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Solubilization of Mouse Liver Microsomal Carboxylesterases

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Abstract

Mammalian liver microsomal carboxylesterases comprise a family of isozymes, few of which have been purified or studied at the molecular level. These enzymes play an important role in the metabolism of drugs, lipids and other xenobiotics. The purpose of this study was to establish conditions for the selective solubilization of these microsomal enzymes.

Solubilization of mouse liver microsomal carboxylesterase was examined with Triton X-100, Lubrol PX, octyl glucoside and 3-(3′-cholamidopropyl)-dimethylammonio-1-propane sulphonate (CHAPS). The solubilized esterase activities were assayed with *p*-nitrophenyl acetate (*p*-NpAc), α-naphthyl acetate (α-NA) and malathion. Triton X-100, Lubrol PX and CHAPS solubilized > 90% of the esterase activity acting on *p*-NpAc and malathion at 0·05–0·10% concentrations, whereas esterase activity acting on α-NA was released at 0·3–1·0% concentrations. Octyl glucoside caused maximum solubilization of esterase activity acting on malathion at 0·3% and *p*-NpAc or α-NA at > 1%.

These detergents solubilized the membrane-bound esterases to varying degrees depending on the concentrations and the substrate used. Octyl glucoside and CHAPS are effective detergents for solubilization due to their high critical micellar concentrations, selectivity, maintenance of high esterase activities and ease of removal by dialysis.

Mammalian liver carboxylesterases (EC 3.1.1.1) are known to play an important role in the metabolism of a wide range of xenobiotics such as drugs, insecticides and herbicides, and endogenous substrates such as lipids, steroids and amino acids (Heymann 1980; Leinweber 1987). These enzymes have broad and overlapping substrate specificities and can hydrolyze compounds containing ester, thioester, carbamate and amide functional groups (Huang et al 1993b, 1996). Recent studies have shown that specific carboxylesterases are involved in the hydrolysis of certain lipids, platelet activating factor and steroids (Leinweber 1987; Parker et al 1996). However, the physiological functions of these enzymes are still not fully understood.

There are multiple forms of liver carboxylesterases that are either free in the lumen or loosely bound to the luminal surface of the endoplasmic reticulum (Harano et al 1988; Robbi et al 1990). Several forms of these enzymes have recently been cloned and sequenced (Robbi et al 1990; Yan et al 1995; Robbi et al 1996; Pindel et al 1997). The C-terminal of these cDNAs contain the HXEL consensus motif which target these enzymes to the lumen of the endoplasmic reticulum. These enzymes can be released from the endoplasmic reticulum by treatments with detergents, phospholipase A, aqueous acetone and alkali (Heymann 1980; Morgan et al 1994), or by lipid peroxidation (Talcott et al 1980).

In this study, we examined the effects of one zwitterionic and three non-ionic detergents at concentrations ranging from 0·05% to 3·0% for the selective solubilization of carboxylesterases from mouse liver microsomes. Solubilization of these enzymes is an essential step for further purification and characterization of these important hydrolytic enzymes.

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Materials and Methods

Reagents

Technical grade (95%) malathion, *O,O*-dimethyl-S-[1,2-di(ethoxycarbonyl)ethyl]-phosphorodithioate, obtained from American Cyanamid Corp. (Princeton, NJ) was further purified by thin-layer chromatography. *p*-Nitrophenol and *p*-nitrophenyl

acetate (p-NpAc) were purchased from Aldrich Chemical Co. (Milwaukee, WI). α-Naphthol, α-naphthyl acetate (α-NA), p-iodonitro-phenyltetrazolium violet (INT), alcohol dehydrogenase, NAD and NAD diaphorase, bovine serum albumin (fraction V), 3-(3′-cholamidopropyl)-dimethylammonio-1-propane sulphonate (CHAPS), Lubrol PX and n-octyl-β-D-glucopyranoside (octyl glucoside) were purchased from the Sigma Chemical Co. (St Louis, MO). Triton X-100 was purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). Bicinchoninic acid (BCA) reagent was purchased from Pierce Chemical Co. (Rockford, IL).

Preparation and solubilization of microsomes

Liver microsomal fractions from mouse were prepared as described previously (Huang et al 1993a, b). All procedures were carried out at 0–4°C. A range of concentrations of Triton X-100, Lubrol PX, octyl glucoside and CHAPS were used to determine the optimal conditions for solubilization of mouse liver microsomal carboxylesterase. The final detergent concentrations were 0·05, 0·1, 0·3, 1·0 and 3·0%. Mouse liver microsomes were diluted to a final protein concentration of 4·0 mg mL^{-1} in 0·1 M Tris-HCl buffer, pH 7·5 containing the appropriate detergents at various concentrations. Enzyme preparations in detergent-free buffers were used for controls. The mixtures were gently shaken for 1 h on a rotating wheel at about 50 rev min^{-1} and then centrifuged at 100 000 g for 1 h. The supernatant fractions were carefully pipetted off and the pellet resuspended in an equivalent volume of buffer. The supernatants and pellets were assayed for esterase activity and protein. Only the esterase activity in the supernatant fraction after solubilization is reported in Table 1. The specific activity was calculated for each detergent concentration. The specific activity for the controls represents the activity in the microsomes incubated in buffer without detergents at 4°C for 1 h. There was no significant esterase activity in the supernatant fraction if microsomes were re-pelleted after incubation in buffer lacking detergent.

Esterase assays with spectrophotometric substrates

The rates of hydrolysis of p-NpAc and α-NA by the mouse liver carboxylesterases were measured in a continuous assay using 96-well microtitre plates (Dynatech Laboratories, Inc., Virginia, VA) with a Vmax plate reader (Molecular Devices, Palo Alto, CA) as described previously (Huang et al 1993a, b). The carboxylesterase activity on malathion was assayed according to the method of Talcott (1979) and modified for use in the microtitre plate reader. The hydrolysis of malathion was coupled to the reduction of a tetrazolium dye (INT) by alcohol dehydrogenase and NADH diaphorase. The enzyme activity, which is equivalent to the reduction of INT, was calculated from the linear portion of the change in absorption at 490 nm using an extinction coefficient of 13·8 mM^{-1} cm^{-1}. The assays were monitored for 5 min in wells containing 300 μL of incubation mixture. The mixture contained 100 μL of enzyme solution (diluted in 0·1 M Tris-HCl buffer, pH 7·5), and 200 μL of reagent mixture in the same buffer. The reaction was started by injecting 1 μL of malathion in acetone (3×10^{-4} M final concentration). As controls, reagent and enzyme blanks were run with reaction mixture without enzyme and substrate, respectively.

The enzyme assays were carried out under conditions where the initial hydrolytic rates were linear with time for the protein and substrate concentrations used. The assay was carried out in quadruplicate. The measured rates were corrected for the spontaneous hydrolysis of the substrate where this was significant.

Protein concentrations were determined using the standard protocol version of the Pierce BCA assay modified for use in the microtitre plate reader. BSA was used as a protein standard.

Results and Discussion

Effective solubilization of carboxylesterase from mouse liver microsomes was achieved with three non-ionic detergents Triton X-100, Lubrol PX and octyl glucoside and a zwitterionic detergent, CHAPS (Table 1). These detergents solubilized the membrane esterases to varying degrees depending on their concentrations and the substrate used. The ability of the detergents to solubilize the esterase was apparently dependent, in part, on their critical micellar concentrations (CMC). Optimal solubilization of proteins generally occurs at or near the CMC for most detergents. The CMC values for Triton X-100, Lubrol PX, octyl glucoside and CHAPS are 0·3 (0·02%), 0·1 (0·006%), 25 (0·73%) and 1·4 (0·49%) mM, respectively (Hjelmeland & Chrambach 1984). Triton X-100, Lubrol PX and CHAPS solubilized > 90% of the carboxylesterase activity acting on p-NpAc and malathion at low concentrations (0·05–0·1%), whereas carboxylesterase activity acting on α-NA was released at higher concentrations (0·3–1·0%). Octyl glucoside caused maximal solubilization of esterase activity acting on malathion at 0·3% and p-NpAc or α-NA at > 1%. Apparent activation at some detergent concentrations was observed. Detergents which can be easily removed, do not denature enzymes and do

Table 1. Solubilization of carboxylesterase activity from mouse liver microsomal fraction by various detergents.

Detergent	Detergent concn (%)	Esterase activity[a]		
		p-NpAc[b]	α-Na[b]	Malathion[c]
Control[d]		4·80 ± 0·37	9·61 ± 0·57	16·29 ± 1·17
Triton X-100	0·05	4·61 ± 0·03	8·20 ± 0·12	20·99 ± 1·34
	0·1	3·68 ± 0·16	7·46 ± 0·10	14·81 ± 0·90
	0·3	3·73 ± 0·09	9·35 ± 0·87	9·54 ± 0·55
	1·0	3·91 ± 0·15	10·97 ± 0·58	13·03 ± 0·82
	3·0	3·06 ± 0·11	10·92 ± 0·60	13·84 ± 0·61
Control[d]		6·03 ± 0·51	12·80 ± 0·70	22·60 ± 0·80
LubrolPX	0·05	7·43 ± 0·08	10·06 ± 0·32	20·43 ± 0·65
	0·1	7·05 ± 0·34	12·58 ± 0·69	15·15 ± 0·83
	0·3	5·52 ± 0·11	10·29 ± 0·23	12·39 ± 0·69
	1·0	6·19 ± 0·26	15·09 ± 0·40	15·66 ± 0·76
	3·0	6·52 ± 0·19	14·17 ± 0·20	20·61 ± 0·89
Control[d]		6·38 ± 0·63	8·57 ± 0·53	26·36 ± 1·42
Octyl glucoside	0·05	0·94 ± 0·09	2·06 ± 0·02	14·17 ± 1·29
	0·1	2·35 ± 0·06	4·73 ± 0·21	17·49 ± 2·97
	0·3	6·12 ± 0·33	8·35 ± 0·49	25·35 ± 1·97
	1·0	7·36 ± 0·10	12·74 ± 0·50	19·63 ± 0·89
	3·0	8·23 ± 0·44	13·00 ± 0·53	22·01 ± 0·96
Control[d]		4·87 ± 0·17	9·45 ± 0·21	16·15 ± 1·32
CHAPS	0·05	2·97 ± 0·12	4·95 ± 0·16	5·37 ± 0·56
	0·1	6·76 ± 0·07	10·41 ± 0·20	22·18 ± 0·45
	0·3	5·64 ± 0·15	11·95 ± 0·09	16·49 ± 1·76
	1·0	5·24 ± 0·08	11·18 ± 0·18	13·92 ± 0·23
	3·0	4·46 ± 0·05	11·31 ± 0·22	11·89 ± 0·28

Mouse liver microsomes were diluted to a final concentration of 4·0 mg mL^{-1} in 0·1 M Tris-HCl buffer pH 7·5 containing the indicated detergents at various concentrations. The mixtures were gently shaken for 1 h at 4°C and then the suspension was centrifuged at 100 000 *g* for 1 h at 4°C. The supernatant fractions and the resuspended pellets were assayed for esterase activity with different substrates. The esterase activity in the supernatant fraction at each detergent concentration is shown. [a]Values are means ± s.d. of four replicates. [b]Rates are expressed in μmol product formed min^{-1} (mg $protein)^{-1}$. *p*-Nitrophenyl acetate (*p*-NpAc); α-naphthyl acetate (α-NA). [c]Rates are expressed in nmol product formed min^{-1} (mg $protein)^{-1}$. [d]Specific activity for controls represents the activity in microsomes incubated in buffer without detergent at 4°C for 1 h and not re-centrifuged. No significant soluble activity was detected with any substrate when control microsomes were re-pelleted and the supernatant monitored. Most catalytic activity was recovered in the resuspended washed microsomes.

not interfere with the UV absorbance of monitoring proteins during purification are generally preferred. Both octyl glucoside and CHAPS appear to satisfy these criteria. These two detergents form small micelles, have high CMCs and are easily removed by simple methods such as dialysis. In addition, they are mild in their solubilizing effects on membrane lipids and integral membrane proteins and do not interfere with the absorbance at 280 nm (Hjelmeland & Chrambach 1984). The solubilized esterase proteins obtained after treatment of mouse liver microsomes with 1% octyl glucoside were characterized with a series of substrates and inhibitors (Huang et al 1996) and further purified using affinity chromatography (Shiotsuki et al 1994). Thus, both CHAPS and octyl glucoside are suitable detergents for releasing and solubilizing the functional membrane esterase proteins. Triton X-100 and Lubrol PX, on the other hand, have low CMCs, a tendency to form large micelles and are difficult to remove without using special resins. Triton X-100 is also known to interfere with protein analysis at 280 nm. The ease with which the liver microsomal carboxylesterases were solubilized by these detergents indicates that these enzymes are weakly bound to the inner surface of the vesicular membrane of the endoplasmic reticulum and remain active after liberation from the membrane.

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