Communications to the Editor

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CIS-TRANS ISOMERIZATION OF A NITROFURAN AF-2 BY RAT LIVER MICROSOMAL PREPARATIONS

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The enzymatic cis-trans isomerization of nitrofurans such as 3-(5-nitro-2-furyl)-2-(2-furyl)acrylamide (AF-2) has been proved to occur via the formation of the corresponding nitro anion radicals. Rat liver microsomes supplemented with NADPH exhibited the cis-trans isomerase activity toward AF-2, which is markedly inhibited by p-chloromercuribenzoic acid, but not by carbon monoxide. Purified rat liver NADPH-cytochrome c reductase supplemented with NADPH also exhibited a significant isomerase activity toward the nitrofuran, whereas purified rat liver NADH-cytochrome b_5 reductase exhibited only a little activity in the presence of NADPH or NADH. These results indicate that mainly NADPH-cytochrome c reductase is involved in the cis-trans isomerization of nitrofurans, i.e. the formation of nitro anion radicals catalyzed by rat liver microsomes.

KEYWORDS ——— nitrofuran derivative; 3-(5-nitro-2-furyl)-2-(2-furyl)-acrylamide (AF-2); cis-trans isomerization; nitro anion radical formation; rat liver microsome; NADPH-cytochrome c reductase; NADH-cytochrome b_5 reductase

Our previous studies[1,2] demonstrated enzymatic cis-trans isomerization of nitrofuran which have an olefinic double bond in the side chain. Xanthine oxidase supplemented with an electron donor could catalyze the cis-trans conversion of 3-(5-nitro-2-furyl)-2-(2-furyl)acrylamide (AF-2) and its related nitrofurans, though the direction of isomerization (cis $\longrightarrow$ trans, cis $\rightleftarrows$ trans or trans $\longrightarrow$ cis) is dependent on the chemical structure of these compounds. Rat liver microsomes could also catalyze the conversion of cis-AF-2 to its trans isomer in the presence of a reduced pyridine nucleotide. And we proposed a new cis-trans isomerization mechanism which is based on the formation of nitro anion radicals. For instance, cis-AF-2 receives a single electron derived from an enzyme system to form the anion radical. Spin density on the olefinic double bond results in free rotation between the olefinic carbons followed by conversion to its thermodynamically more stable trans isomer (Fig.1).

The preliminary experiments[2] using pulse radiolysis technique proved that such isomerization of AF-2 occurs when the nitro anion radical is produced, supporting this postulated mechanism. Furthermore, the electron spin resonance (ESR) spectrum

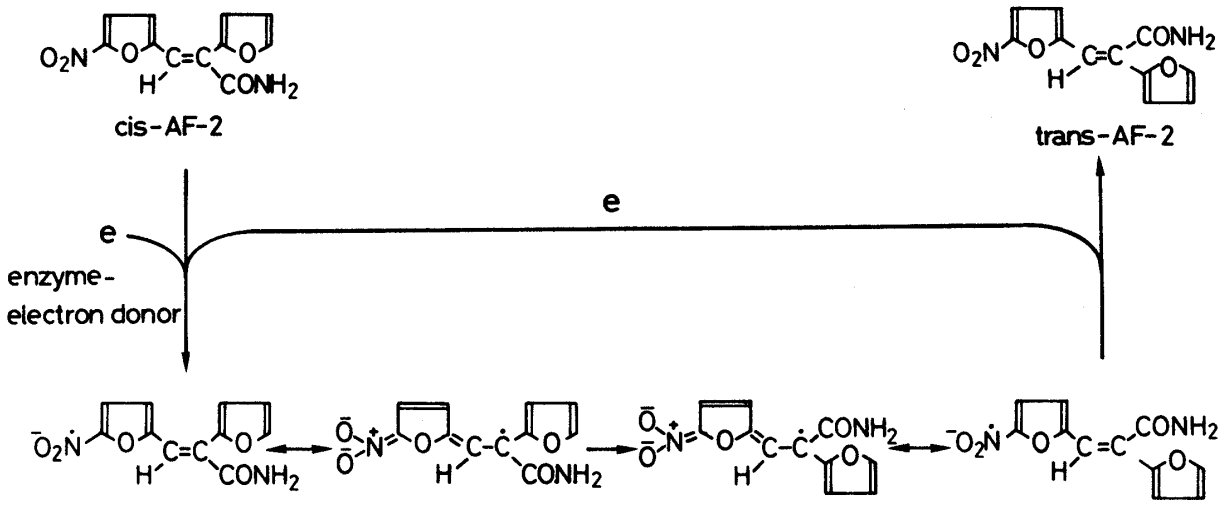

Fig.1. Mechanism for Enzymatic cis-trans Isomerization of 3-(5-Nitro-2-furyl)-2-(2-furyl)acrylamide (AF-2)

of an incubation mixture containing AF-2, rat liver microsomes and an NADPH-generating system provided direct evidence of the formation of the nitro anion radical.[3,4] Mason and his coworkers[5-8] have shown that rat liver microsomal incubation supplemented with NADPH reduces not only nitrofurans, but also other nitro compounds such as p-nitrobenzoic acid and 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (metronidazole) to the corresponding anion radicals, which have recently received considerable attention as possible toxic metabolites. They have suggested that the microsomal flavoproteins, NADPH-cytochrome c reductase and NADH-cytochrome b_5 reductase are the microsomal enzymes responsible for such one-electron reduction of nitro compounds.[5,8] In the presence study, the ability of rat liver microsomal preparations to isomerize cis-AF-2 to its trans isomer was examined in order to explore microsomal one electron-donating enzymes in nitroreduction.

cis-AF-2 (mp 151-152°C) and its trans isomer (mp 176-177°C)[9] were kindly donated by Ueno Fine Chemical Industries, Ltd. Male Wistar strain rats (100-180 g) were used. The liver was homogenized in 4 volumes of 1.15% KCl, the homogenate was centrifuged for 20 min at 9,000 x g, and then the supernatant fraction was centrifuged for 60 min at 105,000 x g. The microsomal fraction was washed by resuspension in the KCl solution and resedimentation for 60 min at 105,000 x g. NADPH-cytochrome c reductase and NADH-cytochrome b_5 reductase were purified from the rat liver microsomes by the methods of Yasukochi and Masters,[10] and Takesue and Omura,[11] respectively. When these flavin enzymes were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the protein staining showed a single band in either case. In the assay of cis-trans isomerase activity a Thunberg-type cuvette was used. A typical incubation mixture consisted of 0.075 μmol of cis-AF-2, 0.15 μmol of a reduced pyridine nucleotide and an enzyme in a final volume of 2.5 ml of 0.2 M phosphate buffer (pH 7.4). The side arm contained a reduced pyridine nucleotide and the cuvette contained all other components. Prior to incubation, the cuvette was evacuated

TABLE I. Ability of Rat Liver Microsomes to catalyze the cis-trans Isomerization of 3-(5-Nitro-2-furyl)-2-(2-furyl)acrylamide (AF-2)

Addition	trans-AF-2 formed (μmol/min/mg protein)
None	0
NADPH	3.94 ± 0.13
NADH	0.08 ± 0.02
NADPH, CO*	3.55 ± 0.30
NADPH, PCMB**	0

Each value represents mean ± S.D. of three experiments.
The incubation mixture contained microsomes equivalent to 10 μg of protein.
* The assay was performed under an atmosphere of carbon monoxide.
** p-Chloromercuribenzoic acid, 1×10^{-5}M

TABLE II. Ability of Rat Liver Microsomal Flavin Enzymes to Catalyze the cis-trans Isomerization of 3-(5-Nitro-2-furyl)-2-(2-furyl)acrylamide (AF-2)

Addition	trans-AF-2 formed (μmol/min/mg protein)	
	NADPH-cytochrome c reductase	NADH-cytochrome b_5 reductase
None	0	0
NADPH	2083.3	1.6
NADH	9.5	1.3
NADPH, PCMB*	16.3	-

Each value represents mean of two experiments.
The incubation mixture contained NADPH-cytochrome c reductase equivalent to 0.68 μg of protein or NADH-cytochrome b_5 reductase equivalent to 12.9 μg of protein.
* p-Chloromercuribenzoic acid, 1×10^{-5}M.

with an aspirator for 3 min and then tightly closed. After the cuvette was preheated for 2 min at 20°C, the reaction was started by mixing the components of the side arm and the cuvette together, and the increase of absorbance at 417 nm (the absorption maximum of trans-AF-2) was recorded. The extinction coefficient of trans-AF-2 was 21.5×10^3 $M^{-1}cm^{-1}$. When p-chloromercuribenzoic acid was used as an inhibitor, the cuvette was preheated for 5 min instead of 2 min. Protein was determined by the method of Lowry et al.[12] with bovine serum albumin as a standard.

As shown in Table I, rat liver microsomes in the presence of NADPH exhibited the cis-trans isomerase activity toward AF-2 under anaerobic conditions. NADH was effective to a much lesser extent, compared with NADPH. The NADPH-linked activity was markedly inhibited by p-chloromercuribenzoic acid (PCMB), but not carbon monoxide. This result agrees with the ESR spectroscopic observation[5] that when p-nitrobenzoate was anaerobically incubated with rat liver microsomes and NADPH, carbon monoxide did not affect the steady-state concentration of the p-nitrobenzoate dianion radical formed. These facts suggested that the microsomal formation of nitro anion radicals is mainly mediated through a flavin enzyme, probably NADPH-cytochrome c reductase. Table II shows the cis-trans isomerase activity toward AF-2 of purified rat liver NADPH-cytochrome c reductase and NADH-cytochrome b_5 reductase. The former enzyme supplemented with NADPH exhibited significant activity which is markedly inhibited by PCMB, whereas the latter enzyme exhibited only a little activity in the presence of NADPH or NADH. From these results, we concluded that NADPH-cytochrome c reductase is mainly involved in the cis-trans isomerization of nitrofurans, i.e. the formation of nitro anion radicals catalyzed by rat liver microsomes.

REFERENCES

1) K. Tatsumi, S. Kitamura, N. Koga, H. Yoshimura and Y. Kato, Biochem.Biophys.Res. Commun., 73, 947 (1976).
2) K. Tatsumi, N. Koga, S. Kitamura, H. Yoshimura, P. Wardman and Y. Kato, Biochim. Biophys.Acta, 567, 75 (1979).
3) B. Kalyanaraman, E. Perez-Reyes and R.P. Mason, Mol.Pharmacol., 16, 1059 (1979).
4) B. Kalyanaraman, R.P. Mason, R. Rowlett and L.D. Kispert, Biochim.Biophys.Acta, 660, 102 (1981).
5) R.P. Mason and J.L. Holtzman, Biochemistry, 14, 1626 (1975).
6) R.P. Mason and J.L. Holtzman, Biochem.Biophys.Res.Commun., 67, 1267 (1975).
7) E. Perez-Reyes, B. Kalyanaraman and R.P. Mason, Mol.Pharmacol., 17, 239 (1980).
8) R.P. Mason, " Free Radicals in Biology," Vol.5, ed. by W.A. Pryor, Academic Press, Inc., San Diego, 1982, pp. 161-222.
9) The cis or trans form of AF-2 means the isomer in which the furan rings attached to the olefinic double bond lie on the same or opposite sides of the molecule, respectively.
10) Y. Yasukochi and B.S.S. Masters, J.Biol.Chem., 251, 5337 (1976).
11) S. Takesue and T. Omura, J.Biochem., 67, 267 (1970).
12) O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.L. Randall, J.Biol.Chem., 193, 265 (1951).

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