THE ENZYMATIC CONVERSION OF SULFOXIDE TO SULFONE : THE OXIDATION OF METHYL TETRAHYDROFURFURYL SULFOXIDE TO THE CORRESPONDING SULFONE BY RAT LIVER MICROSOMES

Takeshi Fujita and Suzuoki-Ziro
The Biological Research Laboratories, Research and Development Division, Takeda Chemical Industries, Ltd., Higashiyodogawa-ku, Osaka, Japan

Received August 4, 1967

Among many drug-metabolizing reactions catalyzed by hepatic microsomes (Gillette, 1966) have been demonstrated S-demethylation, S-methylation, desulfuration, and sulfoxide formation on sulfur-containing drugs (Mazel et al., 1964; Bremer and Greenberg, 1961; Spector and Shideman, 1959; Gillette and Kamm, 1960). However, little is as yet known of the enzymatic oxidation of sulfoxide to its sulfone. Recently Gerhards and Gibian (1967) have briefly reported dimethylsulfoxide is oxidized to dimethylsulfone by rat liver microsomes in the presence of NADPH and molecular oxygen. While studying the enzyme system involved in the metabolism of the ^{35}S-tetrahydrofurfuryl moiety of thiamine tetrahydrofurfuryl disulfide*, which is mostly excreted as the derivatives of S-methyl sulfoxide and sulfone in rat, rabbit and man (Suzuoki-Z. et al., 1967), we have established the occurrence of an enzyme reaction in rat liver microsomes which catalyze the oxidation of methyl tetrahydrofurfuryl sulfoxide (MTFSO) to the corresponding sulfone in the presence of both NADPH and oxygen. The present paper describes experimental evidence for the reaction and some properties of this enzyme system.

* A homologue of thiamine propyl disulfide, a derivative of thiol-type thiamines, whose propyl is replaced by tetrahydrofurfuryl group (see the Merck Index (7th ed.) and also the literature of Kawasaki (1963)).

Methods. Male Sprague-Dawley (JCL) rats, weighing 200 to 300 g, starved overnight were used in all experiments. The microsomes and other cell fractions were isolated by differential centrifugation from 0.25 M sucrose homogenates of liver by a modification of the method of Hogeboom et al.,(1948). Protein was determined by the method of Lowry et al., (1951). The sulfoxide oxidizing activity was measured by determining the radioactivity of ^{35}S-methyl tetrahydrofurfuryl sulfone ($MTFSO_2$) produced from ^{35}S-MTFSO. The standard incubation system contained homogenates or microsomes (ca. 4 to 6 mg protein), 5 mM ^{35}S-MTFSO (about 50,000 cpm), 100 mM Tris-HCl, pH 7.4, 100 mM phosphate, pH 7.4, 50 mM nicotinamide, 1 mM NADP and an NADPH-generating system consisting of 20 mM glucose 6-phosphate, 5 mM $MgCl_2$ and glucose 6-phosphate dehydrogenase sufficient to reduce 3 μmoles NADP per minute, in a total volume of 2.0 ml. The reaction started by addition of cell fractions was carried out for 1 hr under constant shaking in air at 30°C. The reaction mixture was extracted three times with 3 volumes of chloroform-methanol (5:1, v/v) after adding 4 mg of cold $MTFSO_2$ as carrier. The reaction product ^{35}S-$MTFSO_2$ and the unreacted substrate in the extract were mutually separated by thin layer chromatography (silica gel G; benzene-acetone-formic acid = 1:1:0.05, v/v/v). The radioactivity associated with the spot of authentic $MTFSO_2$ (Rf = 0.79) was determined in a Packard Tri-Carb model 3000 liquid scintillation spectrometer.

Results and Discussion. Under the standard assay conditions, the reaction proceeded linearly with time up to at least 1 hr and the rate was proportional to the amount of homogenates up to about 40 mg of protein per vessel (corresponding to 6 mg protein of microsomes). The reaction product was preliminarily identified as $MTFSO_2$ by thin layer chromatography using five different solvent systems. Decisive evidence for

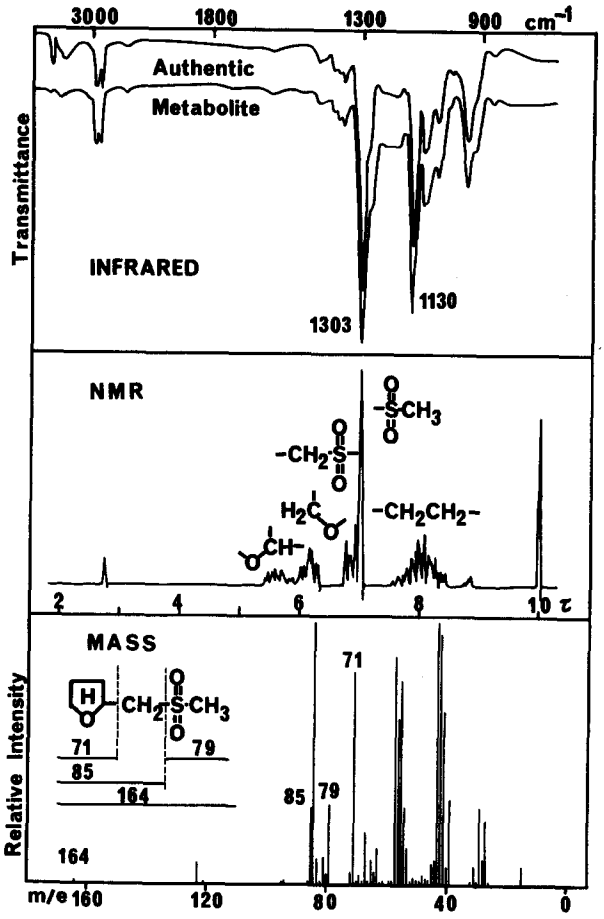

Fig. 1. Spectra of infrared, NMR, and mass of the isolated reaction product.

The incubation media consisted of 795 mg protein of microsomes, 32 mM ^{35}S-MTFSO (257,000 cpm), 0.5 M phosphate buffer, pH 7.4, 50 mM nicotinamide, 2.1 mM NADP, 5 mM $MgCl_2$, 59 mM glucose 6-phosphate and 30 units of glucose 6-phosphate dehydrogenase, in the total volume of 100 ml. The reaction mixture was incubated for 5 hr under constant shaking in air at 30°C, and extracted by chloroform-methanol (5:1, v/v). The reaction product ^{35}S-$MTFSO_2$ thus extracted was purified by silicic acid column chromatography followed by thin layer chromatography and finally crystallized from n-hexane containing a small amount of benzene. About 23 mg of purified ^{35}S-$MTFSO_2$ was obtained.

---

the identity of ^{35}S-$MTFSO_2$ was obtained by the isolation of the reaction product in large scale experiments (see legend to Fig. 1). The infrared, NMR, and mass spectra of the reaction product were completely identical with those of authentic $MTFSO_2$ (Fig. 1).

The activity was mostly found in liver and kidney, and subsequently in adrenal glands. However, heart, brain, skeletal muscle, spleen, and small intestine could scarcely catalyze the oxidation of MTFSO. About 90% of the hepatic activity was found in the microsomal fraction; the specific activity of microsomes (nearly 0.20 mμmoles/min/mg protein) was about 4.5 fold higher than that of whole liver homogenates. Negligible activity was observed in the nuclear, mitochondrial, and soluble fractions.

That the microsomal sulfoxide oxidizing system required both NADPH and molecular oxygen was evident from the data of Table I. NADH as the same concentration was far less effective than NADPH. Ascorbate could not act as an electron donor. The apparent Michaelis constants for NADPH and MTFSO were 5.0×10^{-5} M and 5.6×10^{-3} M, respectively.

The sulfhydryl binding reagents, such as p-chloromercuribenzoate, N-ethylmaleimide and mercuric chloride (0.5 mM), and the redox dyes, such as menadione (0.5 mM) and methylene blue (1 mM) produced nearly complete inhibition of the activity. β-Diethylaminoethyl diphneylpropylacetate hydrochloride (SKF-525A), which is known as inhibitor of microsomal drug enzymes, at 1 mM also blocked the oxidation of sulfoxide. The reaction was not affected by potassium cyanide, sodium azide (5 mM) and o-phenanthroline (1 mM). The addition of an excess of catalase to decompose 1,500 μmoles of hydrogen peroxide per minute had no effect on this enzyme system, indicating that "free" hydrogen peroxide was not involved in the reaction. Although carbon monoxide ($CO-O_2-N_2$ mixture = 5:1:4, v/v/v) inhibited the aniline hydroxylase (50%), the sulfoxide oxidizing activity was scarcely affected by at the same concentration of CO but with higher ratio of CO/O_2 slight inhibition was observed.

It was noticed that the sulfoxide oxidizing activity was strongly accelerated when the reaction was carried out in the presence of high concentration of potassium phosphate. At a phosphate concentration of 0.6 M, the reaction was stimulated about four fold as compared with that

Table I

Requirements for both NADPH and Molecular Oxygen in Sulfoxide Oxidizing Enzyme System by Rat Liver Microsomes

4.3 mg protein of microsomes were incubated under the standard assay conditions as described in Methods.

Experiments	System	mμmoles ^{35}S-MTFSO$_2$ formed per 60 minutes
1	NADPH-generating, 1 mM	62.7
	Minus NADP	0.25
	Minus Glucose 6-phosphate and its Dehydrogenase	0.02
	Minus Oxygen (evacuated)	0.09
	Minus Microsomes plus boiled Microsomes (100°C, 3 min)	0.00
2	NADPH-generating, 1 mM	57.0
	NADPH*, 1 mM	48.1
	NADH-generating,** 1 mM	5.72
	NADH*, 1 mM	8.47
	NAD, 1 mM	0.00
	Ascorbate, 20 mM	0.00

* NADPH or NADH was added at 20-minute intervals to the incubation system.

** 0.1 M ethanol, 1 mM NAD and alcohol dehydrogenase (5.4 units).

in 0.1 M Tris-HCl buffer at the same pH. Moreover, similar activation was also observed with polyanions such as potassium pyrophosphate, sodium sulphate and ATP. However, this phenomenon is neither due to a mere effect of the ionic strength nor of the particular cation, because sodium chloride and potassium chloride were found to be rather inhibitory at higher ionic strengths where polyanions such as phosphate and sulfate were greatly stimulatory. No stimulatory effect was observed on the rates of aniline hydroxylase, microsomal methanol oxidase (Orme-Johnson and Ziegler, 1965), and aminopyrine demethylase in the presence of higher concentrations of phosphate, pyrophosphate and sulfate salts.

We are grateful to Dr. M. Nishikawa and Mr. T. Toga for the synthesis of ^{35}S-MTFSO, and Mr. M. Numata for generous gifts of MTFSO and $MTFSO_2$. We are also indebted to Dr. Y. Asahi, Mr. T. Shima, Mr. A. Ouchida and Mr. K. Shinozaki for the analysis of mass and NMR spectra.

REFERENCES

Bremer, J., and Greenberg, D. M., (1961), Biochim. Biophys. Acta, 46, 217.
Gerhards, E., and Gibian, H., (1967), Ann. N. Y. Acad. Sci., 141, 65.
Gillette, J. R., (1966). Advan. Pharmacol., 4, 219.
Gillette, J. R., and Kamm, J. J., (1960), J. Pharmacol. Exptl. Therap., 130, 262.
Hogeboom, G. H., Schneider, W. C., and Palade, G. E., (1948), J. Biol. Chem., 172, 619.
Kawasaki, C., (1963), Vitamins and Hormones, 21, 69.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., (1951), J. Biol. Chem., 193, 265.
Mazel, P., Henderson, J. F., and Axelrod, J., (1964), J. Pharmacol. Exptl. Therap., 141, 1.
Orme-Johnson, W. H., and Ziegler, D. M., (1965), Biochem. Biophys. Res. Comm., 21, 78.
Spector, E., and Shideman, F. E., (1959), Biochem. Pharmacol., 2, 182.
Suzuoki-Z., Murakami, K., Kikuchi, S., Nishikawa, K., and Numata, M., (1967), J. Pharmacol. Exptl. Therap., in press.