[Chem. Pharm. Bull. 23(1) 240—242 (1975)]

UDC 577.15.04

Effect of Organophosphate Pesticide on Acid Cholesterol Esterase in Rat Liver

Mitsuo Nakagawa, Kayoko Ishikawa, Yasuo Suzuki and Mitsuru Uchiyama[1a]

Pharmaceutical Institute, Tohoku University[1]

(Received September 2, 1974)

Acid cholesterol esterase in the 9000 *g* precipitates of rat liver was stimulated by the addition of organophosphate pesticide, dimethyl dichlorovinyl phosphate (DDVP) *in vitro*. The major portion of the esterase in the 9000 *g* precipitates may be lysosomal esterase. The esterase activity was also stimulated by the osmotic treatment of the 9000 *g* precipitates as well as that in the presence of DDVP. However, the esterase activities in the osmotic treated-9000 *g* precipitates and the supernatant solution obtained by centrifugation after the osmotic treatment of the 9000 *g* precipitates were no longer stimulated by the addition of DDVP.

These results suggest that the stimulatory effect of DDVP may be due to the release of the esterase by the disruption of lysosomal membrane or the translocation of the esterase on lysosomal membrane from a bound type a free one.

Cholesterol ester hydrolyzing enzymes in mammalian liver have been demonstrated in various subcellular fractions.[2–6]

Deykin and Goodman[2] have partially purified a neutral cholesterol esterase from 10000 *g* supernatant fraction of rat liver, and also found cholesterol esterase activity with a somewhat acidic optimal pH in microsomal fraction. The acid cholesterol esterase with a optimal pH 4.5 has also been demostrated in rat and human liver lysosomal fractions.[4,6]

The present communication deals with the effect of dimethyldichlorovinylphosphate (DDVP) on acid cholesterol esterase in rat liver.

Table I shows the extent of hydrolysis of cholesterol palmitate in various subcellular fractions of rat liver. The highest hydrolysis of the cholesterol ester was obtained in the 9000 *g* precipitates. We have confirmed that the hydrolysis of cholesterol palmitate progresses linearly with up to 30 min under the present experimental conditions. Nilsson, *et al.*[6] recently reported that the major portion of the acid cholesterol esterase with a optimal pH of 4.5 is located in lysosomal fraction, and also that the enzyme activity can be almost sedimented by centrifugation at 11700 *g* for 35 min. Accordingly, the major portion of acid cholesterol esterase in the 9000 *g* precipitates may be lysosomal esterase.

Table II shows the effect of DDVP and osmotic treatment on acid cholesterol esterase in the 9000 *g* precipitates. It has been shown that the slightly acidic cholesterol esterase in microsomes is strongly inhibited by diisopropyl fluorophosphate (DFP).[2] However, acid cholesterol esterase in the 9000 *g* precipitates was stimulated by the addition of DDVP. Furthermore, the esterase activity in the 9000 *g* precipitates preincubated in distilled water at 37° for 10 min was also elevated as well as that in the presence of DDVP. However, the esterase activity in the 9000 *g* precipitates after the osmotic treatment was no longer stimulated by the addition of DDVP.

1) Location: *Aobayama, Sendai*; *a*) Present address: *National Institute of Hygienic Sciences, Kamiyoga-1-chome, Setagaya-ku, Tokyo.*

2) D. Deykin and D.S. Goodman, *J. Biol. Chem.*, **237**, 3649 (1962).
3) O. Stein, Y. Stein, D.S. Goodman and N.H. Fidge, *J. Cell. Biol.*, **43**, 410 (1969).
4) K.T. Stokke, *Biochim. Biophys. Acta*, **270**, 156 (1972).
5) K.T. Stokke, *Biochim. Biophys. Acta*, **280**, 329 (1972).
6) A. Nilsson, H. Norden and L. Wilhelmsson, *Biochim. Biophys. Acta*, **296**, 593 (1973).

To further investigate the stimulatory effect of DDVP on the esterase activity, the supernatant fraction obtained by centrifugation after the osmotic treatment of the 9000 *g* precipitates was incubated with DDVP (Table III). The esterase activity in the supernatant solution was not also affected by DDVP.

These results suggest that the stimulatory effect of DDVP on the esterase in the 9000 *g* precipitates may be due to the release of the enzyme from lysosomes by labilizing action of the agent as well as the observation by Barzu, *et al.*[7] in which several lysosomal enzymes are released by the treatment of tetraethyldithiopyrophosphate, rather than the direct action of DDVP to the esterase.

Table I. Cholesterol Esterase Activity in Various Subcellular Fractions of Rat Liver

Fractions	Hydrolysis of cholesterol ester, %
9000 *g* precipiattes	15.4
Microsomes	2.3
Cytoplasm	0

The incubation mixture contained 10 mg protein of each fraction, 5 mg of bovine serum albumin, 4 mμmoles of ^{3}H-cholesterol palmitate (0.1 μci) in acetone (20 μl). The final volume was adjusted to 1 ml with citrate buffer, pH 4.4. Incubation was carried out at 37° for 30 min under N_2. The values were calculated as (^{3}H-cholesterol/^{3}H-cholesterol+^{3}H-cholesterol palmitate)×100.

Table II. Effect of DDVP and Osmotic Treatment on Acid Cholesterol Esterase in the 9000 *g* Precipitates

Enzyme fraction	Hydrolysis of cholesterol ester, %	
	Expt. I	Expt. II
9000 *g* precipitates	16.5 (100)	20.6 (100)
9000 *g* precipitates+5×10^{-4}M DDVP	21.6 (130.9)	26.6 (129.1)
9000 *g* precipitates after Osmotic treatment	24.6 (149.1)	28.8 (139.7)
Osmotic treatment+5×10^{-4}M DDVP	23.7 (143.6)	27.6 (133.9)

The incubation conditions were the same as in Table I. As the osmotic treatment, the 9000*g* precipitates were suspended in distilled water and then incubated at 37° for 10 min under N_2. The protein content of the osmotic treated-precipitates was 10 mg per ml of incubation medium.

Table III. Effect of DDVP on Acid Cholesterol Esterase in the Supernatant Solution after Centrifugation of the Osmotic treated 9000*g* Precipitates

Enzyme fraction	Hydrolysis of cholesterol ester, %	
	Expt. I	Expt. II
Supernatant solution after Osmotic treatment	4.7	19.3
Osmotic treatment+5×10^{-4}M DDVP	4.3	19.0

The incubation conditions and osmotic treatment were the same as in Table II. The supernatant solution was obtained by centrifugation at 9000*g* for 30 min after the osmotic treatment of the 9000*g* precipitates. The protein contents of the supernatant solution used were 4 mg (Experiment I) and 10 mg (Experiment II) per ml of incubation medium. The enzyme preparations for Experiment I an II were different.

7) T. Barzu, B. Cuparencu and A. Hantz, *Biochem. Pharmacol.*, **22**, 185 (1973).

Experimental

Chemical——Cholesterol and ^{3}H-cholesterol were purchased from Kanto Chemical Co. (Tokyo, Japan) and Schwarz/Mann (N.Y., U.S.A.), respectively. Palmitic acid was obtained from Kao Soap Co. (Tokyo, Japan).

^{3}H-Cholesterol palmitate was chemically synthesized according to the method of Deykin and Goodman.[2] The synthesized ^{3}H-cholesterol palmitate gave a single of cholesterol ester by thin-layer chromatography on silica gel G plate with *n*-hexane–ethyl ether–acetic acid (70: 30: 1, v/v) as the developing solvent.

Preparation of Enzyme——Female albino rats of the Wistar strain, weighing 100 to 130 ***g*** were used. The animals were fed *ad libitum* on a commercial diet obtained from Oriental Yeast Co. (Tokyo, Japan). The rats were killed by cervical fracture and livers were excised and homogenized in 3 volumes of cold 0.25M sucrose solution. The homogenates were centrifuged at 1000 ***g*** for 10 min to sediment the nuclei and cell debris. The supernatant fraction was centrifuged at 9000 ***g*** for 30 min and the pellet was washed by a small amounts of 0.25M sucrose solution and then centrifuged as before. Microsomes were sedimented from the 9000 ***g*** supernatant fraction by centrifugation at 104000 ***g*** for 60 min and the soluble supernatant fraction was then removed. The pellet (microsome) was washed with 0.25M sucrose solution by the use of ultracentrifugal procedure.

Enzyme Assay——The incubation mixture contained 10 mg protein of each subcellular fraction, 5 mg of bovine serum albumin. 4 mμmoles (0.1 μci) of ^{3}H-cholesterol palmitate in 20 μl of acetone. In some experiment, DDVP was added to the incubation mixture. The final volume was adjusted to 1 ml with citrate buffer, pH 4.4, ionic strength 0.1. The samples were placed in 15 ml of screwcapped tubes, flushed with N_2, sealed and incubated at 37° for 30 min with mechanical shaking. After incubation, the extraction and separation of lipids, measurements of radioactivity and protein content were conducted as described previously.[8,9]

8) M. Nakagawa and T. Nishida, *Biochim. Biophys. Acta*, **296**, 577 (1973).
9) M. Nakagawa and M. Uchiyama, *Biochem. Pharmacol.*, **23**, 1641 (1974).