J. Biochem. 132, 457–462 (2002)

Regulatory Effects of Divalent Metal Cations on Human Cytosolic Sulfotransferases[1]

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Received June 28, 2002; accepted July 5, 2002

Cytosolic sulfotransferases (STs), traditionally viewed as Phase II drug-metabolizing or detoxifying enzymes, are increasingly being implicated in the metabolism of endogenous biologically-active molecules. Except for studies on changes in their levels of expression and activity in the early stage of development in mammals, very little is known about how these enzymes are regulated. In this study, the regulatory effects of divalent metal cations on the activity of human cytosolic STs were quantitatively evaluated. Results obtained indicate that all nine human cytosolic STs examined are partially or completely inhibited/stimulated by the ten divalent metal cations tested at 10 mM concentration. Compared with the other metal cations, the inhibitory or stimulatory effect of Mg^{2+} and Ca^{2+} on the activities of the human cytosolic STs appeared to be relatively smaller. Concentration-dependent effects of the divalent metal cations were further examined. The IC_{50} or EC_{50} values determined for different divalent metal cations were mostly above their normal physiological concentration ranges. In a few cases, however, IC_{50} values close to the physiological concentrations of certain divalent metal cations were observed. Using the monoamine (M)-form phenol ST (PST) as a model, it was demonstrated that the K_m for dopamine changed only slightly with increasing concentrations of Cd^{2+}, whereas the V_{max} was dramatically decreased.

Key words: divalent metal cations, human cytosolic sulfotransferases, regulatory effects.

Sulfate conjugation is a major pathway *in vivo* for the biotransformation and/or excretion of xenobiotics and endogenous compounds such as steroid and thyroid hormones, catecholamines, cholesterol, bile acids, *etc.* (*1–3*). The responsible enzymes, called the "cytosolic sulfotransferases (STs)," catalyze the transfer of a sulfonate group from the active sulfate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to an acceptor substrate compound containing either a hydroxyl or an amine group (*4*). Sulfate conjugation may result in the inactivation/activation of the substrate compounds or increase their water-solubility, thereby facilitating their removal from the body (*1–3*).

Except during the early stage of development, cytosolic STs in general have been shown to be constitutive enzymes with little known about the regulation of their enzymatic activity (*1*). Although no cofactors have been shown to be required for the functioning of cytosolic STs, studies performed in our laboratory in the past several years have revealed that some divalent metal cations may exert stimulatory or inhibitory effects on cytosolic STs (*5, 6*). Using human monoamine (M)-form phenol ST (M-PST) as a model (*7*), it was shown that the addition of Mn^{2+} to the reaction mixture resulted in a dramatic increase in its Dopa/tyrosine-sulfating activity. To a lesser extent, Co^{2+} and Mg^{2+} could also stimulate the Dopa/tyrosine-sulfating activity of M-PST. In contrast, Ca^{2+} and Cd^{2+} caused an inhibition of this activity. These findings indicate that divalent metal cations may play a significant role in regulating the activity of M-PST. An important issue, therefore, is whether cytosolic STs in general are subject to regulation by divalent metal cations.

We report in this communication a systematic investigation of the effects of a variety of divalent metal cations on the activities of nine human cytosolic STs. The concentrations of different divalent metal cations causing 50% inhibition or enhancement (IC_{50} or EC_{50}) of the activities of individual enzymes were determined. Moreover, using M-PST as a model, kinetic experiments were performed to examine the mode of action of the divalent metal cation, Cd^{2+}.

MATERIALS AND METHODS

Materials—Dopamine, *p*-nitrophenol, adenosine 5′-triphosphate (ATP), Trizma base, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes), 3-[*N*-tris-(hydroxymethyl)methylamino]-propanesulfonic acid (Taps), dehydroepiandrosterone (DHEA), 3,3′,5-triiodo-L-thyronine (sodium salt) (T_3), and estrone (1,3,5[10]-estratrinen-3-ol-17-one) were products of Sigma. The sulfate-activating enzymes, ATP sulfurylase and APS kinase, from *Bacillus stearothermophilus* were kindly provided by Dr. Hiroshi Nakajima of Unitika (Uji). Carrier-free sodium [^{35}S]sulfate was from ICN Biomedicals. Radioactive PAP[^{35}S] was synthesized

[1]This work was supported in part by a grant from the American Heart Association (Texas Affiliate) (MCL), a UTHCT President's Council Research Membership Seed Grant (MCL), and an award from the Naito Foundation (MS).

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Abbreviations: ST, sulfotransferase; PAPS, 3′-phosphoadenosine 5′ phosphosulfate; DHEA, dehydroepiandrosterone.

based on a previously published procedure (*8*). Cellulose thin-layer chromatography (TLC) plates were from EM Science. All other chemicals were of the highest grade commercially available.

Preparation of Purified Human Cytosolic STs—M-form (SULT1A3) and P-form (SULT1A1) PSTs, two SULT1C STs (designated #1 and #2), DHEA (SULT2A1) ST, and thyroid hormone (SULT1B2) ST (TH ST) were cloned, expressed, and purified using the pGEX-2TK Glutathione S-transferase Gene Fusion System based on the procedure previously established (*9, 10*). Estrogen (SULT1E1) ST (EST), SULT2B1a ST, and SULT2B1b ST were cloned, expressed, and purified using the pET23c protein expression system as previously described (*11*).

Determination of the Stimulatory/Inhibitory Effects of Divalent Metal Cations on Human Cytosolic STs—To determine the stimulation/inhibition patterns of divalent metal cations on purified human cytosolic STs, enzymatic assays in the presence or absence of divalent metal cations were performed based on the procedure previously established (*5–7*). The standard assay mixture, in a final volume of 30 μL, contained 10 mM of the divalent cation tested, 14 μM PAP[^{35}S], 100 mM buffer, the enzyme being assayed, and a specified concentration of the acceptor substrate (*cf.* Table I). Controls containing all the reagents, but without divalent metal cation or with 10 mM EDTA, were assayed in parallel. The reaction was started by the addition of the enzyme and allowed to proceed for 10 min at 37°C, followed by heat inactivation of the enzyme at 100°C for 2 min. Aliquots (1 μl) of the final reaction mixtures were spotted onto cellulose TLC plates and subjected to ascending TLC (*cf.* Table I for solvent systems used). After the completion of TLC, the plates were air-dried and subjected to autoradiography for 24 to 48 h. The radiolabeled sulfated products on the plates were located, cut out and eluted in 0.5 ml of water, mixed with 4 ml of Ecolume scintillation fluid, and counted for radioactivity. To determine the concentrations required for 50% inhibition or stimulation (IC_{50} or EC_{50}), a broader concentration range for each divalent metal cation was first tested. After the concentration-dependent inhibitory/stimulatory effect was observed, a narrower range of concentrations was then used for the determination of IC_{50} or EC_{50}.

Kinetic Analysis of the Inhibitory Effects of Cd^{2+} on M-PST—M-PST was chosen as a model for examining the mode of action of the metal ion Cd^{2+}, which exerts a profound inhibitory effect on its dopamine-sulfating activity. Kinetic experiments with varying substrate concentrations and fixed divalent cation concentrations were performed. Fifteen microliter assay mixtures containing a constant concentration of $CdCl_2$, 14 μM PAP[^{35}S] (15 Ci/mmol), 100 mM TAPS (pH 8.0), 0.25 μg enzyme, and specified concentrations of dopamine were prepared. The reactions were allowed to proceed at 37°C for 10 min, and terminated by heating at 100°C for 2 min. The final reaction mixtures were subjected to TLC analysis as described above. The concentrations of $CdCl_2$ tested in different sets of experiments were 0.5, 1, and 2 mM. As a control, 0.1 mM EDTA was used instead of $CdCl_2$. Data on the velocity (v) and corresponding substrate concentration were processed using the Excel program to generate the best fit for the Lineweaver-Burke double-reciprocal plot.

RESULTS AND DISCUSSION

Metal cations are known to play important roles in the function of biological molecules (*12*). Some of them, including manganese, zinc, copper, iron, and cobalt, are essential components of different enzymes, while others, such as magnesium and calcium, are required for the actions of certain enzymes/proteins. In contrast to these biologically useful metal cations, some metal cations that enter the body primarily as environmental contaminants have been shown to exert deleterious effects. For example, lead and mercury are known to act as neurotoxicants (*13, 14*). The targets of the actions of these metal cations *in vivo*, however, require further investigation. As mentioned earlier, our previous studies revealed that some divalent metal cations, including Mn^{2+}, Co^{2+}, Mg^{2+}, Ca^{2+}, and Cd^{2+}, exert stimulatory or inhibitory effects on the Dopa/tyrosine-sulfating activity of human M-PST (*5–7*). The next logical step would be to determine if these metal cations exert similar effects on other cytosolic STs, and whether other divalent metal cations are also capable of exerting stimulatory/inhibitory effects on the activity of cytosolic STs. We therefore decided to carry out a systematic study of the regulatory effects of divalent metal cations on nine human cytosolic STs.

Effects of Divalent Metal Cations on the Activities of Human Cytosolic STs—In the first series of experiments, enzymatic assays using individual human cytosolic STs and their physiological (or preferred) substrates (*cf.* Table I) were carried out in the absence or presence of various divalent metal cations at a concentration of 10 mM. As a control for the counter ion, Cl^-, parallel assays in the presence 20 mM NaCl were also performed. Results obtained are compiled in Table II. The degrees of inhibition or stimulation were calculated by comparing the activities determined in the presence of metal cations with the activities determined in the absence of metal cations. It was noted that the NaCl controls displayed slight stimulatory or inhibitory effects on

TABLE I. **Summary of the buffers and substrates used in the sulfotransferase assays and the solvent systems used for the TLC analyses of sulfated products.**

Enzyme	Buffer	Substrate	TLC Solvent System (*n*-butanol:isopropanol:formic acid:water) (by volume)
M-PST (SULT1A3)	100 mM Taps, pH 8.0	10 μM dopamine	3:1:1:1
P-PST (SULT1A1)	100 mM Taps, pH 8.0	10 μM *p*-nitrophenol	3:1:1:1
DHEA ST (SULT2A1)	100 mM Taps, pH 8.0	5 μM DHEA	2:1:1:2
SULT1C ST #1	100 mM Taps, pH 8.0	25 μM *p*-nitrophenol	3:1:1:1
SULT1C ST #2	100 mM Hepes, pH 7.0	5 μM *p*-nitrophenol	3:1:1:1
EST (SULT1E1)	100 mM Taps, pH 8.0	25 μM estrone	2:1:1:2
SULT2B1a ST	100 mM Taps, pH 8.0	10 μM DHEA	2:1:1:2
SULT2B1b ST	100 mM Taps, pH 8.0	10 μM DHEA	2:1:1:2
TH ST (SULT1B2)	100 mM Taps, pH 8.0	5 μM T_3	ammonium hydroxide: n-propanol (3:2; by volume)

the nine human cytosolic STs tested. After accounting for such effects, presumably due to the counter ion (Cl^-), all nine human cytosolic STs were found to be partially or completely inhibited/stimulated by a majority of the divalent metal cations tested. The addition of equimolar concentrations of EDTA neutralized the inhibitory effects of the divalent metal cations on the dopamine-sulfating activity of M-PST, except for Hg^{2+} which is known to exhibit high-affinity interaction with the sulfhydryl group, and, therefore, causes the irreversible inhibition of proteins (*15*). (Although the data are not shown, similar results for the addition of equimolar EDTA were also found for other cytosolic STs tested.) That the cytosolic STs remained fully active in the presence of EDTA suggests that no divalent metal cations are required as a cofactor for the basal activities of these enzymes. As shown in Table II, while seven of the metal cations exerted complete inhibition in many cases and Mn^{2+} stimulated M-PST, the divalent metal ions Mg^{2+} and Ca^{2+} had relatively minor effects on the nine human cytosolic STs. These results imply that these cytosolic STs in general are less sensitive to the effects of physiologically more abundant metal cations, but are more sensitive to the detrimental effects of other metal cations, many of which may enter the body as environmental contaminants. It should be pointed out that, in contrast to the Dopa/tyrosine-sulfating activity, which is dramatically stimulated by Mn^{2+} (by a factor of two orders of magnitude) (*6, 7*), the dopamine-sulfating activity of M-PST is stimulated less than twofold by Mn^{2+}. This makes sense from the physiological standpoint, since dopamine [the physiological substrate of M-PST (*16, 17*)] plays an important role as a neurotransmitter *in vivo*. Excessive stimulation of the dopamine-sulfating activity of M-PST, leading to the sulfation of dopamine and its inactivation/elimination, may have a detrimental effect. The dopa/tyrosine-sulfating activity, on the other hand, may represent a "xenobiotic-sulfating activity" of M-PST, which may be provoked more easily by Mn^{2+}. (It is to be noted that the Dopa/tyrosine-sulfating activity of M-PST displays stereoselectivity favoring the D-form enantiomer of Dopa/tyrosine (*6, 7*)).

To examine further the inhibitory/stimulatory effects, the activities of the human cytosolic STs in the presence of different concentrations of metal cations were determined. These experiments were performed mostly in two stages, first with a broader and then a narrower concentration range of metal cation. The results compiled in Table III demonstrate that the different human cytosolic STs tested respond to the divalent metal cations differently. For example, when the Fe^{2+} concentration reached 2.0–4.0 mM, it inhibited the activities of P-PST, DHEA ST, SULT2B1a ST, SULT2B1b ST, TH ST, and EST by 50%, while it achieved the same effect on M-PST, SULT1C ST #1, and SULT1C ST #2 at much lower (0.45, 0.06, and 0.04 mM) concentrations. (It is noted that the activity of P-PST in the presence of 10 mM $FeCl_2$ was 33% of the control (Table II) and yet the IC_{50} was 3.0 mM. In contrast, no EST activity was detected in the presence of 10 mM $FeCl_2$, and the IC_{50} (4.8 mM) was higher than that for P-PST. This discrepancy might have been due to the slight yellowish precipitates observed when 10 mM $FeCl_2$ was included in the assay mixture for EST. It could have been that the purified EST was somewhat unstable at higher concentrations of $FeCl_2$ and became denatured.) Co^{2+} inhibited the activities of M-PST, DHEA ST, SULT1C ST #2, and SULT2B1a ST by 50% at concentra-

TABLE II. **Inhibitory/stimulatory effects of divalent metal cations on the activities of human cytosolic STs.**[a]

	M-PST (SULT1A3)		P-PST (SULT1A1)	DHEA ST (SULT2A1)	SULT1C ST #1	SULT1C ST #2	TH ST (SULT1B2)	EST (SULT1E1)	SULT2B1a ST	SULT2B1b ST
Control	6,702 ± 69 (100%)	6,733 ± 44[b] (101%)	3,927 ± 106 (100%)	1,364 ± 73 (100%)	7.3 ± 0.2 (100%)	1,185 ± 93 (100%)	298 ± 17 (100%)	459 ± 10 (100%)	570 ± 8 (100%)	466 ± 121 (100%)
$MgCl_2$	7,134 ± 106 (106%)	6,971 ± 50 (104%)	5,047 ± 168 (129%)	1,661 ± 65 (122%)	3.7 ± 0.2 (51%)	1,119 ± 61 (94%)	290 ± 18 (97%)	247 ± 7 (54%)	239 ± 15 (42%)	655 ± 60 (141%)
$MnCl_2$	11,606 ± 160 (173%)	6,145 ± 119 (92%)	5,069 ± 76 (129%)	683 ± 5 (50%)	3.4 ± 0.7 (47%)	1,072 ± 10 (90%)	437 ± 14 (147%)	99 ± 4 (22%)	416 ± 28 (73%)	153 ± 25 (33%)
$CaCl_2$	6,759 ± 19 (101%)	6,815 ± 25 (102%)	5,049 ± 245 (129%)	1,746 ± 76 (128%)	4.6 ± 0.4 (63%)	1,104 ± 6 (93%)	478 ± 97 (160%)	315 ± 14 (69%)	817 ± 61 (143%)	337 ± 54 (72%)
$CuCl_2$	238 ± 25 (3.6%)	6,533 ± 19 (97%)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$ZnCl_2$	181 ± 13 (2.7%)	6,965 ± 63 (104%)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$FeCl_2$	175 ± 13 (2.6%)	6,295 ± 25 (94%)	1,285 ± 201 (33%)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$CoCl_2$	1,571 ± 31 (23.4%)	7,021 ± 232 (105%)	4,700 ± 117 (120%)	228 ± 15 (17%)	N.D.	171 ± 3 (14%)	66 ± 8 (22%)	90 ± 14 (20%)	56 ± 5 (10%)	39 ± 27 (8%)
$HgCl_2$	188 ± 6 (2.8%)	131 ± 13 (2.0%)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$CdCl_2$	263 ± 44 (3.9%)	6,971 ± 131 (104%)	1,449 ± 31 (37%)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Pb acetate	801 ± 13 (12%)	6,852 ± 31 (102%)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
NaCl	6,896 ± 63 (103%)	6,652 ± 38 (99%)	5,148 ± 305 (131%)	1,364 ± 73 (100%)	6.3 ± 0.3 (86%)	1,132 ± 87 (96%)	312 ± 45 (105%)	480 ± 2 (105%)	764 ± 104 (134%)	481 ± 54 (103%)

[a]The concentration of the divalent metal cations tested was 10 mM, and the concentration of NaCl tested was 20 mM. Specific activities of the STs are expressed as pmo duct produced/min/mg protein. Data shown represent means ± SD of five determinations. N.D. refers to "no activity detected" and, therefore, the complete inhibition of the enzyme. [b]The values shown in this column were derived from assays performed in the presence of 10 mM of the divalent cations tested plus 10 mM EDTA.

TABLE III. **Summary of the IC_{50} or EC_{50} of different divalent metal cations for human cytosolic STs.**[a]

	M-PST (SULT1A3)	P-PST (SULT1A1)	DHEA ST (SULT2A1)	SULT1C ST #1	SULT1C ST #2	TH ST (SULT1A2)	EST (SULT1E1)	SULT2B1a ST	SULT2B1b ST
$MgCl_2$	—[b]	—	EC_{50} = 18 mM	IC_{50} = 12.0 mM	—	—[b]	IC_{50} = 16 mM	IC_{50} = 6.0 mM	IC_{50} = 12 mM
$MnCl_2$	EC_{50} = 1 mM	—	IC_{50} = 10 mM	IC_{50} = 3.5 mM	IC_{50} = 9.5 mM	IC_{50} = 20 mM	IC_{50} = 4.0 mM	IC_{50} = 12 mM	IC_{50} = 2.0 mM
$CaCl_2$	—	—	EC_{50} = 20 mM	IC_{50} = 12 mM	—	—	IC_{50} = 14 mM	—	—
$CuCl_2$	IC_{50} = 0.4 mM	IC_{50} = 0.2 mM	IC_{50} = 0.1 mM	IC_{50} = 0.13 mM	IC_{50} = 7.0 μM	IC_{50} = 0.15 mM	IC_{50} = 0.16 mM	IC_{50} = 50 μM	IC_{50} = 0.16 mM
$ZnCl_2$	IC_{50} = 0.6 mM	IC_{50} = 0.14 mM	IC_{50} = 0.1 mM	IC_{50} = 24 μM	IC_{50} = 7.0 μM	IC_{50} = 80 μM	IC_{50} = 20 μM	IC_{50} = 2.0 μM	IC_{50} = 60 μM
$FeCl_2$	IC_{50} = 0.45 mM	IC_{50} = 3.0 mM	IC_{50} = 2.6 mM	IC_{50} = 60 μM	IC_{50} = 40 μM	IC_{50} = 2.0 mM	IC_{50} = 4.8 mM	IC_{50} = 4.0 mM	IC_{50} = 4.4 mM
$CoCl_2$	IC_{50} = 8.4 mM	—	IC_{50} = 4.5 mM	IC_{50} = 0.3 mM	IC_{50} = 3.0 mM	IC_{50} = 0.2 mM	IC_{50} = 1.2 mM	IC_{50} = 2.6 mM	IC_{50} = 70 μM
$HgCl_2$	IC_{50} = 0.4 mM	IC_{50} = 0.38 mM	IC_{50} = 0.15 mM	IC_{50} = 0.22 mM	IC_{50} = 30 μM	IC_{50} = 80 μM	IC_{50} = 3 μM	IC_{50} = 0.3 μM	IC_{50} = 0.16 mM
$CdCl_2$	IC_{50} = 0.7 mM	IC_{50} = 7.0 mM	IC_{50} = 0.14 mM	IC_{50} = 0.31 mM	IC_{50} = 0.32 mM	IC_{50} = 0.24 mM	IC_{50} = 0.18 mM	IC_{50} = 5.0 μM	IC_{50} = 0.25 mM
Pbacetate	IC_{50} = 3.7 mM	IC_{50} = 1.2 mM	IC_{50} = 0.2 mM	IC_{50} = 1.2 mM	IC_{50} = 50 μM	IC_{50} = 0.6 mM	IC_{50} = 1.2 mM	IC_{50} = 0.1 mM	IC_{50} = 0.4 mM

[a]Data shown were derived from three determinations. [b]IC_{50} or EC_{50} could not be determined in the concentration range of the divalent cation tested.

tions of 8.4, 4.5, 3.0, and 2.6 mM, respectively, while it inhibited the activities of SULT2B1b ST, TH ST, EST, and SULT1C ST #1 by 50% at concentrations of 0.07, 0.2, 1.2, and 0.3 mM, respectively. Interestingly, P-PST appeared to be virtually insensitive to the effect of Co^{2+}. Cd^{2+} inhibited the activity of P-PST by 50% at a concentration of 7.0 mM, but it achieved the same effect on M-PST, DHEA ST, SULT1C ST #2, SULT2B1b ST, TH ST, EST, and SULT1C ST #1 at concentrations of 0.7, 0.14, 0.32, 0.25, 0.24, 0.18, and 0.31 mM, respectively. These IC_{50} values are all above the normal physiological concentration ranges of the divalent metal cations tested (*18*), implying that human cytosolic STs, in general, are not easily influenced by these metal cations *in vivo*. In a few cases, however, IC_{50} values close to the physiological concentration ranges were ob-served. For example, SULT2B1a ST was found to be ex-tremely sensitive to Zn^{2+}, Hg^{2+}, and Cd^{2+}, with 50% of its activity being inhibited at concentrations of, respectively, 0.3, 2.0, and 5.0 μM. Similarly, 50% of the activity of SULT1C ST #2 was inhibited by Cu^{2+} or Zn^{2+} at a concentration of 7.0 μM; and 50% of the activity of EST was inhibited by Hg^{2+} at a concentration of 3.0 μM. These results indicate that these latter cytosolic STs may, in fact, be vulnerable to the inhibitory effects of certain divalent metal cations under physiological conditions. It should be cautioned that the physiological concentration ranges refer to the levels in human serum. The levels in tissues may be quite different and may differ depending on the tissue or organ, some of which tend to selectively concentrate certain metal cations (*18*). Also, in view of the fact that these are cytosolic enzymes and some of the metal ions may be sequestered in organelles such as mitochondria (*19*), any process that disrupts this sequestration (as may be caused by apoptosis or the oxidative damage that is believed to accompany many neurodegenerative disorders) (*20*) may affect the activity of these STs and the processes they regulate. In short, it is possible that relatively small increases or decreases in the serum levels of some metal ions may have important consequences for the activity of many of these STs and the processes they regulate. Moreover, some disorders (e.g., the neurodegenerative disorders) may be partly mediated via changes in the cytosolic concentrations of some metal ions, with subsequent effects on the activity of enzymes such as the cytosolic STs.

Investigation of the Mode of Action of the Divalent Metal Cation Cd^{2+} using M-PST as a Model—An important issue is the modes of action of divalent metal cations. Since M-PST is the best characterized human cytosolic ST and plays an important role in the homeostatic regulation of dopamine metabolism (*16, 17*), we decided to use it as a model in this study. The effects of Cd^{2+} on the kinetics of dopamine sulfation by M-PST were examined. Enzymatic assays using varying concentrations of the substrate, dopamine, in the presence of fixed concentrations of $CdCl_2$ were performed. Data obtained were used to generate a Lineweaver-Burk double-reciprocal plot (Fig. 1). A striking feature of the double-reciprocal plot generated is that the lines corresponding to the various concentrations of $CdCl_2$ tested, while crossing the *Y*-axis at different positions, appear to converge within a narrow region on the *X*-axis. These results indicated that the K_m value of M-PST for dopamine changes only slightly in the presence of the $CdCl_2$ concentrations tested, whereas the V_{max} decreases dramatically with increasing concentrations of Cd^{2+}. The values of K_m and V_{max}, as well as V_{max}/K_m, calculated from the Lineweaver-Burk double-reciprocal plots are compiled in Table IV. These data seem to point to a noncompetitive-type of inhibition in which the divalent cation, Cd^{2+}, and the substrate, dopamine, may bind independently at different sites on M-PST. The complex of the enzyme, Cd^{2+}, and dopamine, however, may be catalytically inactive. It is possible that Cd^{2+} may cause a change in the conformation of the M-PST-dopamine complex, and thereby prevent the proper positioning of the catalytic center, leading to the inactivation of the enzyme (*21*). It will be important to find out which amino acid residue(s) in M-PST is(are) responsible for Cd^{2+}binding.

The present study represents the first systematic investi-

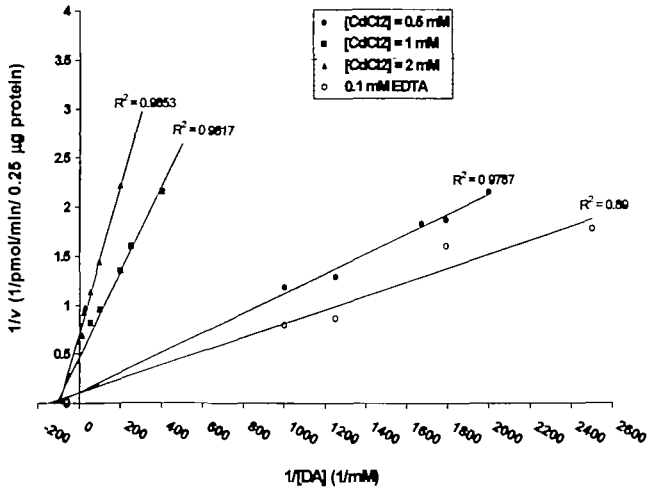

Fig. 1. **Lineweaver-Burk double-reciprocal plot of M-PST with dopamine as the substrate in the presence of different concentration of $CdCl_2$.** The concentrations of dopamine are expressed in μM and velocities are expressed as pmol of product formed/min/0.25 μg enzyme. Each data point represents the mean value derived from five determinations.

TABLE IV. **Kinetic constants of M-PST at different concentrations of $cdCl_2$ with dopamine as substrate.**[a]

$CdCl_2$ (mM)	K_m (μM)	V_{max} (pmol/min/ 0.25 μg protein)	V_{max}/K_m
0.5	4.5 ± 1.0	4.3 ± 0.9	0.96
1	6.2 ± 0.8	1.8 ± 0.1	0.29
2	11.9 ± 0.3	1.5 ± 0.1	0.13
0.1 mM EDTA	9.6 ± 3.9	15.6 ± 6.6	1.63

[a]Data shown represent means ± SD of five determinations.

gation of the role of divalent metal cations in regulating the activities of human cytosolic STs. Cytosolic STs are traditionally viewed as Phase II drug–metabolizing or detoxifying enzymes that are important in the inactivation and removal of xenobiotic compounds (*1–3*). It is becoming increasingly clear, however, that these enzymes are also involved in the metabolism of endogenous compounds that function as neurotransmitters, hormones, *etc.* (*22–24*). To some extent, the role of an enzyme may depend on its location. M-PST, for example, is found in the upper gastrointestinal (GI) tract, brain, platelets, and lung (*25*). While in the brain it may play a role in regulating the levels of dopamine, in the GI tract it may detoxify potentially lethal dietary catecholamines and help to regulate the gut-blood barrier (*24*). The presence of various metal ions of dietary, therapeutic, or environmental origin, by affecting the activity of the cytosolic STs at the various interfaces, such as gut, lung, *etc.*, is likely to disrupt the integrity of various barriers and have physiological sequelae. Fluctuations in the levels of various metal ions *in vivo* will also affect the functioning of other enzymes including the cytosolic STs, *e.g.*, brain M-PST, which play a role in the regulation of endogenous compounds. In some extreme cases, environmental or occupational heavy metal poisoning has been documented (*26–28*). Individuals intoxicated with, for example, lead or mercury have been reported to exhibit neurological symptoms (*13, 14*). In view of the fact that M-PST is involved in the homeostatic regulation of monoamine neurotransmitters (*17*), particularly dopamine, as well as in controlling the entry of toxic catecholamines in the gut (*24*), it is possible that this enzyme may be one of the targets of heavy metal contaminants producing their neurological effects. Manganese poisoning or manganism, has been documented in people working in manganese mines, and this disorder mimics some of the features of Parkinson's disease (*29, 30*). Parkinsonism is characterized by greatly lowered brain dopamine levels (31), and it is likely that the Parkinsonian symptoms of manganism are mediated through a stimulatory effect of divalent manganese ions on brain M-PST, resulting in the removal of dopamine (*5–7*).

In general, the cytosolic STs have been considered housekeeping enzymes and, except during the early stage of development, very little is known with regard to the regulation of their expression or activity (*1–3*). That various divalent metal cations are capable of inhibiting or stimulating the activities of human cytosolic STs presents a new issue for consideration in understanding the functioning of these enzymes. More studies are warranted to fully elucidate the effects of divalent metal cations on cytosolic STs in the context of toxicology, endocrinology, neurology, *etc.*

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