OXIDATION OF AMINES WITH 4a-FlEt-OOH: AN ENZYME MODEL OF FAD-CONTAINING MONOOXYGENASE

Shigeru Oae*[1], Kaoru Ogawa Asada, Toshiaki Yoshimura[2], and Ken Fujimori

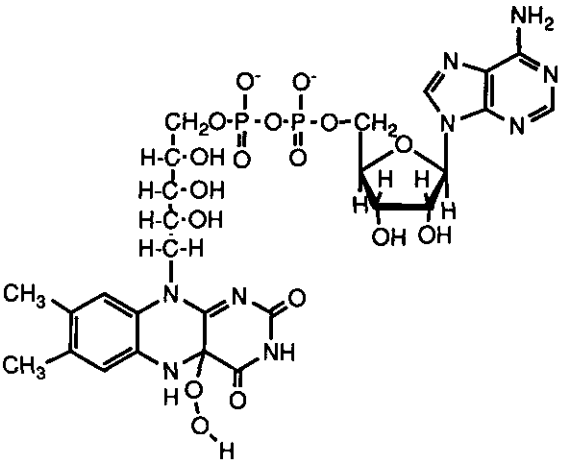
Department of Chemistry, The University of Tsukuba, Tsukuba, Ibaraki-ken 305, Japan

Abstract-Unlike the real enzyme, FAD-containing monooxygenase, 4a-FlEt-OOH, oxidizes most of common primary, secondary and tertiary water-soluble amines, such as *N*-methylmorpholine and n-octylamine. Both kinetic rates and products of the oxidation were obtained. The plots of the rates *vs.* pKa values gave three different correlations lines depending upon the types of amines.

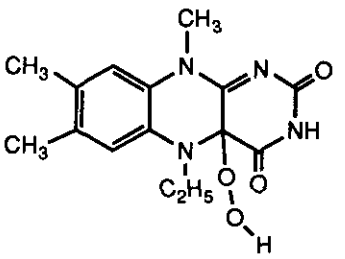
FAD-containing monooxygenase, discovered by D. M. Ziegler,[3] is an oxygenase which is widely distributed in mammalian organs, especially rich in liver, and plays an important role in the metabolism of a variety of xenobiotic substances such as drugs and toxic compounds along with cytochrome P-450.[4] This enzyme is known to oxidize amines and organosulfur compounds,[5] suggested[6] and indeed proved to be electrophilic.[4] In this enzyme the essential part is flavin which is responsible for the oxygenation and the 4a-hydroxyperoxide is considered to be the actual active center of the oxygenation, whereas the adenine part is considered to play a role in binding to the protein wall.

The oxygenation with this enzyme is presumed to be electrophilic,[4] since the 4a-hydroperoxide in the enzyme can be regarded as an organic hydroperoxide, much the same as 4a-FlEt-OOH, as a model compound of the real enzyme. Indeed, we have shown earlier that the oxidation of both sulfides and dimethylaniline to their corresponding oxides by 4a-FlEt-OOH is a typical electrophilic oxidation.[7] One interesting difference between the oxygenation with FAD-monooxygenase and the oxidation with the enzyme model, 4a-FlEt-OOH, may be the extremely fast rate of oxygenation of both amines and sulfides with the real enzyme and the rate-determining step is not the oxidation but the last step of dehydration.[5b, 8] Another would be why n-amine which is less soluble in aqueous media than some tertiary amines, can act as a catalyst without being oxidized. Then a question may arises as to what

would happen to such primary amines in the oxidation with the model system, 4a-FlEt-OOH. This study has revealed that all these amines are oxidized to the corresponding derivatives with 4a-FlEt-OOH in dioxane.



Flavin Adenine Dinucleotide (FAD)



4a-FlEt-OOH

C. Kemal and T. C. Bruice (1976)

FAD-containing monooxygenase is known to oxidize tertiary and secondary amines, but primary amines are known to be not oxidized by this enzyme. However, the oxygenation of tertiary and secondary amines becomes faster in the presence of a primary amine such as n-octylamine. Actually, unlike the real enzyme. 4a-FlEt-OOH oxidized all kinds of amines and the representative data are shown in Table 1.

Table 1. Products for the Oxidations of Amines with 4a-FlEt-OOH in Dioxane (30°C).

Substrates	Products	
$PhCH_2NH_2$	$PhCH{=}N{-}OH$	(90 %)
	$PhCH_2NH{-}OH$	(trace)
$PhCH_2NHCH_3$ [8]	$PhCH_2N(OH){-}CH_3$	(100 %)
$PhCH_2N(OH){-}CH_3$	$PhCH{=}N(O){-}CH_3$	(53 %)
	$PhCH_2{-}N{=}CH_2$ (O)	(24 %)
$PhN(CH_3)_2$	$PhN(O)(CH_3)_2$	(100 %)

Formations of the hydroxylamine from the secondary amine, i.e., *N*-benzyl-*N*-methylhydroxylamine, the corresponding nitrones from *N*-benzyl-*N*-methylhydroxylamine and dimethylaniline oxide from dimethylaniline by the oxidation with 4a-FlEt-OOH in dioxane, are expected, since the real enzyme also is known to oxygenate these amines in the same way.[4] However, the formations of both benzyl- and benzalhydroxylamines from *N*-benzylamine by the oxidation with 4a-FlEt-OOH is unexpected. Obviously, the formation of the unsaturated hydroxylamine is due to the secondary oxidation of *N*-benzylhydroxylamine formed initially. Since the real enzyme is known to oxygenate soft nucleophiles such as divalent sulfur compounds and amines and colloquially called as SNOX, we have compared the relative rates of oxidation of both representative amines and organosulfur compounds in the second-order reactions of the oxidation and the results are shown in Table 2.

Table 2. Second Order Rate Constants for the Reactions of Amines and Organosulfur Compounds with 4a-FlEt-OOH in Dioxane.

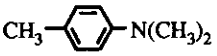
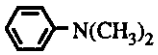
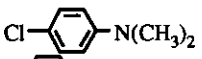
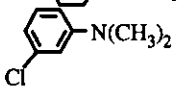
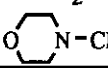
$$\text{Nu:} + \text{4a-FlEt-OOH} \xrightarrow[\text{dioxane}]{30^{\circ}C} \text{Nu}\rightarrow\text{O} + \text{4a-FlEt-OH}$$

substrates	k_2 (l mol^{-1} sec^{-1})	relative rate
$PhSCH_3$	1.72×10^{-2}	35
$PhS(O)CH_3$	5.54×10^{-4}	1.1
PhSH	1.21×10^{-2}	24
$PhN(CH_3)_2$	4.97×10^{-4}	1.0
$PhCH_2NHCH_3$[8]	1.23×10^{-2}	25
$PhCH_2N(OH)CH_3$[8]	9.33×10^{-3}	19
$PhCH_2NH_2$	6.85×10^{-5}	0.14
$CH_3(CH_2)_3NH_2$	3.24×10^{-4}	0.65
$CH_3CH_2CH(NH_2)CH_3$	1.70×10^{-5}	0.034

When the rate of the oxidation of dimethylaniline is taken as unity, those of thioanisole, *N*-benzylmethylamine and the hydroxylamine are nearly in the same order, however, those of primary amines and even that of secondary butylamine are quite low. But they still react. The main difference between this oxidant and the real enzyme is that the enzymic oxygenation requires the substrate to be fit inside the cavity quite well, however this oxidant does not discriminate the size. However, it is still a mystery why n-octylamine acts as a catalyst and unreactive in the real enzyme oxygenation.

The kinetic rates are tabulated along with the known pKa values[9] of various amines in Table 3, expecting to see some correlations.

Table 3. Second Order Rate Constants for the Reactions of Primary and Secondary Amines with 4a-FlEt-OOH in Dioxane (30 °C).

amines	k_2 (l mol $^{-1}$ sec $^{-1}$)	pKa
$CH_3(CH_2)_3NH_2$	3.25×10^{-4}	10.60
$CH_3CH_2CH(NH_2)CH_3$	1.70×10^{-5}	10.56
$CH_3(CH_2)_7NH_2$	1.87×10^{-4}	10.57
$CH_2{=}CHCH_2NH_2$	6.05×10^{-5}	9.69
$PhCH_2NH_2$	6.85×10^{-5}	9.62
$CH_3O(CH_2)_3NH_2$	3.23×10^{-5}	9.45
	1.23×10^{-3}	5.89
	7.76×10^{-4}	5.61
	4.97×10^{-4}	5.18
	2.48×10^{-4}	4.40
	1.98×10^{-4}	3.84
$(C_2H_5)_3N$	2.12×10^{-1}	10.7
$PhCH_2N(CH_3)_2$	6.21×10^{-2}	9.03
 [8]	1.90×10^{-2}	7.57

However, the nucleophilic attack of the amino nitrogen is dependent on the size of the steric bulkiness around the nitrogen atom. Thus, three different correlations have been found as shown in Figure 1. There is a marked deviation with the branched primary amine, i.e., 2-aminobutane, the only one branched primary amine which we observed. Perhaps, another correlation would be observed with these secondary amines. Differences of reactivities of both primary and tertiary amines in the oxidation with 4a-FlEt-OOH are indicated by β-values, i.e., β=0.38 for tertiary amines and β=0.8 for primary amines. From these data, the pKa is found to affect the reactivity of the primary amine more than the tertiary amine.

Although chemical behavior of amines in the electrophilic oxidation with 4a-FlEt-OOH is somewhat different from that of oxygenation with the real enzyme, both are electrophilic in nature. However,

unlike iron-porphilins which are considered to be good model compounds for cytochrome P-450, 4a-FlEt-OOH cannot be a good model compound to mimic the role of FAD-containing monooxygenase.

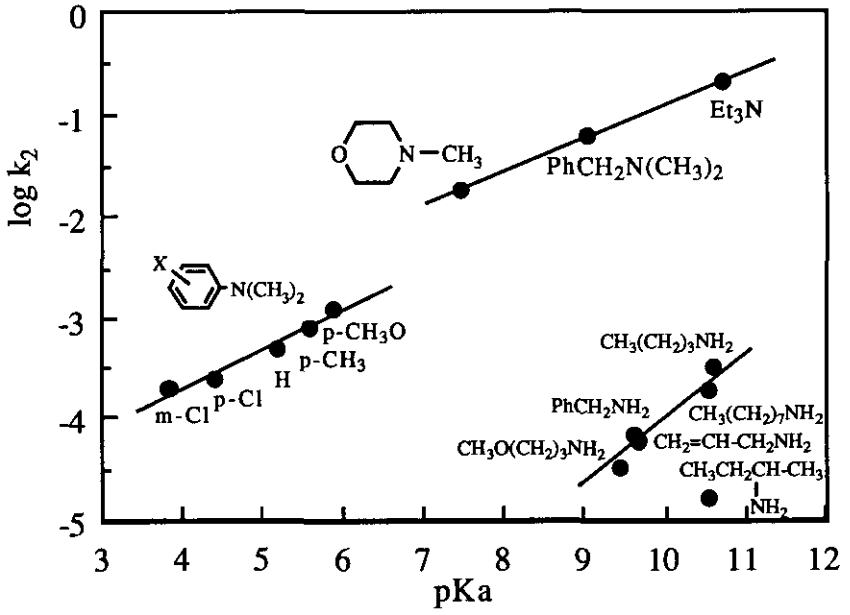


Figure 1. Plots of log k_2 for the Reactions of 4a-FlEt-OOH with Amines *vs.* pKa of the Amine in H_2O.

EXPERIMENTAL

Materials. Following commercially available compounds were obtained from Wako Chemicals Co., Namely, 30% hydrogen peroxide, thioanisole, thiophenol, *N*,*N*-dimethylaniline, *N*,*N*-dimethyltoluidine, n-octylamine, n-butylamine, sec-butylamine, *N*,*N*-dimethylbenzylamine, *N*-methylbenzylamine, triethylamine, 3-methoxypropylamine and allylamine. Thioanisyl oxide was made by the standard oxidation with hydrogen peroxide in acetic acid,[10] while *N*-methyl-*N*-benzylhydroxylamine was synthesized from benzaldehyde and *N*-methylhydroxylamine according to the known method,[11] mp 41-42 °C. 4a-FlEt-OOH was synthesized according to the procedures of Yoneda *et al.*[12] and T. C. Bruice used.[6] 5-Ethyl-4a-hydroperoxy-3-methyllumiflavin we prepared had the correct analytical values in all aspects, and had mp 144-145 °C. Dioxane we used for the kinetic studies, was refluxed under dry nitrogen over sodium and benzophenone until the intensely blue color of the benzophenone anion radical persisted. Distillation of dioxane from the blue solution was followed by several freeze-thaw.

Kinetic Experiment. Kinetic runs were conducted with a solution (3 ml) of an aliquot amine (1.7-4.6 mol/dm^3) in dry dioxane in a uv cell which was incubated at 30 °C for five minutes. To this was

added 50 µl solution of 4a-FlEt-OOH (1.5×10^{-3} mol/dm^3) in dioxane and the disappearance absorption due to 4a-FlEt-OOH at 365 nm was monitored and pseudo-first order rate constants were obtained at various concentrations of amines as well as other substrates.

Analyses of the Products. Analyses of $PhCH_2NHOH$ and PhCh=N-OH formed in the reaction of benzylamine with 4a-FlEt-OOH were carried out by initial isolation of products with thin layer chromatography using Merk Kieselgel GF_{254}, (AcOEt: benzene= 1:1 as eluent) and subsequent identification by measurements of its nmr spectra.

REFERENCES

1. Present address: Institute of Heteroatom Chemistry, 2509 Hirao, Mihara-cho, Minamikawachi-gun, Osaka-fu 587, Japan.
2. Present address: Department of Applied Chemistry, Toyama University, 3190 Gofuku, Toyama City 930, Japan.
3. a) D. M. Ziegler, D. Jollow, and D. Cook, Properties of a Purified Microsomal Mixed Function Amine Oxidase, in 'Flavins and Flavoproteins,' ed. by H. Kamin, Univ. Park Press, Baltimore, MD, 1971, pp. 504-522; b) L. L. Poulsen, R. M. Hysop, and D. M. Ziegler, Biochem. Pharm., 1974, **23**, 3431.
4. a) S. Oae, A. Mikami, T. Matsuura, K. Ogawa-Asada, Y. Watanabe, K. Fujimori, and T. Iyanagi, Biochem. Biophysics Res. Commun., 1985, **131**, 567; b) S. Oae and K. Fujimori, J. Synth. Org. Chem. Jpn., 1983, **41**, 846.
5. a) D. M. Ziegler and L. L. Poulsen, 'Method in Enzymology', 1978, **52**, 142; b) D. M. Ziegler and L. L. Poulsen, J. Biol. Chem., 1979, **254**, 6449.
6. a) C. Kemal and T. C. Bruice, Proc. Natl. Acad. Sci. USA, 1976, **73**, 995, 1977, **74**, 405; b) T. C. Bruice, Chapter 44, in 'Flavin and Flavoproteins,' ed. by V. Massey and C. H. Williams, Elsevier, 1982, p. 265; D. P. Ballou, ibid., Chapter 48, p. 301.
7. S. Oae, K. Asada, and T. Yoshimura, Tetrahedron Lett., 1983, **24**, 1256.
8. N. B. Beaty and D. P. Ballou, J. Biol. Chem., 1981, **256**, 4611, 4619.
9. Kagaku Binran (Tables of Physico-Chemical Constants) Third Ed. II, Chem. Soc, Japan, Maruzen, Tokyo, 1983, pp. 337-345.
10. L. I. Denisova and V. A. Batyakina, Kim. Fram. Zh., 1970, **4**, 9.
11. J. Meisenheimer and H. Denner, Chem. Ber., 1932, **65B**, 1799.
12. F. Yoneda, Y. Sakuma, M. Ichiba, and K. Shinomura, J. Am. Chem. Soc., 1976, **98**, 830.

Received, 2nd September, 1991