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SHORT COMMUNICATIONS

N-Deacetylase activity in subcellular fractions of mouse liver

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It is generally known that various aromatic amines and drugs are metabolized via acetylation [1, 2]. Conversely, deacetylations are commonly observed with aromatic arylacetamides and drugs. A mammalian liver *N*-deacetylase enzyme has been studied in mouse, rat, hamster, guinea pig and dog [3–6]. This enzyme is responsible for *N*-deacetylation of various aromatic arylacetamides such as 4-acetylaminobiphenyl, 2-acetylaminofluorene and 2-acetylaminonaphthalene, and drugs such as acetanilide and phenacetin [6–8]. It was reported that the *N*-deacetylase enzyme activity is present mostly in the microsomal fraction of liver from various animal species. However, quantitative data on relative *N*-deacetylase enzyme activity in various subcellular fractions of mouse liver, when the monoarylacetamide or the diarylacetamide are substrates, are still lacking. This communication presents the results of studies on the intracellular distribution of *N*-deacetylase enzyme activity in mouse liver *in vitro*, using 2-acetylaminofluorene and 2,4-diacetylaminotoluene as substrates.

Female Swiss albino mice (30–35 g), obtained from the Animal Production Center, Faculty of Science, Mahidol University, Bangkok, Thailand, were used. The animals were housed in groups of five in aluminum tubs and maintained on a 12-hr (6:00 a.m. to 6:00 p.m.) light schedule at 25°. Water and rat chow (Gold Coin Ltd., Singapore) were provided *ad lib.* Prior to the start of the experiments, there were fasted overnight with water *ad lib.*

2,4-Toluenediamine was purchased from the Aldrich Chemical Co., Milwaukee, WI, and purified before use. 4-Acetylamino-2-aminotoluene, 2-acetylamino-4-aminotoluene and 2,4-diacetylaminotoluene were prepared in this laboratory [9]. 2-Acetylaminofluorene was purchased from the Sigma Chemical Co., St. Louis, MO. 2-Aminofluorene was obtained from Dr. Elizabeth K. Weisburger, Carcinogen Metabolism and Toxicology Branch, National Cancer Institute, Bethesda, MD. All other chemicals were reagent grade.

Liver subcellular fractions were prepared from female Swiss albino mice according to the modified methods of Jarvinen *et al.* [5] and Lower and Bryan [6]. The incubation mixture contained 0.5 μmole of substrate (2-acetylaminofluorene or 2,4-diacetylaminotoluene) dissolved in 0.5 ml of 50% methanol, 1.0 μmole Tris–HCl buffer (pH 7.0 for 2,4-diacetylaminotoluene and pH 7.4 for 2-acetylaminofluorene at 37°), and tissue preparation equivalent to 50 mg wet wt of liver. After incubation of the mixture at 37° for 30 min, or longer in certain instances, the reaction was terminated by the addition of 2.0 ml of a solution of *p*-dimethylaminobenzaldehyde (500 mg in 50 ml ethanol diluted with 50 ml of 1 M acetate–HCl buffer at pH 1.4). The terminated incubation mixtures were centrifuged at 15,000 *g* for 10 min in a Sorvall RC2-B centrifuge at ambient temperature and allowed to sit for an additional 20 min. The optical densities of the supernatant fractions were read at 455 nm. The amount of protein in the subcellular fractions was determined by the method of Lowry *et al.* [10]. Enzyme activity was estimated by quantitating the amount of arylamine generated in the incubated mixtures. The products were chromatographed by thin-layer chromatography (t.l.c.).

The products of *N*-deacetylation of 2-acetylaminofluorene and 2,4-diacetylaminotoluene were identified on

Table 1. *N*-Deacetylation of 2-acetylaminofluorene (2AAF) and 2,4-diacetylaminotoluene (2,4DA) by subcellular fractions of mouse liver

	Enzymatic activity*			
	2-Aminofluorene (nmoles)		2-Acetylamino-4-aminotoluene (nmoles)	
Liver subcellular fraction	Per 50 mg wet wt liver in 30 min	Per mg protein in 60 min.	Per 50 mg wet wt liver in 30 min	Per mg protein in 60 min
Whole homogenate	83.4 ± 3.2	29.3 ± 2.1	102.4 ± 7.0	35.1 ± 2.7
Supernatant (10,000 *g*)	71.5 ± 4.9	32.5 ± 2.9	97.7 ± 5.9	34.3 ± 4.0
Nuclei and mitochondria	9.6 ± 0.6	29.2 ± 4.1	0	0
Cytosol	7.8 ± 0.5	4.9 ± 0.4	97.4 ± 7.8	43.7 ± 5.6
Microsomes (105,000 *g*)	37.4 ± 2.4	70.7 ± 9.3	6.3 ± 1.3	18.0 ± 1.7
Cytosol + microsomes	68.6 ± 3.5	67.6 ± 1.7		
Boiled cytosol† + microsomes	40.7 ± 1.5			
Cofactors‡ + microsomes	38.0 ± 2.0	79.7 ± 4.1		

* Enzyme was assayed using 2AAF and 2,4DA as substrates as described in the text. The enzyme activity was determined in terms of nmoles of 2-aminofluorene (2AF) and 2-acetylamino-4-aminotoluene (2MA) per 50 mg wet wt of liver formed in 30 min. All results are presented as means ± S.E. Each value is based upon four to eight determinations.

† Cytosol was boiled for 10 min.

‡ Cofactor premix consisted of 0.02 μmoles NADP, 0.25 μmoles G-6-P, 0.25 μmoles $MgCl_2$ and 0.03 Sigma units G-6-PDH.

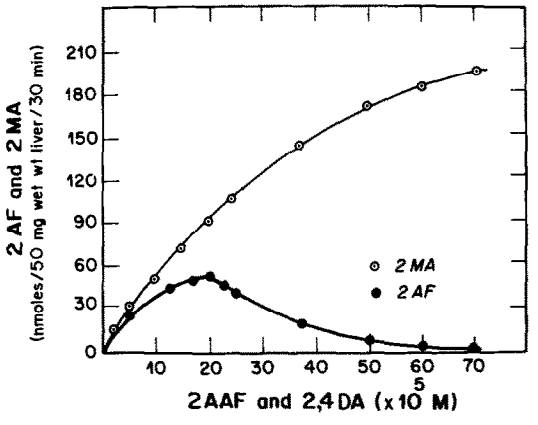

Fig. 1. *N*-Deacetylase enzyme activity of mouse liver cytosol and microsomes incubated with various concentrations of substrates. The enzyme activity was determined in terms of nmoles of 2-aminofluorene (2AF) and 2-acetylamino-4-aminotoluene (2MA) per 50 mg wet wt of liver in 30 min. The data are presented as mean values of the pooled samples from four animals. Abbreviations: 2AAF = 2-acetylaminofluorene, and 2,4DA = 2,4-diacetylaminotoluene.

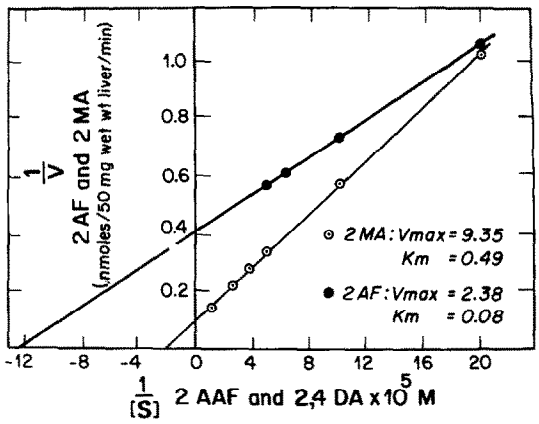

Fig. 2. Lineweaver–Burk plots for *N*-deacetylation of 2-acetylaminofluorene and 2,4-diacetylaminotoluene by mouse liver cytosol and microsomes. The enzyme activity was determined in terms of nmoles of 2-aminofluorene (2AF) and 2-acetylamino-4-aminotoluene (2MA) per 50 mg wet wt of liver in 30 min. The data are presented as mean values of the pooled samples from four animals; 1/V represents activity in nmoles per 50 mg wet wt of liver per min. Abbreviations: 2AAF = 2-acetylaminofluorene, and 2,4DA = 2,4-diacetylaminotoluene.

t.l.c. Under u.v. light, an ethyl acetate extract showed only one major spot which had an R_f value corresponding with that of 2-aminofluorene or 2-acetylamino-4-aminotoluene.

The subcellular distribution of *N*-deacetylase enzyme activity, when 2-acetylaminofluorene and 2,4-diacetylaminotoluene were substrates, is summarized in Table 1. With 2-acetylaminofluorene as substrate, most of the *N*-deacetylase activity was in the supernatant fraction. After further separation of the supernatant fraction, *N*-deacetylase combined activity in the cytosol and microsomes, when assayed separately, was only 63.2 per cent of the supernatant fraction. However, after recombination of the cytosol and microsomes, there was an increase in the *N*-deacetylase activity to the value of the supernatant fraction, whereas recombination of the boiled cytosol and microsomes caused no significant change ($P > 0.05$) in *N*-deacetylase activity from the value in microsomes. Microsomal *N*-deacetylase enzyme activity assayed in the presence of cofactors was not changed.

N-Deacetylase enzyme activity with 2,4-diacetylaminotoluene as substrate was somewhat different from the activity with 2-acetylaminofluorene. *N*-Deacetylase enzyme activity in whole homogenate was higher (19.0 per cent) when 2,4-diacetylaminotoluene was used as a substrate; most of the enzyme activity was localized in the supernatant fraction as well as the cytosol. Microsomes had a small amount of *N*-deacetylase enzyme activity (6.5 per cent). 2-Acetylamino-4-aminotoluene production by *N*-acetylase in the microsomes to incubation time up to 50 min, whereas 2-aminofluorene production by *N*-deacetylase in the cytosol was gradually increased with a longer incubation time. Accordingly, it was found that 2-acetylamino-4-aminotoluene was a major product of 2,4-diacetylaminotoluene, though the incubation time was continued for 60 min at pH 7.0 and for 30 min at pH 4.0 to 9.0. The effect of pH on the *N*-deacetylase enzyme activity in the cytosol and microsomes was also observed. The optimum pH for *N*-deacetylase enzyme activity in the microsomes was 7.4 and the activity decreased sharply to about 95 or 20 per cent at pH 9.0 and 4.0, respectively; the optimum pH for *N*-deacetylase in the cytosol was 7.0 with a gradual decrease of the enzyme activity to approximately 90 or 75 per cent at higher or lower pH values, respectively.

In Figs. 1 and 2, *N*-deacetylase enzyme in the cytosol and microsomes was incubated with various concentrations of 2-acetylaminofluorene and 2,4-diacetylaminotoluene, and the relationships between reaction velocity and substrate concentrations were plotted by the method of Lineweaver and Burk [11]. *N*-Deacetylase enzyme in the microsomes was inhibited by a substrate of 2-acetylaminofluorene at concentrations higher than 2.0×10^{-4}M. *N*-Deacetylase enzyme in the microsomes was found to have an apparent K_m (0.8×10^{-6}M) for 2-acetylaminofluorene lower than that of the *N*-deacetylase enzyme in the cytosol for 2,4-diacetylaminotoluene (K_m of 4.9×10^{-6}M).

2-Acetylaminofluorene was mostly *N*-deacetylated by mouse liver microsomes to 2-aminofluorene. This microsomal *N*-deacetylase enzyme did not require cofactors. In addition, higher concentrations of the cofactors inhibited the *N*-deacetylase enzyme activity (unpublished data). These results are similar to those in a previous report in which dog and rodent microsomal *N*-deacetylase enzyme did not require cofactors [6]. However, *N*-deacetylase enzyme activity was increased to the value in the supernatant fraction when the cytosol was added to the microsomes, but no change in activity was observed with boiled cytosol. These findings indicate that there may be a cofactor present in the cytosol that is required for the greater activity of this microsomal *N*-deacetylase enzyme. In view of the thermolability of the cofactor, it may be a peptide. However, identification of this cofactor requires further investigation.

2-Acetylamino-4-aminotoluene was a major product formed by *N*-deacetylation of 2,4-diacetylaminotoluene in mouse liver cytosol. These results indicate that the 2,4-diacetylaminotoluene in *N*-deacetylated to a greater extent at the *p*-position than at the *o*-position. Conversely, it was reported recently that mouse liver cytosol could also *N*-acetylate 2,4-diaminotoluene to a greater extent at the *p*-position than at the *o*-position [12]. However, other products, such as 4-acetylamino-2-aminotoluene and 2,4-diaminotoluene, were probably formed *in vitro* to such a small extent that they could not be detected by thin-layer chromatography.

In the present experiment, 2-acetylaminofluorene was *N*-deacetylated primarily by mouse liver microsomes, whereas 2,4-diacetylaminotoluene was *N*-deacetylated primarily by mouse liver cytosol. These finding suggest that mouse liver has at least two *N*-deacetylase enzyme systems, a microsomal *N*-deacetylase enzyme which is more specific for the monoarylacetamide and a cytosol *N*-deacetylase enzyme which is more specific for the diarylacetamide. Furthermore, formation of the products was directly proportional to the incubation times up to 60 min only for 2,4-diacetylaminotoluene. The effect of pH on *N*-deacetylase activity was greater for 2-acetylaminofluorene. In addition, the K_m of *N*-deacetylase enzyme for 2-acetylaminofluorene (0.8×10^{-6}M) was lower than that for 2,4-diacetylaminotoluene. These results, therefore, support the possible conclusion that there are two separate *N*-deacetylase enzyme systems in mouse liver.

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Department of Physiology, Faculty of Science, Mahidol University, Bangkok 4, Thailand

THIRAYUDH GLINSUKON
AURANUCH LOOKYEE
CHAIVAT TOSKULKAO
RONALD C. SHANK*

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* Visiting Professor, Department of Physiology, Faculty of Science, Mahidol University, Bangkok 4, Thailand, during the month of February, 1978. Current address: Department of Community and Environmental Medicine, California College of Medicine, University of California in Irvine, Irvine, CA 92717, U.S.A.

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Induction of the hepatic cytochrome P-450-dependent mono-oxygenase system in young and geriatric rats

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It has long been recognized that aging is associated with alterations in pharmacological variables which may be responsible for changes in the susceptibility to toxic effects of xenobiotics in senescent animals [1–3]. Investigations of drug metabolism *in vivo* have demonstrated that metabolic efficiency of rodents declines with advancing age [4–6]. Moreover, an age-related decline in metabolic activities has been observed in *in vitro* studies of the metabolism of a number of xenobiotics in hepatic microsomal preparations from rats of increasing age [7–9]. This decrease in activity has been shown to parallel the apparent decrease in the levels of components of the hepatic cytochrome P-450-dependent mixed function oxygenase (MFO) enzyme system [10]. Indeed, the decrease in drug-metabolizing activity in geriatric animals has been attributed specifically to an age-related loss of hepatic microsomal NADPH-cytochrome *c* reductase activity [6]. In contrast, Birnbaum and Baird [11] have reported no differences in NADPH-cytochrome *c* reductase activity in young and old rats.

The activities and components of the hepatic MFO enzyme system are sensitive to a wide variety of agents, being induced by a number of chemicals (typically the barbiturates and polycyclic hydrocarbons). However, controversy exists in the literature with regard to the inducibility of this enzyme system in senescent rats. Kato and Takanaka [10] showed that phenobarbital-induced elevation of MFO activities was markedly higher in young rats than in senescent rats. Recent reports, however, demonstrated no such age-related differences in the sensitivity of the functional components of the hepatic microsomal drug-metabolizing enzyme system following either phenobarbital or 3-methylcholanthrene treatment [6, 11].

The hepatic MFO enzyme system is a major determinant of toxicity of drugs and environmental agents. It is important, therefore, to define clearly any age-related differences in the responses of this enzyme system to xenobiotics, since such age-related differences may be a determining factor in the altered susceptibility to toxic effects of foreign chemicals in senescence.

In this paper, the effects of phenobarbital (PB) and β-naphthoflavone (BNF) on the induction of the hepatic MFO enzyme system were examined in both young adult and geriatric rats. BNF is a non-carcinogenic inducer which has inductive properties similar to 3-methylcholanthrene [12].

Male Fischer 344 rats (young adults, 10 weeks; geriatrics, 100 weeks) were housed in the same environmentally controlled animal room (20°, 50% humidity and 12-hr light cycles) and maintained *ad lib.* on Purina Laboratory Chow and tap water. The animals were treated for 4 consecutive days with daily i.p. doses of either PB (80 mg/kg) in 0.9% (w/v) saline (1 ml/kg) or BNF (80 mg/kg) in corn oil (10 ml/kg), and appropriate controls were treated in parallel. The animals were starved for 24 hr prior to being