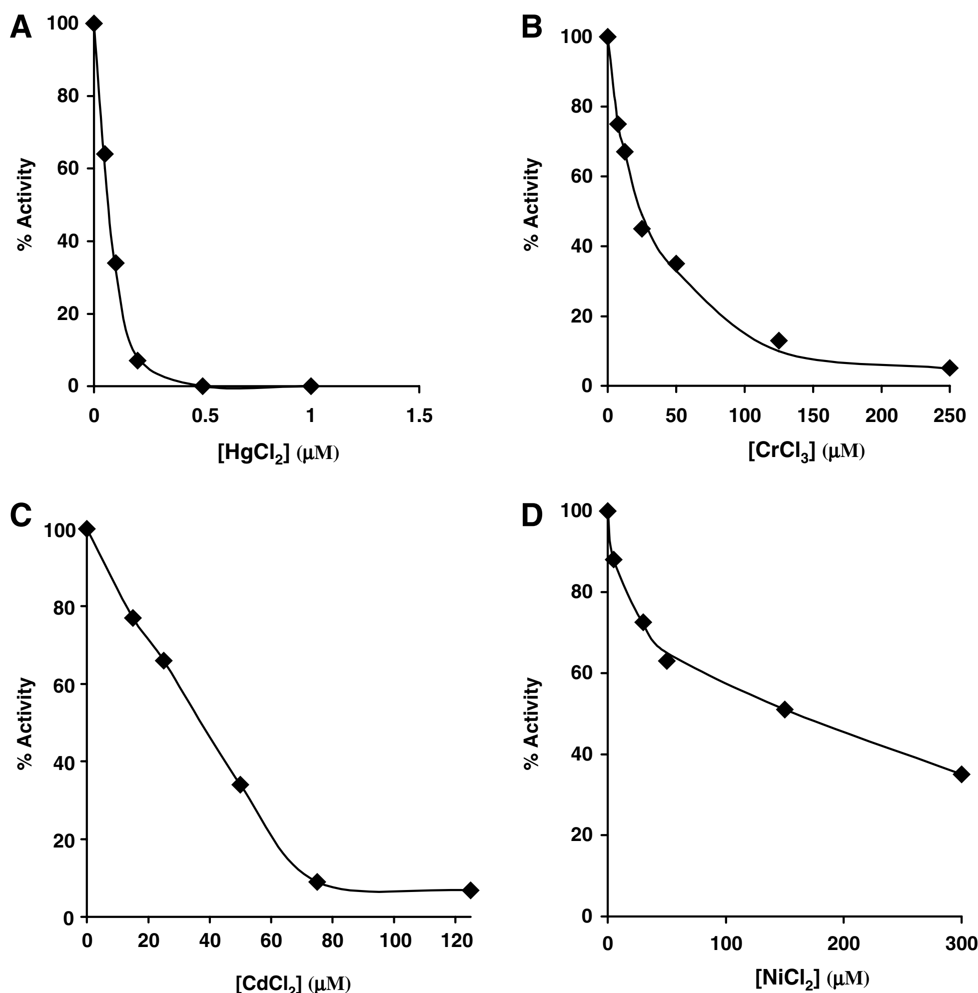

Fig. 1. In vitro effects of $HgCl_2$ (A), $CrCl_3$ (B), $CdCl_2$ (C) and $NiCl_2$ (D) on purified NADPH-cytochrome P450 reductase. The points are average of duplicate determinations.

Table 1
Inhibitory effects of metal ions on NADPH-cytochrome P450 reductase

Metal ions	% inhibition at 50 μM metal ions	IC_{50} values of metal ions (μM)
Hg^{+2}	100	0.07
Cr^{+3}	65	24
Cd^{+2}	66	33
Ni^{+2}	37	143
Zn^{+2}	No inhibition	–

IC_{50} as μM represents the metal ion concentration exhibiting 50% inhibition of the initial cytochrome P450 reductase activity.

stant for inhibitor binding). Both of these plots suggest the inhibition manner to be apparently noncompetitive. From the intersecting point of the three lines, the K_i value was determined to be 0.048 μM (Table 2).

In the determination of the inhibition mechanism of cadmium on NADPH-cytochrome P450 reductase, the concentration of the cadmium was held at three different values (15 μM, 25 μM and 50 μM) at the activity assay mixture, while the substrate concentrations were varied (10 μM, 20 μM, 40 μM). Analysis of both Lineweaver–Burk plot and Dixon plot indicated that the mode of inhibition of the fish liver cytochrome P450 reductase by cadmium was noncompetitive (Fig. 3). From the intersecting point of the three lines in Dixon plot, the K_i value was determined to be 73 μM (Table 2).

Inhibition profile of chromium was determined at three different concentration of chromium (7.5 μM, 12.5 μM and 25 μM), while the substrate concentrations were varied (20 μM, 40 μM, 80 μM). Analysis of both Lineweaver–Burk plot and Dixon plot indicated that the mode of inhibition of the fish liver cytochrome P4560 reductase by chromium was noncompetitive (Fig. 4). In Dixon plot, the three lines crossed apparently at a point on the *X*-axis (Fig. 4). The K_i value was determined to be 18 μM from the intersecting point of the three lines in this plot (Table 2).

Inhibition profile of nickel was also determined at four different concentrations of nickel (30 μM, 60 μM, 150 μM and 300 μM) at the activity assay mixture, while the substrate concentrations were varied (10 μM, 20 μM, 40 μM). Analysis of both Lineweaver–Burk plot and Dixon plot suggest the inhibition manner to be apparently noncompetitive (Fig. 5). $1/v$ vs $[I]$ plot (Dixon plot) gave the three lines crossing apparently at a point on the *X*-axis. From the

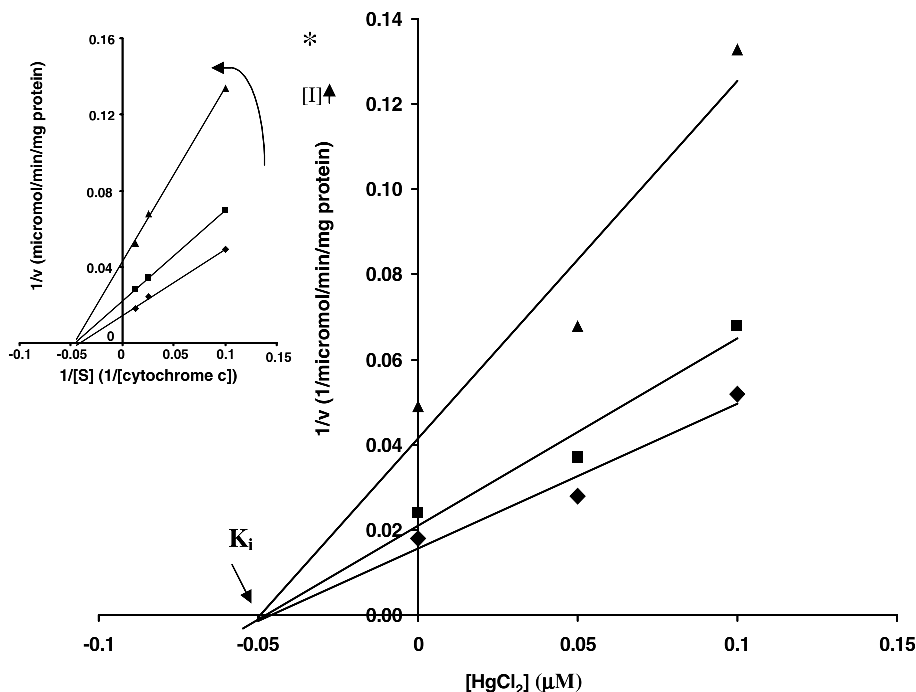

Fig. 2. Dixon plot of purified mullet NADPH-cytochrome P450 reductase at different concentrations of mercury using substrate concentrations of 10 μM (▲), 40 μM (■) and 80 μM (◆). * Lineweaver–Burk plot of purified mullet NADPH-cytochrome P450 reductase at 0.05 μM (■) and 0.1 μM (▲) concentrations of mercury.

Table 2
Mechanism of inhibition of NADPH-cytochrome P450 reductase by metal ions and apparent K_i values

Metal ions	K_i (μM)	Type of inhibition
Hg^{+2}	0.048 ± 0.0002	Noncompetitive
Cr^{+3}	17.8 ± 0.8	Noncompetitive
Cd^{+2}	72.8 ± 3.5	Noncompetitive
Ni^{+2}	329 ± 50.3	Noncompetitive

K_i values were calculated from the Dixon plots of metal ions from Figs. 2–5.

intersecting point of the three lines, the K_i value was determined to be 329 μM (Table 2).

4. Discussion

Microsomal NADPH cytochrome P450 reductase contains one molecule each of FAD and FMN. These flavins participate in electron transport from NADPH to cytochrome P450 in a phospholipids environment. It is encoded by a single gene in all species examined. Knocking out the cytochrome P450 reductase gene in mice leads to embryonic lethality, and missense mutations cause disordered steroidogenesis, ambiguous genitalia, and Antley-Bixler syndrome (Shen et al., 2002; Otto et al., 2003; Huang et al., 2005; Iyanagi, 2005).

Some metal ions may affect enzymes, either as inhibitor or activators of enzyme activity. The mechanism of this effect is not clear for most of the enzymes. Concentrations of the metal ions might be the important factors in regulating the activities of CYP dependent monooxygenases and of their essential component NADPH-cytochrome P450 reductase. Indeed, information on the effect of heavy metals on microsomal and purified cytochrome P450 reductase activity is limited. In vivo treatment of rats with cadmium increased rat liver microsomal cytochrome P450 reductase activity after 6 h and afterward decreased significantly (Casalino et al., 2000). Kim et al. (2002) examined the effects of Zn^{+2}, Mg^{+2}, Mn^{+2}, Ca^{+2}, Co^{+2} and Cu^{+2} on purified rat cytochrome P450 reductase and observed that only Cu^{+2} inhibited NADPH-dependent P450 reductase activity, whereas, Zn^{+2}, Mg^{+2}, Mn^{+2}, Ca^{+2} and Co^{+2} had no apparent effects on P450 reductase activity. The present study showed that mercury, cadmium, nickel and chromium ions were strong inhibitors of NADPH-cytochrome P450 reductase. However, the affinities of all the metal ions to NADPH-cytochrome P450 reductase were not at the same degree. Of the metal ions tested, Hg^{+2} exhibited much higher inhibitory effect at lower concentrations; so it was evidently a more potent inhibitor than the others. The potency of the metals to inhibit NADPH-cytochrome P450 reductase enzyme follow the sequence of Hg^{+2}, Cr^{+3}, Cd^{+2}, Ni^{+2}. Both Lineweaver–Burk plot and Dixon plots of the kinetic data showed that the mode of inhibition for tested metal ions were noncompetitive. K_i values of Hg^{+2}, Cr^{+3}, Cd^{+2}, and Ni^{+2} were calculated from Dixon plots as 0.048 μM, 18 μM, 73 μM and 329 μM, respectively. The calculated K_i values were more clearly stated that the most effective inhibitor was mercury. The noncompetitive mode of inhibition of cytochrome P450 reductase suggests that the affinity of NADPH-cytochrome P450 reductase to its substrate, cytochrome *c*, does not change in the presence of these metal ions. The presence of

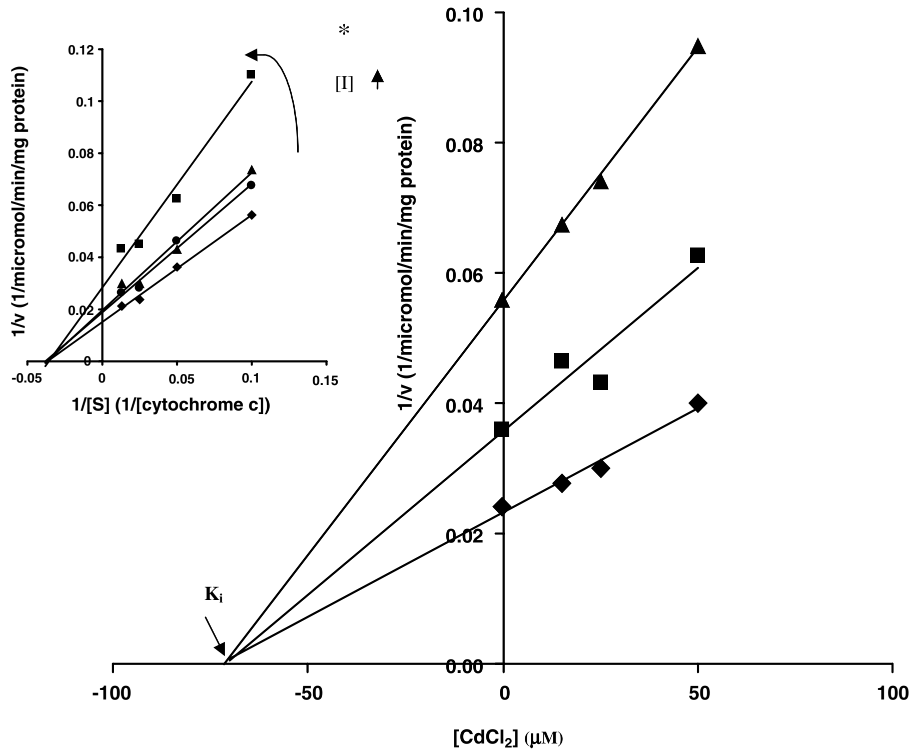

Fig. 3. Dixon plot of purified mullet NADPH-cytochrome P450 reductase at different concentrations of cadmium using substrate concentrations of 10 μM (▲), 20 μM (■) and 40 μM (◆). * Lineweaver–Burk plot of purified mullet NADPH-cytochrome P450 reductase at 15 μM (●), 25 μM (▲) and 50 μM (■) concentrations of cadmium.

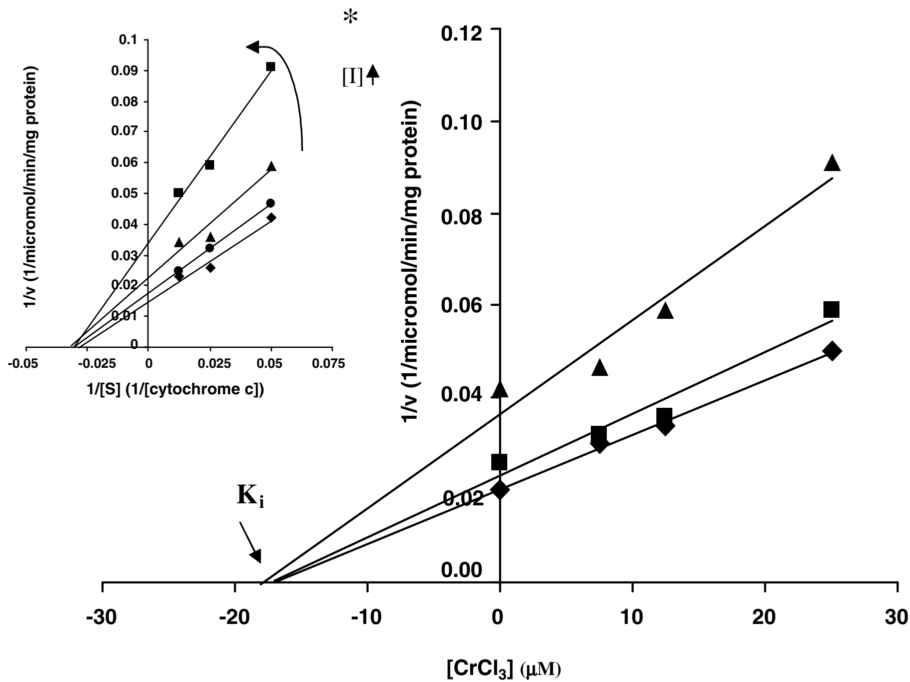

Fig. 4. Dixon plot of purified mullet NADPH-cytochrome P450 reductase at different concentrations of chromium using substrate concentrations of 20 μM (▲), 40 μM (■) and 80 μM (◆). * Lineweaver–Burk plot of purified mullet NADPH-cytochrome P450 reductase at 7.5 μM (●), 12.5 μM (▲) and 25 μM (■) concentrations of chromium.

metal ions causes reduction in the maximum activity of NADPH-cytochrome P450 reductase, which indicates an alteration in the structure of the enzyme. The metal ions tested in this study may exhibit different inhibition modes with different enzymes. For examples, the activity of aniline 4-hydroxylase is competitively inhibited by Hg^{+2}, Ni^{+2}, Cd^{+2}

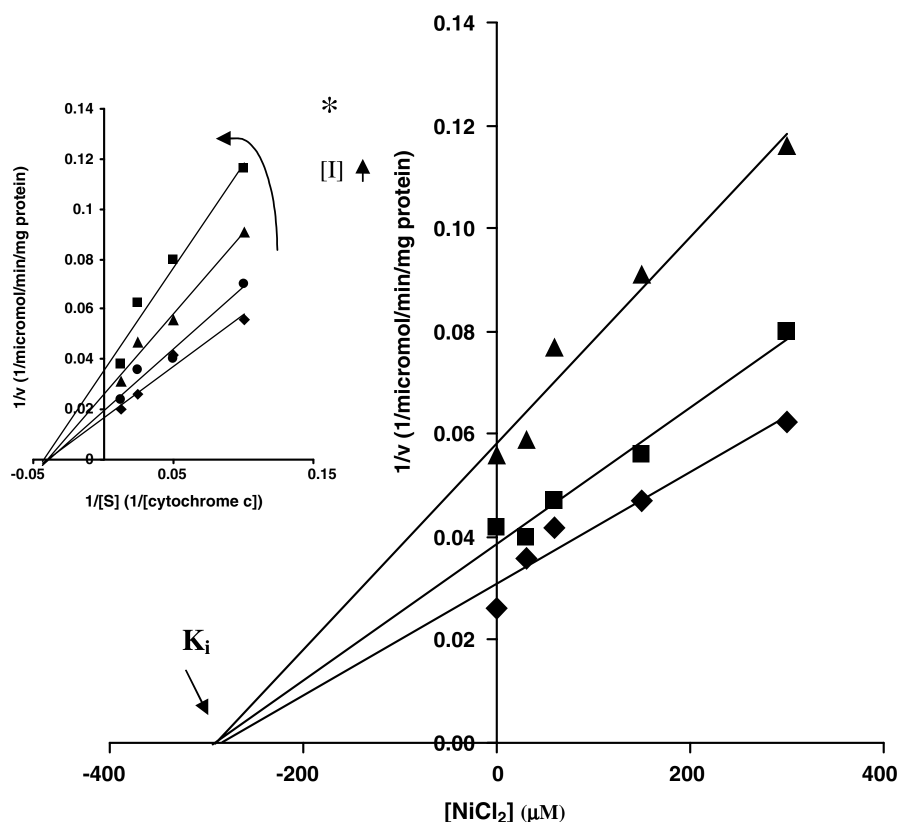

Fig. 5. Dixon plot of purified mullet NADPH-cytochrome P450 reductase at different concentrations of nickel using substrate concentrations of 10 μM (▲), 20 μM (■) and 40 μM (◆). * Lineweaver–Burk plot of purified mullet NADPH-cytochrome P450 reductase at 30 μM (●), 150 μM (▲) and 300 μM (■) concentrations of nickel.

and Zn^{+2} (Guner and Colak, 1996). Competitive mode of inhibition of collagenase has been reported by the dimer of Cr(III) (Gayatri et al., 2000). Cd^{+2} is a mixed type inhibitor of human serum butyrylcholinesterase (Sarkarati et al., 1999). However, it is a competitive inhibitor of sheep brain glutathione reductase (Acan and Tezcan, 1995). In competitive inhibition, increasing substrate concentration may overcome inhibition, whereas in noncompetitive inhibition, shape of the enzyme is altered to prevent the substrate that combines with it. Therefore noncompetitive inhibition is much severe. The structure of cytochrome P450 reductase is composed of four structural domains: the NH_2-terminal membrane domain, the FMN-binding domain, the FAD-binding domain and the NADPH-binding domain (Arinç, 1995; Gutierrez et al., 2003). It has been shown that, in cytochrome P450 reductase, NADPH binding is important in controlling the rate of internal electron transfer (Gutierrez et al., 2003). NADPH-cytochrome P450 reductase contains one essential cysteine residue at or near NADPH binding site (Hanui et al., 1986). The metal binding site may be in the NADPH binding domain of the NADPH-cytochrome P450 reductase.

Heavy metals are stable and persistent environmental contaminants and tend to accumulate. Their presence in environment has increased in some areas to levels which threaten the health of aquatic and terrestrial organisms, including man. The toxicity of metal ions is known in a variety of vertebrate species, but relatively little is known about the underlying molecular mechanism. Chronic exposure of fish to sub-lethal levels of heavy metals impairs growth and alters lipid metabolism in fish (Levesque et al., 2002). The gonads size and hormone levels are also altered in the fish from the metal-contaminated lakes (Levesque et al., 2002). It is well known that cytochrome P450-dependent monooxgenases have roles in the synthesis of fatty acids and steroids. Since cytochrome P450 reductase is essential for cytochrome P450 activity, inhibition of this enzyme may be expected to inhibit the metabolism of these endogenous compounds. Reduced synthesis of steroids and lipids may affect the growth and reproduction capacities of economically important fish species. In addition, inhibition of NADPH-cytochrome P450 reductase activity in fish may cause the accumulation of toxic xenobiotics such as organic carcinogenic pollutants and may lead to adverse effects which in turn may cause changes at the population and the community level in the years to come. Fish is a main food source in many part of the world. Contamination of the environment and food sources with metal ions may cause similar bioaccumulation and effects in human as in fish. Cytochrome P450 proteins together with cytochrome P450 reductase enzyme in humans are enzymes that are used to synthesize cholesterol, steroids, and other important endogenous substrates such as prostacyclins and thromboxane A2. In addition, cytochrome P450 isoforms in humans

are responsible from the oxidative metabolism of the majority of drugs and xenobiotics. The inhibition of cytochrome P450 reductase may cause prolongation of xenobiotic action, an increase or decrease in xenobiotic toxicity, and may affect the metabolism of endogenous compounds such as steroids and fatty acid in humans.

In conclusion, the results of this study clearly indicated that the effects of metal ions on cytochrome P450 reductase enzyme activity were concentration dependent. Nanomolar concentration of mercury and micromolar concentration of chromium, cadmium and nickel may disrupt overall cytochrome P450 reductase catalyzed reactions, including P450 functions.

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