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ONE ELECTRON TRANSFER MECHANISM IN THE ENZYMATIC OXYGENATION OF SULFOXIDE TO SULFONE PROMOTED BY A RECONSTITUTED SYSTEM WITH PURIFIED CYTOCHROME P-450

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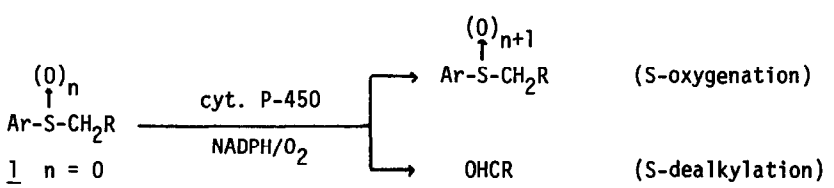
A kinetic study on enzymatic S-oxygenation of sulfoxides to sulfones was carried out by a reconstituted system with purified cytochrome P-450. A linear correlation observed between log(Vmax)'s and the one-electron oxidation potentials of sulfoxides suggests that the oxygenation of sulfoxides proceeds *via* one electron transfer process to the active "oxenoid" intermediate of the enzyme.

Recently, the mechanism of the oxidative N-demethylation of tertiary amines promoted by a few enzymes has been examined and a one-electron transfer process from the tri-valent nitrogen to the enzymes to produce the corresponding aminium radical intermediate has been suggested.[1-3] Meanwhile, we have revealed that the oxygenation of sulfides with purified cytochrome P-450 proceeds *via* one-electron transfer from the di-valent sulfides to the active "oxenoid" intermediate of cytochrome P-450.[4,5] The generation of a sulfenium radical intermediate is supported by both kinetic study[4,5] and its chemical reactivities.[6] In the oxygenation of sulfoxides with microsomal cytochrome P-450 a similar mode of oxygenation mechanism was suggested, since the S-dealkylation reaction of some sulfoxides having an acidic α-methylene is found to proceed. On the other hand, they are oxidized by H_2O_2 or *m*-chloroperbenzoic acid to to give only sulfones.[7]



(1)

1 n = 0

2 n = 1

R: H, Ph, CN, C_6H_4-NO_2-*p*, COPh

Furthermore, we have demonstrated a kinetic study to be a useful method to examine the mechanism of enzymatic oxygenation.[4,5]

In this paper, we wish to report a kinetic evidence to support one-electron transfer mechanism in the oxygenation of sulfoxides to sulfones[8] by the purified cytochrome P-450.

MATERIALS: Purified cytochrome P-450 was isolated from hepatic microsomes of phenobarbital treated male rabbit according to the procedure reported.[9] NADPH-cytochrome P-450 reductase was purified by the method of Iyanagi.[10]

KINETIC CONDITION: The reconstituted system [1.0 nmol of cytochrome P-450, 1.5 nmol of reductase in 0.1 ml of phosphate buffer (pH 7.25, 0.1 M) containing 0.2% of Emalgen 913] was allowed to stand at 24.5° for 5 min in UV micro-cell, and then was diluted by 0.9 ml of phosphate buffer (pH 7.4, 0.2 M) containing 100 nmol of NADPH. After the substrate-independent consumption of NADPH by following absorbance at 340 nm, a methanolic solution of a substrate (2 - 30 µl, 20 mM) was introduced into the UV micro-cell and incubated at 24.5° to initiate the reaction. The oxygenation of the sulfoxide to the sulfone was monitored at several time intervals by following the consumption of NADPH.

Cyclic voltammograms of sulfoxides were obtained according to the procedure described in previous papers.[4,5]

A substrate-dependent NADPH consumption was observed when the methanolic solution of sulfoxide (2, 40 - 600 µM) was incubated in the reconstituted system with the purified cytochrome P-450. Vmax and Km values were calculated from a double reciprocal plot of 1/V *vs.* 1/[S] where V is the rate of NADPH consumption. A few typical kinetic results are listed in Table 1.

Table 1 Comparison of kinetics of oxidation of NADPH in the reconstituted system of purified cytochrome P-450 with one-electron oxidation potential (Ep) of sulfoxides.

substrate p-X-C_6H_4-S(O)-CH_3		Km (µM)	Vmax (NADPH nmol/min/nmol P-450)	Ep[a] (volt *vs.* SCE)
2a	X = MeO	78	3.2	1.75
b	Me	364	2.7	1.86
c	H	74	2.3	1.97
d	Cl	58	2.1	2.05

a) Oxidation potentials listed are the first peak potentials of the sulfoxides measured in 0.1 M of n-Bu_4NClO_4/CH_3CN.

When an electron-donating group was substituted on aromatic ring of the sulfoxide, the Vmax value of the oxygenation was found to increase and log(Vmax)'s are correlated nicely with both Hammett σ^+-values ($\rho^+ = -0.2$) and one-electron oxidation potentials of the corresponding sulfoxides. Meanwhile, the effect of substituents in the oxidation of diphenylsulfoxide by *m*-chloroperbenzoic acid was characterized by a value of $\rho = -0.54$ as a typical electrophilic oxidation.[11]

In the oxygenation of *para*-substituted thioanisole derivatives with purified cytochrome P-450, a similar result was obtained ($\rho^+ = -0.16$). The rather small Vmax values of sulfoxides than those of sulfides would be due to the higher oxidation potentials of sulfoxides and/or larger hydrophobicity of sulfides.[12,13]

Table 2 shows the effects of catalase and CO on the activity for the formation of sulfone (3) in the oxygenation with the reconstituted cytochrome P-450 system. No inhibition was observed when catalase was added, while 50% CO gas was found to inhibit the oxygenation of the sulfoxide. These observations suggest that the oxygenation of sulfoxides to sulfones takes place with the "oxenoid" intermediate $(FeO)^{3+}$ but not with hydrogen peroxide.

When 14 mM (200 x Km) of phenyl methyl sulfoxide (2c) was incubated in the reconstituted system, only the sulfone was obtained (measured by GLC and TLC) and the activity of 2.8 (product nmol/min/nmol P-450) suggests that the oxidation rates of NADPH (Vmax's in Table 1) correspond with the oxygenation rates of sulfoxides ro sulfones.

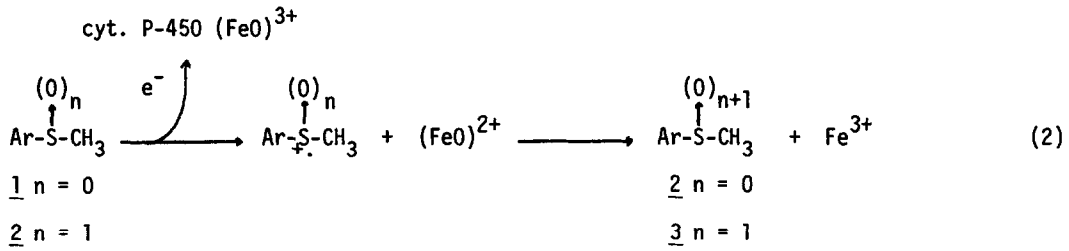
The linear correlationship between the one-electron oxidation potentials of sulfoxides

Table 2 Activity for the formation of sulfone in the reconstituted system with a purified cytochrome P-450

reaction	system	activity[a,b] (product nmol/min/nmol P-450)	relative rate
Ph-S-CH_3 ⟶ Ph-S-CH_3 (O ↓, O O)	cyt. P-450 / reductase / NADPH / O_2	2.8	100
	+ catalase[c]	2.7	96
	+ 50% CO-O_2	1.3	46

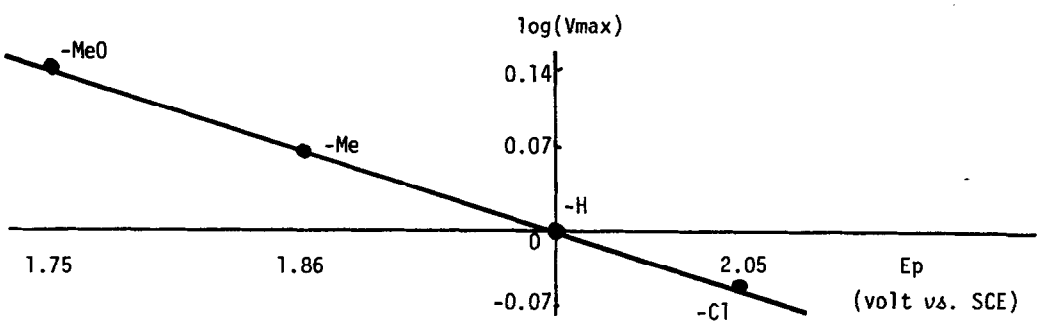
a) Product (sulfone, 3c) was measured by GLC. b) The oxygenation was carried out for 20 min at 20°. c) 180 nM of catalase was added (0.18 eq. to cytochrome P-450).

and log(Vmax)'s suggests that the S-oxygenation of sulfoxides to sulfones is initiated by one-electron transfer from tri-valent sulfoxides to the "oxenoid" intermediate of cytochrome P-450 as illustrated in eq. 2.



(2)

Fig Relationship between log(Vmax) and Ep of *para*-substituted phenyl methyl sulfoxides



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