Involvement of a Novel Mouse Hepatic Esterase (ES46.5K) in the Hydrolysis of Phthalate Esters

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Introduction

The major metabolic pathway of phthalate esters, primary plasticizers used in polyvinyl chloride products, is hydrolysis of diester to monoester which is suspected to be hepatocarcinogenic and teratogenic after chronic exposure or high dosage.^{1,2)} We recently purified and characterized a novel 46.5-kilodalton esterase from mouse hepatic microsomes.³⁾ This novel esterase (ES46.5K) efficiently hydrolyzes 11-acetoxy- Δ^8 -THC, acetate of an active metabolite of Δ^8 -THC, although the physiological role of ES46.5K is not clear at present. The present study describes the participation of ES46.5K in hydrolysis of phthalate esters.

Methods

Male ddN mice (25–35 g) were used, and various organ homogenates (brain, lung, heart, liver, pancreas, spleen, adipose tissue, intestine, kidney and testis) and hepatic microsomes were prepared. ES46.5K was purified from hepatic microsomes of male ddN mice by the methods described previously.³⁾ Phthalate esters [diethyl (DEP), dibutyl (DBP), diisobutyl (DIBP), dioctyl (DOP) and diethylhexyl (DEHP) *etc.*] were incubated with tissue homogenates under various conditions. Monoester metabolites formed were determined by gas-chromatography (GC). Kinetic parameters were determined from double reciprocal plots of enzyme activity and substrate concentration. Inhibitory effects of several esterase inhibitors were determined to know the contribution of ES46.5K in the hydrolysis activity in various organs.

Results and Discussion

Kinetic analyses indicated that K_M (μM) for DEP, DBP, DIBP and DOP were 703, 408, 105 and 381, respectively, and V_{max} ($\mu\text{mol/min/mg protein}$) were 0.41, 0.34, 0.18 and 0.02, in the hepatic microsomes, whereas the values in ES46.5K were 68, 7, 17 and 418, and 19.8, 19.5, 9.6 and 19.3. V_{max}/K_M ratios of ES46.5K were 200–3300 times higher than those of hepatic microsomes. ES46.5K did not hydrolyze phthalates having ring structure such as dicyclohexyl phthalate and diphenyl phthalate. Phthalate ester hydrolysis activities for liver, pancreas, intestine and kidney were much higher than those for brain, heart, spleen and testis. DBP hydrolysis activity for pancreas was higher than that for all organs, thus organ differences were observed in these activities. Bis-*p*-nitrophenylphosphate (1.0 mM), which is known to be a typical inhibitor of carboxylesterase, completely inhibited the activity of carboxylesterase from porcine liver, whereas it exhibited a somewhat lower inhibitory effect on ES46.5K and hepatic homogenates. The present study suggests that ES46.5K contributes to hydrolysis of phthalate esters.

References

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