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POLARITY IN AROMATIC HYDROXYLATION BY HEMIN-THIOL MODEL SYSTEMS FOR CYTOCHROME P-450

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The polarity at heme site of cytochrome P-450 monooxygenases, which have strongly hydrophobic nature (1) and catalyze the hydroxylation of substrates in drug metabolism, is supposed to play an important role in the activation of oxygen, and in the binding and hydroxylation of substrates. However, there is no indication of the degree of polarity at heme site in the hydroxylation by cytochrome P-450 monooxygenases, moreover it is difficult to estimate the polarity with this enzyme. To solve this problem, the study of hydroxylation by model systems for cytochrome P-450 monooxygenases would present a useful method. Recently, we have suggested that several ferrous ion-thiol and hemin-thiol systems are possible model systems closer to cytochrome P-450 monooxygenases from the results of studies on hydroxylation of aniline and on methyl migration during hydroxylation of p-toluidine (NIH shift) (2,3).While many model systems are available to evaluate the active site of cytochrome P-450 monooxygenases (4,5), the development of probes for polarity in the hydroxylation has received little attention so far.

Our purpose has been to study the hydroxylation of aniline by above mentioned model systems in various ratios of acetone-water binary solvent in order to quantitate the relationship between the

polarity of solvent and the content of hydroxylation, and to estimate the polarity in the heme site of cytochrome P-450 monooxygenases.

The reactions were carried out by the same procedure previously reported (2) (pH 6.0, 40°, 2 hours). The reaction volume was adjusted to 10 ml with various ratio of acetone and water. The reaction products, p- and o-aminophenol (p- and o-AP), were determined with LC. Dielectric constants in acetone-water binary mixtures were measured at 40° according to conventional method.

As is apparent from Fig. 1(A), the maximum content of hydroxylation was obtained in 60% acetone when, in the presence of hemin, cysteine was used as a thiol, which was presumed in the past (6) and was proved recently (7,8) to be a necessary axial ligand for iron in cytochrome P-450. Similar result was observed with hemin-cysteine methyl ester system, in which the maximum hydroxylation was obtained in 80% acetone. In these systems, the changes in ratio of para to ortho isomers (p/o ratio)were found to increase with the content of acetone to water. On the other hand, in the system such as hemin-thiosalicylic acid (Fig. 1(B)) and in the system containing ferrous ion such as ferrous ion-cysteine or ferrous ion-cysteine methyl ester instead of hemin, the hydroxylations were scarcely influenced by the polarity of the solvent.

It is noteworthy that there occurred the dependence of both hydroxylation and p/o ratio upon the polarity of solvent only in the systems containing both hemin and biologically significant thiol compound such as cysteine or cysteine methyl ester.

The desirable dielectric constants of solvent for the maximum hydroxylation of aniline by the model systems ranged 30.0(80% acetone) to 40.0(60% acetone). The range corresponds to the value from 80.7 to 85.5 by Kosower's empirical solvent polarity scale Z (9), which made it possible to estimate the polarity of the environment around

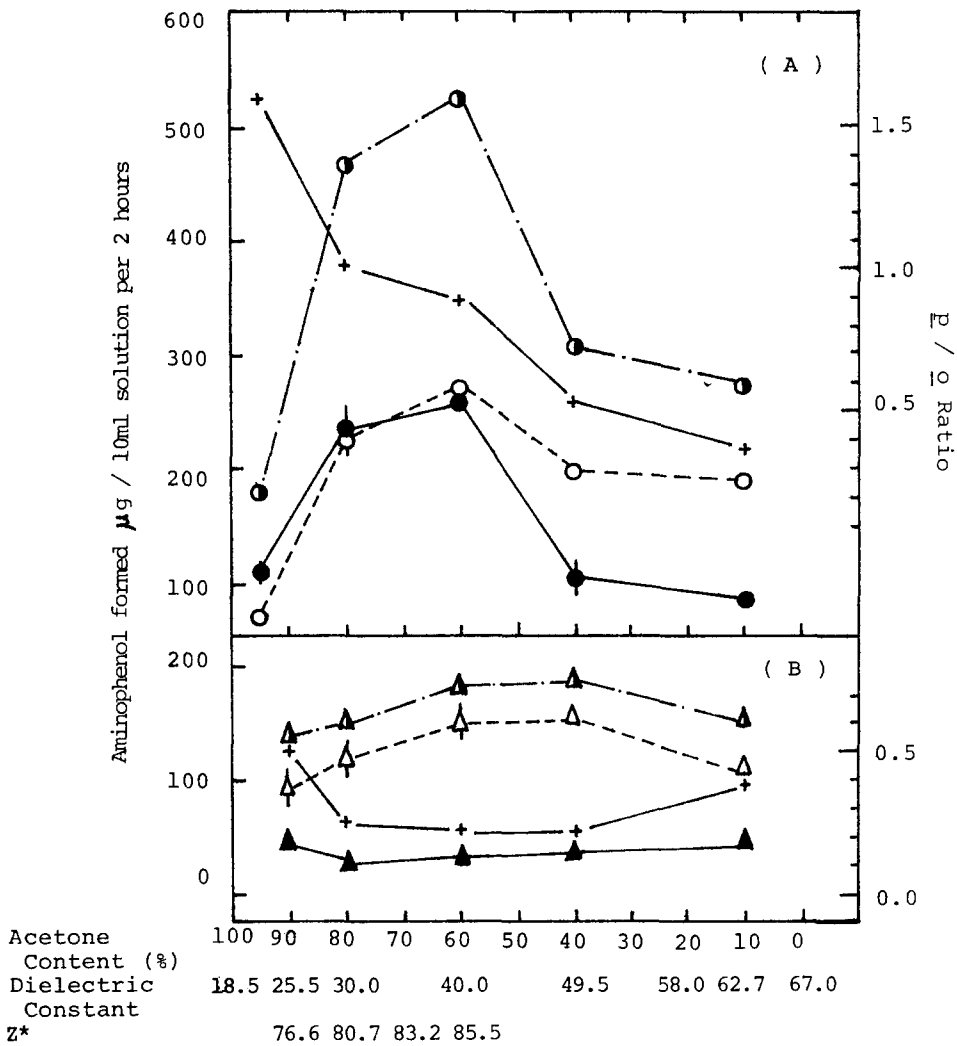

Figure 1. Formation of p- and o-Aminophenol, and Ratio of p- to o-Aminophenol in Hemin-Thiol Systems.

thiol : hemin chloride : aniline = 10^{-1}M : 10^{-3}M : 10^{-1}M
(pH 6.0, 40°, 2 hr.)
thiol compound : (A) cysteine; ●— p-AP, --○-- o-AP, --◑-- p-AP+o-AP, -+- p/o ratio
(B) thiosalicylic acid; ▲— p-AP, --△-- o-AP, --▲-- p-AP+o-AP, —+— p/o ratio

Each point in p-AP and o-AP is the mean ± S.D. of four experiments. The other ib the mean of four experiments.
* data from reference (9)

substrates without knowledge of the precise composition of the environment (10). It seems worthy to note that this range lies within the estimated Z value range (80 to 88) for protein sites with various solvents (10).

From the facts described above, it may be reasonable to take the dielectric constant range 30～40 as temporary standard for the most effective polarity in the hydroxylation of aniline.

In conclusion at this point, the presented result with the possible model systems for cytochrome P-450 such as hemin-cysteine and hemin-cysteine methyl ester system suggests that the heme site in aromatic hydroxylation by cytochrome P-450 monooxygenases has similar polarity.

Further investigations are in progress and the results will be reported in the near future.

References

(1) Y.Imai and R.Sato, Biochem.Biophys.Res.Commun., 60, 8 (1974).
(2) H.Sakurai and S.Ogawa, Biochem.Pharmacol., 24,1257 (1975).
(3) H.Sakurai and M.Kito, Biochem.Pharmacol.,24,1647 (1975).
(4) V.Ullich and Hj.Staudinger, in "Concepts in Biochemical Pharmacology", Vol.XVIII, Springer-Verlag, p.251 (1971).
(5) D.M.Jerina, Chemtech., 120 (1973).
(6) H.A.O.Hill, A.Röder, and R.J.P.Williams, Struct. Bonding (Berlin), 8, 123 (1970).
(7) J.P.Collman, T.N.Sorrell, and B.M.Hoffman, J.Am.Chem. Soc., 97, 913 (1975).
(8) S.Koch, S.C.Tang, R.H.Holm, R.B.Frankel, and J.A.Ibers, J.Am.Chem.Soc., 97, 916 (1975).
(9) E.M.Kosower, J.Am.Chem.Soc., 80, 3253 (1958).
(10) D.C.Tuner and L.Brand, Biochemistry, 7, 3381 (1968).