

Department of Chemistry,
Faculty of Science,
The University of Tokyo
Bunkyo-ku, Tokyo

TATSUSHI MURAE
TAKAHIKO TSUYUKI
TAKEYOSHI TAKAHASHI

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Substrate Induced Difference Spectrum of Microsomal P-450 in Human Liver

Substrate-P-450 difference spectra, which are induced by addition of various substrates into suspension of liver microsomes, have been reported to classify into two or three groups. In rat liver microsomes, hexobarbital, aminopyrine and SKF525-A cause type I difference spectrum and aniline causes type II difference spectrum.¹⁻³⁾

Schenkman, *et al.*⁴⁾ and Kitagawa, *et al.*⁵⁾ have reported that hexobarbital shows similar type II difference spectrum when it is added into microsomes treated with 3-methylcholanthrene.

Additionally, the author, *et al.*⁶⁾ has reported that hexobarbital shows similar type I spectrum when low concentrations of the substrate are used and similar type II spectrum when rather high concentrations of the substrate are employed in 3-methylcholanthrene treated microsomes.

Studies on some activities of drug-metabolizing enzymes in human liver have been realized by Kuntzman, *et al.*,⁷⁾ Creaven, *et al.*,^{8,9)} and the authors.¹⁰⁾

In the present paper, we wish to report substrate induced P-450 difference spectra in human liver microsomes.

Adult human livers which were isolated in judicial or administrative dissection within 24 hr after death were used, and they were from individuals without any history of drug poisoning or liver illness. About 50 g of the livers were sliced and washed more than three times with ice-cold 1.15% KCl solution to remove blood. The slices were homogenized with 2 volumes of ice-cold 1.15% KCl solution. The homogenate was centrifuged at -2.0° — 2.0° , $9000 \times g$ for 20 min. The supernatant was recentrifuged at $105000 \times g$ for 1 hr, and the microsomal pellet was suspended in 0.1M phosphate buffer pH 7.5. The suspension was used for measurements of P-450 content and P-450-substrate difference spectra.

P-450 content was measured by the method according to Omura, *et al.*¹¹⁾ Microsomal protein was determined according to the method of Lowry, *et al.*¹²⁾

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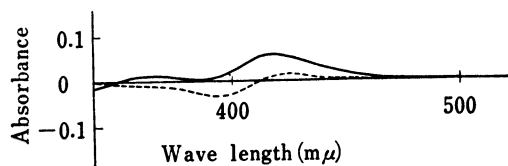


Fig. 1. Aniline Difference Spectrum with Microsome from Human Liver

(protein concentration: 2.97 mg/ml, P-450: O.D. 450—490 $m\mu$ 0.0125)
 -----: 2.67 mM, ———: 6.67 mM

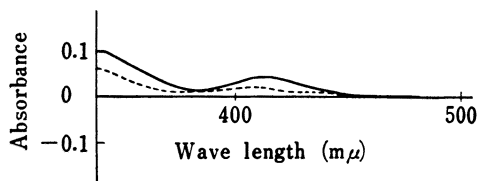


Fig. 2. Hexobarbital Difference Spectrum with Microsome from Human Liver

(protein concentration: 2.69 mg/ml, P-450: O.D. 450—490 $m\mu$ 0.0125)
 -----: 1.0 mM, ———: 5.0 mM

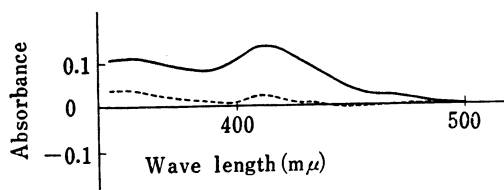


Fig. 3. Aminopyrine Difference Spectrum with Microsome from Human Liver

(protein concentration: 2.69 mg/ml, P-450: O.D. 450—490 $m\mu$ 0.0125)
 -----: 1.0 mM, ———: 5.0 mM

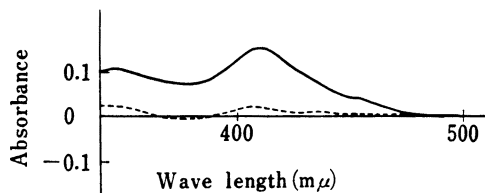


Fig. 4. SKF 525-A Difference Spectrum with Microsome from Human Liver

(protein concentration: 2.69 mg/ml, P-450: O.D. 450—490 $m\mu$ 0.0125)
 -----: 0.05 mM, ———: 0.5 mM

As shown in Fig. 1 and Fig. 2, aniline-P-450 difference spectrum in human liver microsomes was type II, and this was similar to that obtained in rat liver microsomes. However, hexobarbital induced difference spectrum was type II in human liver microsomes, and this was different from that in rat liver microsomes. The type II difference spectrum induced by hexobarbital had an absorption peak at about 412 $m\mu$ and a trough at about 380 $m\mu$. Similar type II difference spectrum was also observed when aminopyrine and SKF525-A were used as substrates (Fig. 3, Fig. 4). These type II difference spectra in human liver microsomes were observed in 3 samples employed. A possible interaction of hemoglobin remained in liver microsomal fraction was examined using standard human hemoglobin at a concentration of 0.05 mg per ml. Hemoglobin did not show similar difference spectra when hexobarbital, aminopyrine and SKF525-A were used. Therefore, type II difference spectra in human liver microsomes induced by hexobarbital, aminopyrine and SKF525-A seemed to be caused not by hemoglobin but by P-450.

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*Department of Biochemical Pharmacology,
 Faculty of Pharmaceutical Sciences,
 University of Chiba
 1-33 Yayoi-cho, Chiba-shi, Chiba*

TETSUYA KAMATAKI
 MITSUKAZU KITADA
 HARUO KITAGAWA

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