CATATAN SINGKAT

Oxydative desulfuration of [^{14}C]-fenitrothion by liver microsomes of some species of fishes

[Oksidasi desulfurasi [^{14}C]-fenitrothion pada mikrosom liver dari beberapa spesies ikan]

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Abstract

A study was made of the oxydative desulfuration of [^{14}C]-fenitrothion by liver microsomes of some species of fishes. The preliminary research had been carried out to obtain the optimal condition of fenitrothion assay of the liver microsomes of every test spesies, including : pH, temperature, pre incubation time, incubation time, trypsin inhibitor, NADH and NADPH effect. The hepatic microsomes of treated and control were subjected to fenitrothion oxydative desulfuration assay, protein and cytochrome P-450 content and another enzyme activities.

Key words: cytochrome P-450, enzyme activities, fenitrothion, oxydative desulfuration, fishes, microsomes, protein.

Abstrak

Suatu studi telah dilakukan tentang oksidasi desulfurasi dari [^{14}C]-fenitrothion yang berlangsung pada mikrosom liver dari beberapa spesies ikan. Penelitian pendahuluan telah dilakukan untuk mengetahui kondisi optimum pada suatu uji fenitrothion yang berlangsung pada liver dari spesies uji meliputi beberapa parameter seperti: pH, suhu, waktu pra-inkubasi, waktu inkubasi, inhibitor tripsin, serta pengaruh dari NADH dan NADPH. Mikrosom liver dari kontrol dan perlakuan telah digunakan pada uji oksidasi desulfurasi fenitrothion untik mendeterminasi kandungan protein dan sitokrom P-450.

Kata penting: aktivitas enzim, fenitrothion, ikan, mikrosom, oksidasi desulfurasi, protein, sitokrom P-450.

Introduction

One of the most important roles of the fish liver is to detoxicate various foreign compounds which could exert toxicity to animals. The liver takes in these compounds from the portal vein and oxidized many of them to more polar metabolites, which are further metabolized by epoxide hydrate and/or conjugating enzyme to water soluble metabolites to be excreted out of the fish body safely and rapidly (Lumban Batu, 1991).

Liver microsomal cytochrome P-450 is a key enzyme which participates in the initial step of this detoxication process, and it was expected that the fish microsomes had a function of the oxydation of drugs (Lumban Batu, 1992).

The organophosphorous compounds one major group insectisides, and a certain portion there of may be transported to the aquatic environment resulting either from the actual use on paddy fields or from the unavoidable transmittance to waterways.

Fenitrothion [Sumithion, 0,0-dimethyl O-(3-methyl 4- nitrophenyl) phosphorothioate is a broad-spectrum insecticide used extensively throughtout the world for the control of agricultural and forest pest, and since it is rather highly toxic to some aquatic organism.

By the introduction of a methyl group to methyl parathion of the m – position in the benzene ring, the toxicity of product, i.e. fenitrothion

to mammals decreased by the factors of between 4.4 to 54,3 compared with that of methyl parathion in oral administration, where as the toxicity to fishes and shell did not so differ (Lumban Batu, 2001a, 2001b).

The present study was undertaken to clarify the relation of drug-metabolizing enzyme activities and fenitrothion metabolites formation activities, in order to confirm the toxicant resistant of fishes to fenitrothion and to discuss the interelationship between the fish fenitrothion treated liver microsomes.

Materials and methods

Special chemicals

In this investigation radioactive [ring-U-^{14}C] FS was utilized. The specific activity of the preparation was 30 µCi/ml supplied by the Institute for Biological Science Sumitomo Chemical Co. Ltd. Non radioactive fenitrothion (FS) metabolite (fenitrooxon (FO), des-methyl fenitrothion (DMFS), des-methyl fenitrooxon (DMFO) and 3-methyl-4 nitrophenol (3-M-4-NP) were offered by the Institute. Glucose-6-phosphatase dehydrogenase (G6PDH), glucose-6-phosphatase (G-6-P), nicotinamide adenin dinucleotide (NADH), nicotinamide adenin dinucleotide phosphatase hydrogenase (NADPH) were all from Sigma Chemical Co., USA, and the other reagent were of the highest grade commercially available.

Animals

Common carp, *Cyprinus carpio* of both sexes were purchased from fish farmer, and aclimatized in aquarium at least for one week prior to use for the experiment. Rainbow trout, *Salmo gairdneri*; redsea bream, *Chrysophrys major*; yellow tail, *Seriola quinquerdinata*; eel, *Anguilla japonica*; nile tilapia, *Oreochromis niloticus*; and ayu, *Plecoglosus altivelis* also used in this study.

Preparation of microsomal fraction

All of these fish studied were killed by a blow to the head, and livers were removed immediately, cut into slices and washed with ice-cold 0,9% NaCl solution, removing the connective tissues.

The livers slices were homogenized with 9 volumes of ice-cold 0.25 M succrose-10 mm Tris (hydroxymethyl) amino methane (Tris)-HCl buffer solution (pH 7.5) by a Potter-Elvehjem type glass-teffon homogenizer. The homogenate was centrifuged at 10,000x g for 15 min, and the resulting supernatant was sentrifused at 100,000x g for 60 min. All the procedures for microsome preparation were performed at 0 to 5 ^{o}C (Lumban Batu, 1998).

The microsomal pellets obtained were resuspended in the adequate amount of 0.25 M Succrose-10 mM Tris-HCl buffer solution (pH 7.5), containing 0.1 mM EDTA, adjusting the final concentration equivalent to 0.25 g wet liver per ml. The microsomal preparations were subjected to fenitrothion oxidative desulfuration activities (Lumban Batu, 2001).

Fenitrothion oxidative desulfuration assay was performed by the procedure shown in Fig. 1. [^{14}C]-FS benzene extractable was streated onto a precoated Merck Silica Gel Plates, layer thickness 0.25 mm, Merck 60 F_{254} Co-Chromatography (TLC); and then, was developed with two types of developer systems: I. Toluene-ethylformate-formic acid=(5:7:1 v/v) and II. Ethyl acetate-benzene: (1:4 v/v).

The plate was inspected under UV light for fluorecent band, which were marked, and cut off. Fractions of FS, FO, DMFS and 3-M-4-NP

cut off and put into vials for scintillation counting with 15 ml of ACS II scintillation (Amersham) as a counting medium. And then, subjected to the measurement of their radioactivity by liquid scintillation counter (ALOKA, LSC-900, LSC).

The amount of [^{14}C]-FS and its metabolite found in its benzene-extractable were calculated from their radioactivities on the basis of the specific activity of [^{14}C]-FS (46 dpm mol^{-1}) used in this experiment.

Results

The preliminary research had been carried out to obtain the optimal condition of assay of liver microsomes of every test species, including: pH, temperature, incubation time, pre-incubation time, trypsin inhibitor, NADH and NADPH effect. Requirements for microsomal fenitrothion oxidative desulfuration activity in liver are given in Table 1.

Figure 2 shown the rate of formation of microsomal metabolites in the presence of NADPH. The formation of FO was almost linear from 0 to 20 min and from 21 to 40 min was almost constant, while the rate of formation of FO decreased after 41 min.

Under in vitro condition the metabolism of [^{14}C]-FS was examined. During the incubation for 20 min, FS was metabolized in liver microsomes through oxidation immediately and its metabolites was enzymatically formed. Activities of FS and its metabolites of liver microsomes of some species of fish shown in Figure 3.

Discussions

Enzyme activity was reduced appreciably by omission of NADH and NADPH but clearly that the native protein, oxygen and NADPH are required for full activity in liver microsomes. Substitution of chemically reduced NADH for the NADPH generating system stimulated activi-

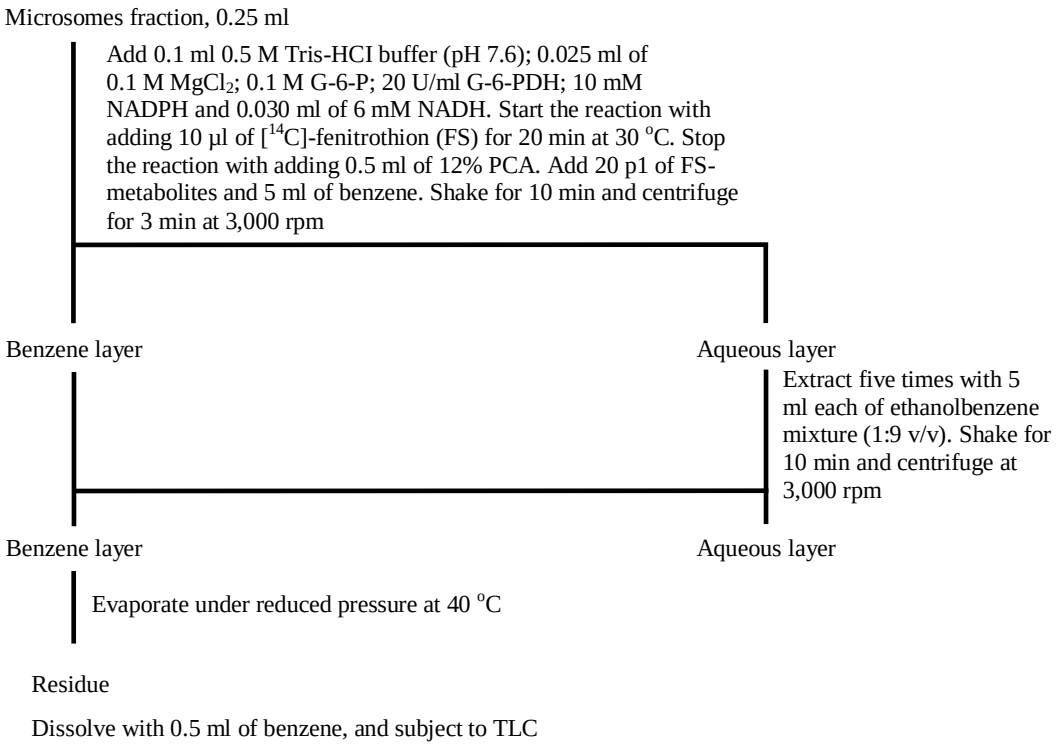

Figure 1. Oxydative desulfuration of [^{14}C]-fenitrothion by microsomes

Table 1. Effect of reduced pyridine nucleotide on oxidative desulfuration of fenitrithion by carp liver microsomes

Treatment	Activity (ρmol mg^{-1}-P min^{-1})			
	A	%	B	%
NADPH (25 µl) + NADH (30 µl)	36	100	65	100
NADPH (25 µl) + buffer (30 µl)	24	66	48	74
NADP (25 µl) + NADH (30 µl)	33	92	59	92
NADP (25 µl) + buffer (30 µl)	23	64	52	80
NADH (30 µl) + buffer (25 µl)	9	25	14	22
Buffer (55 µl)	2	5	4	6

Note:
1. All of treatment received FS in 10 µl acetone solution; 0.025 ml of 0.1 MgCl2; 0.1 M G-6-P; 20 U/ml G6PDH; 10 mM NADPH and of 6 mM and 0.25 ml carp liver microsomes, NADH.
2. Activity is ρmol of FO product formed/mg-P/min.
3. The incubations were carried out at 30 C for 20 minutes
   A: Without pre-incubation time,
   B: With 5 min per-incubation

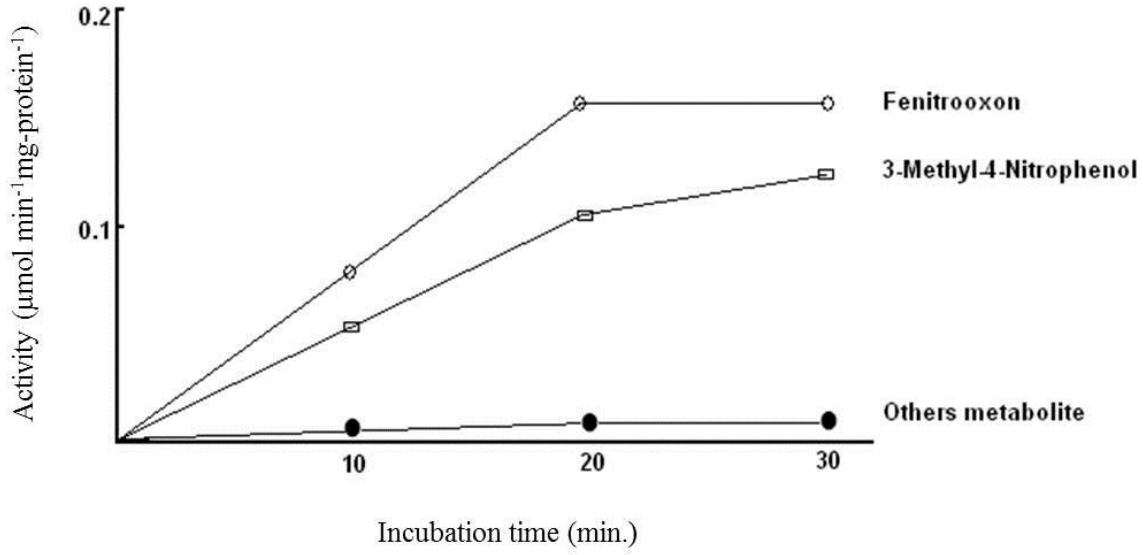

Figure 2. Role of formation of fenitrooxon, 3-methyl-4-nitrophenol and other metabolite by liver microsome of carp

ty considerably, confirming the reguirement for reduced form of the coenzym. Preformed NADH or a NADH generating system could not replace NADH, optimal level of NADPH and NADH for maximum fenitrothion oxidative desulfuration activity were 10 mM and 6 mM, respectively. The isocitric dehydrogenase NADPH-generating system was somewhat more effective than the other in the microsomes preparations (Tabel 1).

The effect of pH on fenitrothion oxidative desulfuration activity by carp liver microsomes was confirmed. Some differences in pH dependence were seen with the fish liver microsomes using different species. The most studied activity was fenitrothion oxidative desulfuration which showed the pH optimum for this desufuration was 7.6 in Tris-HCl buffer. The temperature optima for fenitrothion oxidative desulfuration ac-

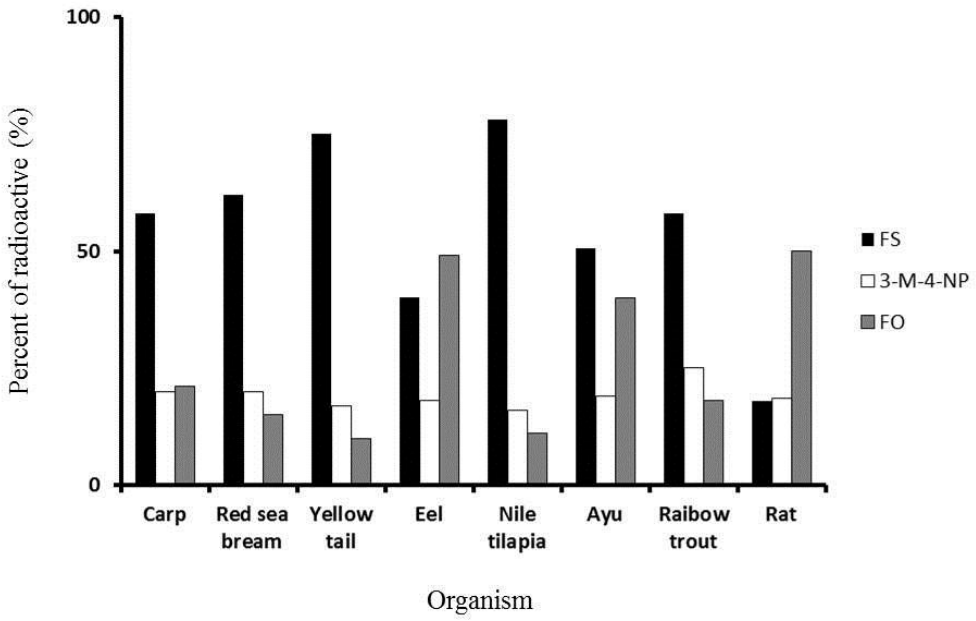

Figure 3. Percent of radioactives of fenitrothion ad its metabolites in fishes and rat liver microsomes

tivity and NADPH-cytochrome-c-reductase were quite similar, near 30 ^{o}C. Extending the incubation time for fenitrooxon oxidative desulfuration activity for 1 hr, confirmed a temperature optimum near 30 ^{o}C for this activity. The thermal lability of carp hepatic cytochrome P-450 systems was assesed with benzo(a)pyrene hydroxylase (Lumban Batu, 2010). There was marked inactivation by incubation for 20 min at temperature above 40 ^{o}C, and at 43 ^{o}C only 30% of original activity remained, indicating clearly that the system in carp hepatic microsomes was quite susceptible to thermal inactivation, more so than in mammals.

On the other hand, the activity of FS formation decreased with prolonged of the incubation time. The activities formation of DMFS and DMFO were also increased with the incubation time. On the contrary to those metabolite, the activity formation of FO was decreased at 80 min of incubation time. This results suggest that the degradation pathway of FS in the liver microsomes of the carp to yield DMFS, FO to yield DMFS, involving to 3-M-4-NP from them. Both FO and DMFO were yield to 3-M-4-NP. Therefore, degration pathways of FO in carp liver microsomes is presumed as follows: FO-DMFO-3-M-4-NP and FO-3-M-4-NP. 3-Methyl-4 Nitrophenol was found as a major metabolites for red sea bream (21.57%); yellow tail (16.70%); ayu (21.55%); and rainbow trout (18.63%), but for carp, eel and rat, FO was found as a major metabolites. The major metabolites of FS in eel was FO (45.61%), this percentage was found a little less than rat (47.60%). On the other hand, the methylated metabolites (DMFS about 2% for carp and ayu, and less than 1% for the other species and DMFO was less than 1%).

Disappearance of FS and formation of its metabolite under in vitro condition of some fishes liver microsomes can performed oxidative desulfuration of FS to FO, O,O-dimethyl O-(3-methyl-4-nitrophenol-phosphate), oxydation of the m-methyl group of FO in the presence of NADPH and hydrolytic cleavage of FO to 3-M-4-NP with Mg^{2+} ion as a cofactor. In the preliminary investigations was found that NADPH was the best cofactor compared with NADH.

The FS oxidation activity was substantially increased when the NADP was replaced by an equal amount of NADPH. Thus, NADPH and NADP gave an activity some 10% higher than that observed with the quantity as NADP and NADPH, in both 5 min pre-incubation and without pre-incubation time.

The difference in the rate of formation of FO and 3-M-4-NP may be mediated by many types of cytochrome P-450, one type of this responsible for the FO formation and the other type for 3-M-4-NP formation. The two major metabolic reaction of FS were:

1. The arylation metabolism resulting in the splitting of the P-O aryl bond of FS and FO to give 3-M-4-NP and
2. Dealkylation metabolism resulting in the cleavage of the CH_3-O-P alkyl bond of FS and FO to give the corresponding desmethyl metabolites (DMFS and DMFO).

The result of this study sugggest that these seven species of fishes studied can oxidized FS by drug oxydative system on their liver microsomes, as rat do, but at much lower.

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