

Studies on the P-450 Difference Spectra induced by Lysergic Acid Diethylamide in Rat Liver Microsomes¹⁾

TETSUKICHI NIWAGUCHI and TAKAKO INOUE

National Research Institute of Police Science²⁾

(Received November 15, 1974)

The nature of LSD-induced difference spectra in rat liver microsomes was investigated. The gradual shift from the type I spectrum to the modified type II spectrum with increasing concentration of LSD in hepatic microsomes suggested a composite change of two spectra. The type I component of LSD was obtained by subtracting the spectra in the presence of both the type I compound and LSD from the spectra of LSD alone. The spectral dissociation constant obtained from the spectra of the type I component of LSD was almost exactly equal to the K_m value obtained from the disappearance of LSD in the enzymatic transformation.

In the previous paper it was shown that *d*-lysergic acid diethylamide (LSD) was transformed to *d*-lysergic acid monoethylamide (LAE) and *d*-N⁶-demethyl-lysergic acid diethylamide (norLSD) by enzyme in hepatic microsomes supplemented with NADPH and oxygen,³⁾ and that the type of LSD-induced spectral change with rat hepatic microsomal cytochrome P-450 shifted gradually from the type I to the modified type II with increasing concentrations of LSD.⁴⁾ It is the purpose of the present study to clarify the relationship between metabolism by rat liver microsomes and LSD-binding with cytochrome P-450.

Experimental

Chemicals—LSD was prepared from *d*-lysergic acid by the method of Nakahara and Niwaguchi.⁵⁾ NADPH and hexobarbital were obtained from the Sigma Chemical Company and Dainippon Pharmaceutical Co., Ltd., respectively. SKF 525-A was kindly supplied by Professor Kitagawa, University of Chiba.

Preparation of Microsomal Fraction—Male rats weighing 120–150 g were killed by decapitation. Livers were quickly removed and homogenized with 2 volumes of ice-cold isotonic KCl solution in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at $9000 \times g$ for 20 minutes in a Marusan 50-B refrigerated centrifuge, and the supernatant was centrifuged at $105000 \times g$ for 60 minutes in a Marusan 50 S-2 ultracentrifuge. The pellet obtained was washed with isotonic KCl solution, and recentrifuged at $105000 \times g$ for 60 minutes. The microsomal pellet was resuspended in 0.1 M phosphate buffer pH 7.4 to give a concentration of 1 g of liver in 2 ml. All procedures were carried out at 0–4°. Microsomal protein was determined by the method of Lowry, *et al.*⁶⁾

Preparation of Incubation Mixture—Since maximal enzyme activity was obtained in the presence of NADPH, nicotinamide and $MgCl_2$ as cofactors in preliminary experiments, the incubation mixture contained 3 ml of microsomal fraction, 0.15–2.50 μ moles of LSD, 1 μ mole of NADPH, 100 μ moles of nicotinamide, 100 μ moles of $MgCl_2$, 0.5 ml of 0.8 M phosphate buffer pH 7.4, and water to make a final volume of 5 ml. Incubations were conducted in 30 ml Erlenmeyer flasks for 10 minutes at 37° with shaking.

Analytical Procedures—Unchanged substrate was extracted and determined by the method of Niwaguchi and Inoue, as previously described.^{3,7)} Difference spectra were obtained with a Shimadzu MPS-5000

- 1) A part of this work was reported at the 94th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, April 1974.
- 2) Location: *Sanban-cho, Chiyoda-ku, Tokyo.*
- 3) T. Niwaguchi, T. Inoue, and Y. Nakahara, *Biochem. Pharmacol.*, **23**, 1073 (1974).
- 4) T. Niwaguchi, T. Inoue, and Y. Nakahara, "Proc. 4th Symposium on Drug Metabolism and Action," ed. by H. Ozawa, Sendai, 1972, pp. 127–136.
- 5) Y. Nakahara and T. Niwaguchi, *Yakugaku Zasshi*, **94**, 407 (1974).
- 6) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
- 7) T. Niwaguchi and T. Inoue, *J. Chromatog.*, **59**, 127 (1971).

recording spectrometer. The same microsomal suspension (2 mg protein/ml) was divided into two cuvettes. LSD solution was added to the sample cuvette and equal volume of aqueous medium was added to the reference cuvette. The spectral dissociation constant, K_s was obtained as follows: the reciprocal of the difference of absorbance between a peak and a trough in each difference spectrum was plotted against the reciprocal of the concentration of LSD, and the x intercept was equivalent to $-(1/K_s)$.

Result and Discussion

Increasing concentration of LSD from 0.01 to 0.03 mM in the microsomal suspension enhanced the amplitude of the type I spectrum with peak near 385 nm and trough near 410 nm. When 0.05 to 0.10 mM LSD was added to the suspension, the wavelength of either the peak or the trough changed gradually to the modified type II spectrum as shown in Fig. 1 (Curves S_1). The amplitude of the modified type II spectra was enhanced with increasing LSD concentration. This finding was similar to the observation with agroclavine⁸⁾ or allyl-substituted barbiturates⁹⁾ interacted with rat liver microsomes. The gradual shift from the type I spectrum to the modified type II spectrum variety upon increasing

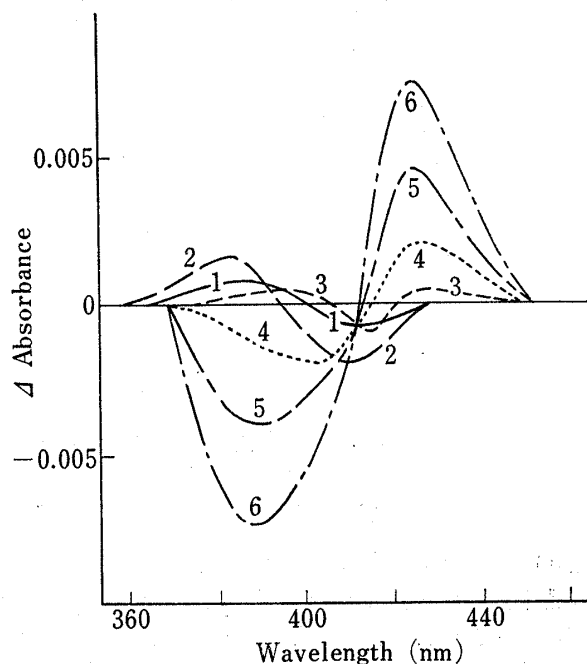


Fig. 1. P-450 Difference Spectra induced by Various Concentrations of LSD (Curves S_1)

microsomal protein: 2 mg/ml, concentration of LSD: 1) 0.01 mM; 2) 0.03 mM; 3) 0.05 mM; 4) 0.1 mM; 5) 0.3 mM; 6) 0.7 mM

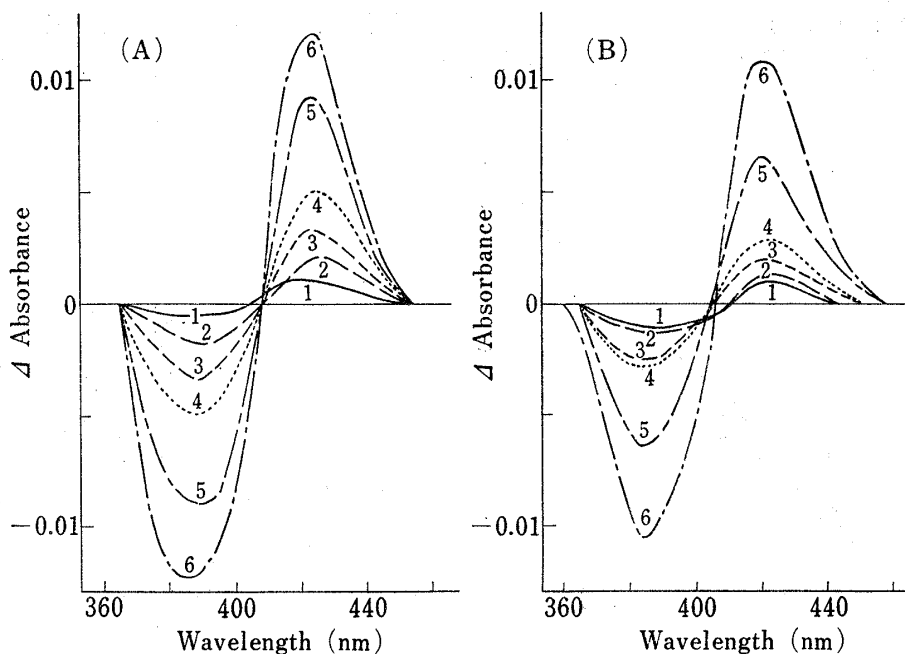


Fig. 2. Effect of the Type I Compound on the LSD-induced Spectral Change

(A) 0.2 μ M of SKF 525-A was used as the type I compound (Curves S_{2S}).

(B) 3.0 mM of hexobarbital was used as the type I compound (Curves S_{2H}).

Microsomal protein: 2 mg/ml, concentration of LSD: 1) 0.01 mM; 2) 0.03 mM; 3) 0.05 mM; 4) 0.1 mM; 5) 0.3 mM; 6) 0.7 mM

8) B.J. Wilson and S. Orrenius, *Biochem. Biophys. Acta*, **261**, 94 (1972).

9) I. Jansson, S. Orrenius, L. Ernster, and J.B. Schenkman, *Arch. Biochem. Biophys.*, **151**, 391 (1972).

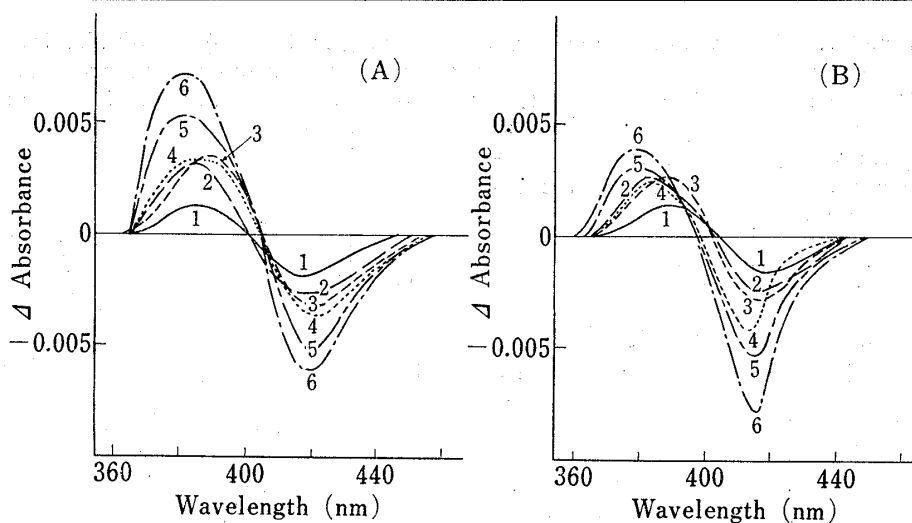


Fig. 3. The Type I Component of LSD-induced Spectral Change

(A) Curves S_{2S} were subtracted from Curves S_1 at each concentration of LSD (Curves S_{2S}).
 (B) Curves S_{2H} were subtracted from Curves S_1 at each concentration of LSD (Curves S_{2H}).

LSD concentration suggested that a composite change of two spectra might be present, and that the microsomal cytochrome P-450 in rat liver might have two or more binding sites to LSD. In order to confirm this possibility, LSD induced difference spectra with rat liver microsomes were measured using type I compounds such as SKF 525-A and hexobarbital. Prior to the addition of LSD to the sample cuvette, 0.2 μM of SKF 525-A, which was high enough to produce the type I spectral change, or 3.0 mM of hexobarbital, which caused the maximal type I spectrum, was added to both reference and sample cuvettes.

Under these conditions, the modified type II spectra were obtained even at less than 0.03 mM LSD, and the amplitude of the spectra was enhanced with increasing concentrations of LSD, as shown in Fig. 2 (Curves S_{2S} and S_{2H}). It was considered that the type I spectral component of LSD was eliminated by the addition of the type I compound to microsomes.¹⁰⁾

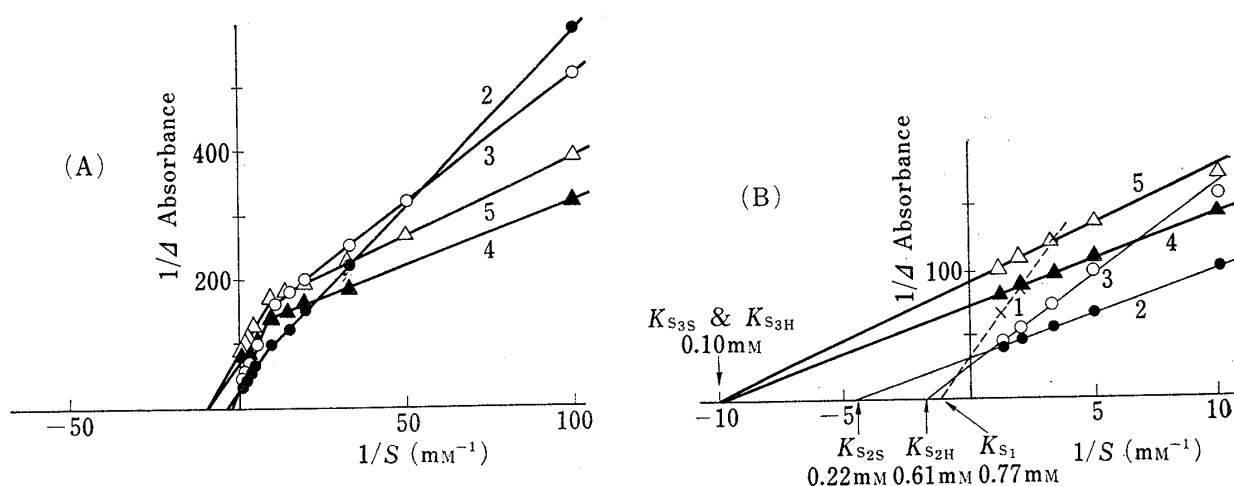


Fig. 4. Double Reciprocal Plot of the LSD-induced Spectral Change in the Presence and Absence of the Type I Compound

(A) full picture, (B) expansion of the higher LSD level
 1) obtained from Curves S_1 , 2) obtained from Curves S_{2S}
 3) obtained from Curves S_{2H} , 4) obtained from Curves S_{3S}
 5) obtained from Curves S_{3H} .

10) J.B. Schenkman, *Biochemistry*, 9, 2081 (1970).

When the spectra in the presence of both the type I compound and LSD were subtracted from the spectra obtained with the same concentration of LSD alone to find the difference between two spectra, the type I spectra were obtained as shown in Fig. 3 (Curves S_{3S} and S_{3H}).

The double reciprocal plots of the spectral changes at different substrate levels in the absence and the presence of the type I compound, were shown in Fig. 4. The same fashion was performed on the subtracted spectra. The spectra dissociation constants, K_{S1} , K_{S2S} , K_{S2H} , K_{S3S} and K_{S3H} were obtained from the curves S_1 , S_{2S} , S_{2H} , S_{3S} and S_{3H} , respectively. Of those, K_{S1} value was obtained from the spectra of LSD in concentrations high enough to cause the modified type II spectral change. It was considered that this K_{S1} value showed the apparent affinity of LSD to cytochrome P-450, while K_{S3S} and K_{S3H} values the affinity of LSD to the type I binding site, and K_{S2S} and K_{S2H} values reflected the affinity between LSD and binding site other than the type I.

The fact that the double reciprocal plots obtained from curves S_{2S} , S_{2H} , S_{3S} and S_{3H} were slightly convex suggested a facilitation of interaction by higher LSD concentration. Those K_s values were compared with K_m value for LSD determined from the disappearance of LSD in the transformation (Fig. 5). As the result, the K_m value (0.10 mM) showed no apparent relationship to the K_{S1} , K_{S2S} and K_{S2H} values (0.77, 0.22 and 0.61 mM), but was almost exactly equal to the K_{S3S} and K_{S3H} values (0.1 mM). This suggested that the type I component in cytochrome P-450-LSD complex might be rather active for the enzymatic transformation of LSD.

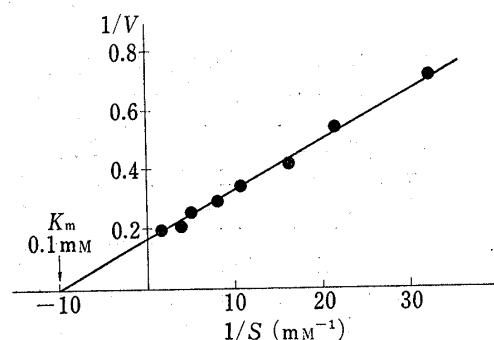


Fig. 5. Lineweaver-Burk Plot for the Determination of the Michaelis-constant

Metabolism rate of LSD was represented as nmoles LSD metabolized per mg microsomal protein per 10 min.