RESEARCH COMMUNICATION

Selective inhibition of Zn^{2+} -glycerophosphocholine cholinephosphodiesterase by tellurium tetrachloride

Dai-Eun SOK* and Mee Ree KIM††

*Daejeon Machine Depot, P.O. Box 35, Daejeon, Korea, and †Department of Food and Nutrition, Chungnam National University, Daejeon 305-764, Korea

A Zn^{2+} -glycerophosphocholine cholinephosphodiesterase (EC 3.1.4.38) purified from mouse brain was found to be reversibly inhibited by tellurium tetrachloride. This effect was characterized by a competitive pattern of inhibition, with apparent K_i values of 0.7 μM and 1.5 μM for the hydrolysis of *p*-nitrophenylphosphocholine and glycerophosphocholine respectively. Interestingly, the inhibitory effect of tellurium tetrachloride was found to be greatly potentiated by tetramethylammonium salt, indicative of a synergistic interaction between the two compounds. Additionally, it was observed that the effect of tellurium tetrachloride was not affected by a number of other metal ions, and was more pronounced at neutral pH, suggesting that the inhibitory role of the tellurium tetrachloride may be of importance under physiological conditions. Thus Zn^{2+} -glycerophosphocholine cholinephosphodiesterase is proposed to be one of the target enzymes which is susceptible to the inhibitory effect of tellurium tetrachloride.

INTRODUCTION

A glycerophosphocholine (GPC) cholinephosphodiesterase (EC 3.1.4.38) has been reported to be present in brain tissue (Abra & Quinn, 1976). More recently, another type of GPC cholinephosphodiesterase, which shows an absolute requirement for Zn^{2+} and the capability to convert *p*-nitrophenylphosphocholine (*p*-NPPC) into *p*-nitrophenol and phosphocholine, was found to be associated with the myelins (Kanfer & McCartney, 1989a). Subsequently this *p*-NPPC-hydrolysing phosphodiesterase was solubilized from brain membranes by phosphatidylinositol-specific phospholipase C (Sok & Kim, 1991), and purified to give a homogeneous protein (Sok & Kim, 1992). The latter study showed that *p*-NPPC phosphodiesterase and Zn^{2+} -GPC cholinephosphodiesterase activities resided in the same protein. Zn^{2+} -GPC cholinephosphodiesterase activity was observed to increase during development (Kanfer & McCartney, 1989b), and was proposed to be involved in the myelin membrane homeostasis which is fundamental to the maintenance of a myelin sheath around axons.

In tellurium-induced neuropathy, it has been suggested that ingested tellurium induces peripheral demyelination and decreases remyelination in developing rats by inhibiting the synthesis of cholesterol (Harry *et al.*, 1989; Goodrum *et al.*, 1990). Also, the alteration of lipid metabolism in myelins was suggested to be the cause of demyelination. It was subsequently proposed that demyelination of peripheral nerves exposed to tellurium was due to inhibition of squalene epoxidation, a step in cholesterol biosynthesis (Wagner-Recio *et al.*, 1991). However, the direct inhibition of squalene epoxidase by tellurium ions was not clearly shown. Therefore it was suggested that the toxicity of tellurium or its products of metabolism is due partly to the inhibition of myelin-bound enzymes responsible for myelination.

In the present study, we demonstrate that tellurium tetrachloride selectively inhibits Zn^{2+} -GPC cholinephosphodiesterase,

and propose that the phosphodiesterase may be a target enzyme for the toxic effect of tellurium tetrachloride.

MATERIALS AND METHODS

Materials

p-NPPC, GPC, glycerol kinase, glycerophosphate oxidase, horseradish peroxidase, glycerol, ATP, phosphatidylinositol-specific phospholipase C (*Bacillus cereus*, type III) and EDTA were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Tellurium tetrachloride and tetramethylammonium chloride were obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.).

Enzyme assay

p-NPPC phosphodiesterase activity was determined by measuring the amount of *p*-nitrophenol released from the hydrolysis of *p*-NPPC, as described previously (Sok & Kim, 1990). Unless otherwise described, assays were conducted in 1 ml of 0.1 M-glycine buffer, pH 10, containing 150 μM -*p*-NPPC at 20 °C. GPC cholinephosphodiesterase activity was measured by a modification of the enzyme-coupled assay (Baldwin & Cornatzer, 1968; McGowan *et al.*, 1983). The enzyme was incubated with 1.25 mM-GPC at 20 °C in 1 ml of 0.1 M-glycine buffer, pH 10. Then the above mixture was adjusted to pH 7.0 and mixed with 1 ml of 0.1 M-phosphate buffer, pH 7.0, containing glycerol kinase (1 unit), glycerophosphate oxidase (2 units), horseradish peroxidase (2 units), ATP (0.3 μmol), MgCl_2 (3 μmol), 4-aminoantipyrine (3 μmol) and sodium *N*-ethyl-*N*-(3-sulphopropyl) *m*-anisidine, and the increase in absorbance at 540 nm was measured. The quantitative measurement of glycerol was performed using the calibration curve obtained with standard glycerol (0–240 nmol/ml).

Preparation of purified GPC cholinephosphodiesterase

The phosphodiesterase was solubilized from brain membranes by microbial phospholipase C as described (Sok & Kim, 1991),

Abbreviations used: GPC, glycerophosphocholine; *p*-NPPC, *p*-nitrophenylphosphocholine.

†† To whom correspondence should be addressed.

and purified by sequential chromatography on concanavalin A-Sepharose and CM-Sephadex columns, and by PAGE analysis. The enzyme protein (specific activity 270 $\mu\text{mol/h}$ per mg) behaved as a homogeneous component in gel electrophoresis, and GPC cholinephosphodiesterase activity and *p*-NPPC phosphodiesterase activity were found to co-migrate in Sephadex G-150 gel chromatography (Sok & Kim, 1992).

Enzymic analysis

The kinetic analyses were performed as described in the legends to the Figures. Values are means of two or three experiments.

RESULTS AND DISCUSSION

When GPC cholinephosphodiesterase purified from mouse brain was preincubated with 1 mM-EDTA prior to exposure to *p*-NPPC, time-dependent inhibition of the enzyme was observed. A

Fig. 1. Time course for the inhibition of the purified GPC cholinephosphodiesterase by EDTA or Te^{4+}

(a) The phosphodiesterase (0.15 μg) was preincubated with 1 mM-EDTA in 1 ml of 0.1 M-glycine buffer, pH 10. After the preincubation times (1, 2, 5, 10 or 30 min) indicated, 50 μl of 3 mM-*p*-NPPC (●) or 25 mM-GPC (▲) was added to the reaction mixture and the remaining enzyme activity was assayed as described in the Materials and methods section. Incubation of the enzyme with EDTA was performed separately in the presence of *p*-NPPC under conditions described above (○), and the activity assay was started after 10 or 30 min. Samples incubated in the absence of inhibitor were assigned 100% activity. Values are means of two or three experiments. (b) Phosphodiesterase was preincubated with 15 μM - Te^{4+} for 1, 2, 5, 10 or 30 min, and the remaining activity was measured as described in (a): ●, *p*-NPPC; ▲, GPC.

similar pattern of inhibition was observed using GPC as the substrate (Fig. 1a). The EDTA-inactivated phosphodiesterase was found to be re-activated by Zn^{2+} only. These results reaffirm the previous observation that the purified phosphodiesterase is a Zn^{2+} -requiring enzyme (Sok & Kim, 1992). Meanwhile, the simultaneous incubation of the enzyme with EDTA in the presence of either *p*-NPPC or GPC led to no significant inactivation of the enzyme. Thus it seems that, in the presence of substrate, the enzyme is protected from inactivation by EDTA, an observation characteristic of Zn^{2+} -metalloenzymes (Orning *et al.*, 1991).

After having established the chelation of Zn^{2+} by EDTA in the catalytic site of the phosphodiesterase, we investigated the inhibitory effect of various cations on the Zn^{2+} -GPC cholinephosphodiesterase. As demonstrated in Table 1, it was found that while tellurium tetrachloride at concentrations lower than 20 μM greatly inhibited the hydrolysis of *p*-NPPC by the phosphodiesterase, the other ions had little or no effect on *p*-NPPC hydrolysis even at 100 μM . These results suggest that there may be a selective interaction between tellurium tetrachloride and the active site of the phosphodiesterase. Such a selectivity was further supported by the observation that microbial phospholipase C, which requires Zn^{2+} , was not inhibited significantly by Te^{4+} .

To investigate the nature of the inhibitory effect of Te^{4+} , the phosphodiesterase was preincubated with Te^{4+} and subsequently assayed for enzymic activity using GPC or *p*-NPPC as substrate. Fig. 1(b) indicates that the inhibitory effect of Te^{4+} seems to be

Table 1. Effect of various cations on Zn^{2+} -requiring GPC cholinephosphodiesterase

The phosphodiesterase was incubated with 150 μM -*p*-NPPC in 0.1 M-glycine buffer, pH 10, containing the appropriate cation, and assayed as described in the Materials and methods section.

Ion	Concn. (mM)	Relative inhibition (%)
Pb^{2+}	1	0
Cd^{2+}	1	0
Mn^{2+}	1	0
Se^{4+}	0.1	< 5
Te^{4+}	0.005	33 ± 4
	0.015	55 ± 1

Fig. 2. Double-reciprocal plots of the inhibition of purified phosphodiesterase by tellurium tetrachloride

(a) Hydrolysis of *p*-NPPC (7.5–150 μM) by the phosphodiesterase (0.15 μg) was measured in either the presence or the absence of Te^{4+} (5 μM or 15 μM) as described in the Materials and methods section. (b) Hydrolysis of GPC (40–1250 μM) was measured in either the presence or the absence of Te^{4+} (5 μM or 10 μM).

Fig. 3. Effect of tetramethylammonium chloride on the inhibitory action of Te^{4+}

The phosphodiesterase (0.15 μg) was incubated with 150 μM - p -NPPC in the presence of various concentrations (2–20 μM) of Te^{4+} in 0.1 M-glycine buffer, pH 10 (○). The inhibitory effect of Te^{4+} was investigated separately in the presence of 2 mM-tetramethylammonium chloride (●). Enzyme activity was measured as described in the Materials and methods section; the relative activity is expressed as a percentage of control.

instantaneous and independent of the preincubation time, suggestive of reversible interaction of the phosphodiesterase by Te^{4+} . Moreover, it was observed that the inhibitory effect of Te^{4+} was not affected by 1 mM- Zn^{2+} , $-\text{Co}^{2+}$, $-\text{Cd}^{2+}$, $-\text{Mn}^{2+}$ or $-\text{Ca}^{2+}$. Based on these results, the possibility that Te^{4+} exerts its inhibitory action by replacing Zn^{2+} in the active site of the enzyme is excluded. Instead, it is proposed that the binding subsite in the active site of the phosphodiesterase may be occupied selectively by Te^{4+} .

Subsequently we examined the mechanism by which the phosphodiesterase is inhibited by Te^{4+} . When the inhibitory effect of Te^{4+} was analysed by a Lineweaver-Burk plot (Fig. 2), a typical competitive pattern of inhibition was observed. Apparent K_i values for Te^{4+} were estimated to be 0.7 μM and 1.5 μM for the hydrolysis of p -NPPC and GPC respectively. These results indicate that Te^{4+} is a potent competitive inhibitor of Zn^{2+} -GPC cholinephosphodiesterase.

To examine the properties of a binding site for Te^{4+} , incubation of the enzyme with Te^{4+} was carried out in the presence of tetramethylammonium chloride, which was proposed to compete with Te^{4+} for the same binding site in the active site of the enzyme. Interestingly, the inhibitory action of Te^{4+} was greatly potentiated by tetramethylammonium ion (Fig. 3), although the latter showed no significant inhibitory effect itself (< 5%), even at 2 mM. A similar but less pronounced potentiating effect was observed with more physiological cationic compounds such as choline and acetylcholine. These results indicate that there is a synergistic interaction between Te^{4+} and cationic compounds in inhibition of the phosphodiesterase. Thus it is suggested that the binding site for Te^{4+} may be distinguished from an anionic binding site for the tetramethylammonium ion.

The inhibitory effect of Te^{4+} on the hydrolysis of p -NPPC was

Fig. 4. Effect of pH on the inhibitory effect of Te^{4+}

The effect of Te^{4+} (15 μM) on the phosphodiesterase-catalysed hydrolysis of p -NPPC was examined at various pH values under conditions similar to those described for Fig. 1; Tris buffer, pH 7.4 and pH 8.0; glycine buffer, pH 9.0, pH 10.0 and pH 10.5.

examined at various pH values, and it was found that this effect was somewhat larger at lower pH (Fig. 4). This might be related to the observation that the binding affinity of p -NPPC for the enzyme was larger at lower pH (D.-E. Sok & M. R. Kim, unpublished work). In a related experiment to determine whether the inhibitory effect of Te^{4+} is important *in vivo*, it was found that the hydrolysis of GPC by the phosphodiesterase was greatly inhibited (> 60%) by 5 μM - Te^{4+} in 10 mM-phosphate buffer (pH 7.4) containing GPC at 0.25 mM, close to the physiological concentration (Kanfer & McCartney, 1989a). Accordingly, it is proposed that Zn^{2+} -GPC cholinephosphodiesterase may be susceptible to the inhibitory action of Te^{4+} *in vivo*.

Although the toxicity of Te^{4+} was suggested to be due in part to the inhibition of squalene epoxidase in the synthesis of cholesterol (Wagner-Recio *et al.*, 1991), the direct inhibition of the phosphodiesterase by Te^{4+} *in vivo* was still questioned by these authors. Alternatively, Te^{4+} may be involved in neuropathy by interfering directly with the synthesis or metabolism of phospholipids. Our present data provide positive evidence that a Zn^{2+} -GPC cholinephosphodiesterase is one of the target enzymes affected by tellurium tetrachloride under physiological conditions.

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