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ORTHO- AND PARA-SELECTIVITY IN AROMATIC HYDROXYLATION BY IRON-THIOL AND HEMIN-THIOL COMPLEXES

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The main function of cytochrome P-450 in monooxygenases is the activation of oxygen and the hydroxylation of substrates. Both reactions have been actively studied in model systems in order to elucidate the mechanism of enzymic hydroxylation (1). Recently, Ullrich (2) reported that non-enzymic hydroxylation occurs with a ferrous ion-thiosalicylic acid-oxygen system in acetone, and that the pattern of products is very similar to that obtained in enzymic reactions with liver microsomal fractions. However, there is as yet no demonstration for model compounds which can mimic the regioselective aromatic hydroxylation observed in biological systems (3). This paper describes the hydroxylation of aniline by several ferrous ion-thiol and hemin-thiol complexes, which show a considerable regioselectivity in the conversion of aniline to aminophenols.

In a typical experiment, the reaction mixture contained the following reagents ; thiol compound 10^{-1}M, ferrous sulphate heptahydrate or hemin chloride 10^{-2}M or 10^{-3}M, aniline 10^{-1}M and sodium hydroxide. Ferrous sulphate was dissolved in water and hemin chloride in 1M sodium hydroxide. The reaction mixture was adjusted to 10ml with acetone (80%). With vigorous shaking in air, the reactions were carried out at pH 4 and 6 at 40° and stopped at 1, 2, and 4 hours by the addition of 0.5ml of 2N hydrochloric acid. The reaction products were identified by use of TLC and LC, and determined with LC by the modification of Brodie's method (4).

Table 1 (A and B) shows the results of the hydroxylation with the ferrous ion-thiosalicylic acid or cysteine methyl ester-oxygen systems. Of interest is the change in *para* and *ortho* isomers (p/o ratio) in the products with reaction time, pH and types of thiol compounds. No *meta* isomer was detected in these systems. It is clear that the interaction of the thiol group and iron plays an essential role in aromatic hydroxylation, since the hydroxylation is completely inhibited by methylation of the thiol group.

On the other hand, Bayer et al (5) and Föder et al (6) have shown that a thiol group could be responsible for the low spin state of the cytochrome and

hemin, respectively. Further, Tsai et al (7) have indicated that a cysteine thiol group of the protein may be an axial ligand for heme iron in cytochrome P-450 from Pseudomonas putida, and that the thiol group would be important in the reactivity of cytochrome P-450.

In order to design a model system closer to cytochrome P-450, several hemin-thiol compound-oxygen systems were investigated. In the presence of hemin, hydroxylation was increased about 5 (1 hour)~9 times (4 hours) of that in the

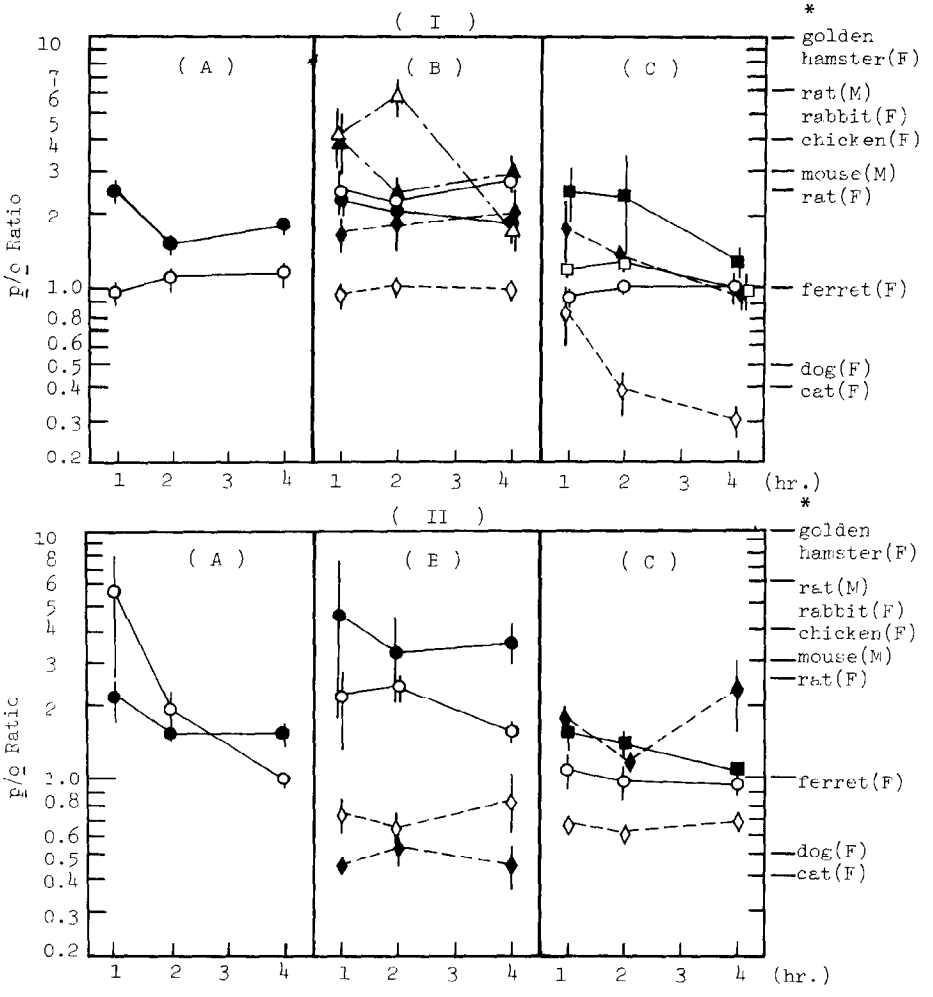

Fig. 1. Ratio of p- to o-Aminophenol formed in the Hemin-Thiol (I) and Ferrous Ion-Thiol (II) Model Systems.

thiol compound : ferrous ion or hemin : aniline = 10^{-1}M : 10^{-3}M : 10^{-1}M
thiol compound ;
(A) cysteine : —○— pH 4, —●— pH 6
(B) cysteine methyl ester : —○— pH 4, —●— pH 6
cysteamine : --△-- pH 4, --▲-- pH 6
o-aminobenzenethiol : --◇-- pH 4, --◆-- pH 6
(C) N-acetyl-cysteine : —○— pH 4, —●— pH 6
β-mercaptopropionic acid : —□— pH 4, —■— pH 6
thiosalicylic acid : --◇-- pH 4, --◆-- pH 6
* data from reference (3)
Each point is the mean ± S.D. of four experiments.

Table 1. Hydroxylation of Aniline by Model Systems

System	pH	Time (hr.)	p-Aminophenol (μ g)	o-Aminophenol (μ g)	p-Aminophenol + o-Aminophenol (μ g)	p/o Ratio
(A) Thiosalicylic Acid - Fe(II)	4	1	427 ± 90	619 ±140	1046 ±229	0.69 ±0.01
	4	2	475 ± 80	763 ± 91	1237 ±170	0.62 ±0.04*
	4	4	689 ± 61	988 ± 95	1677 ±146	0.70 ±0.04*
	6	1	120 ± 12	69 ± 6	189 ± 16	1.76 ±0.16*
	6	2	99 ± 13	76 ± 3	175 ± 16	1.30 ±0.14
	6	4	192 ± 51	83 ± 6	274 ± 47	2.36 ±0.70
(B) Cysteine Methyl Ester-Fe(II)	4	1	117 ± 15	63 ± 29	181 ± 43	2.17 ±0.70*
	4	2	119 ± 24	50 ± 7	169 ± 31	2.35 ±0.21
	4	4	106 ± 17	65 ± 9	171 ± 26	1.63 ±0.08
(C) Cysteine Methyl Ester-Hemin	4	1	705 ± 70	305 ± 74	1010 ±129	2.41 ±0.49*
	4	2	733 ± 47	328 ± 19	1061 ± 61	2.24 ±0.02
	4	4	1204 ±132	442 ± 74	1646 ±206	2.75 ±0.14

* Significantly different from the value obtained with System A (pH 4, 1hr.) ($P<0.01$). Values are means ± S.D. of four experiments.

presence of ferrous ion (B and C in Table 1). In the system containing thiosalicylic acid, however, the increase in hydroxylation activity in the presence of hemin was not observed.

Fig. 1 shows the results of regioselective hydroxylation of aniline with various thiol compounds, compared with the reported data (3) on aniline metabolism in several species of animal.

On the basis of these data, it is suggested that the aliphatic thiol compounds containing SH and NH_2 as coordinating groups may promote hydroxylation at the para- rather than the ortho-position, whereas aromatic thiol compounds such as thiosalicylic acid and o-aminobenzenethiol promote hydroxylation at the ortho- rather than the para-position.

Thus, it may be possible by selection of thiol compounds to control the ortho- and para-selectivities in aromatic hydroxylation.

In order to study the scope and limitation of the presently reported systems as a monooxygenase model, further investigations are under way by use of suitably substituted substrates, and the results will be reported in the near future.

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