

This Week's Citation Classic®

Lu A Y H & Coon M J. Role of hemoprotein P-450 in fatty acid hydrocarbon-hydroxylation in a soluble enzyme system from liver microsomes. *J. Biol. Chem.* 243:1331-2, 1968.

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This paper described the isolation from solubilized liver microsomes of three components—an NADPH-dependent reductase, cytochrome P-450, and a heat-stable, organic solvent-extractable factor. All three components are required for the reconstitution of (ω -hydroxylation of lauric acid. This study represents the first report of the resolution and reconstitution of a microsomal mixed-function oxidase system in which cytochrome P-450 is catalytically functional. [The *SCF* indicates that this paper has been cited in more than 425 publications.]

From Microsomes to Purified Cytochrome P-450

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In February 1966, after completing my PhD thesis at the University of North Carolina, Chapel Hill, I arrived at the University of Michigan, Ann Arbor, for postdoctoral work with Minor J. (Jud) Coon in the Department of Biological Chemistry. At that time, Jud's laboratory was investigating the enzyme components involved in the ω -hydroxylation of hydrocarbons and fatty acids in *Pseudomonas oieovorans*.¹ He suggested that I study the liver microsomal fatty acid ω -hydroxylase system and purify the enzyme component(s).

When I first started this investigation, neither Jud nor I was aware that cytochrome P-450 was involved in microsomal hydroxylation. In fact, we had no knowledge at that time that the cytochrome P-450 system was being intensively studied in the field of drug metabolism and that many laboratories had attempted to solubilize the system without much luck.

I tried every solubilization method I knew and each failed attempt was followed by more ex-

periments and longer working hours. About a year after I started this work, I succeeded by using deoxycholate in the presence of glycerol, dithiothreitol, and EDTA. I repeated the experiment several times before I showed the data to Jud and hoped that the results were good enough for publication. Although excited by my progress on the project, Jud pointed out that my job was not only to solubilize, but also to resolve and reconstitute the system. A publication had to await further progress.

Resolution of the solubilized preparations proved to be as difficult as the initial solubilization step. I incorporated detergent into every fractionation step and followed the activity by recombining all the fractions—a strategy not used by other investigators at that time.

Near the end of 1967, we achieved resolution of the system, and the three components were identified as P-450, reductase, and a heat-stable factor. The first CO spectrum of the solubilized cytochrome P-450 was actually recorded after the system was reconstituted. We wrote up the results, and this short manuscript was promptly accepted for publication. It was my first paper, received by the journal on January 12, which coincidentally is my birthday.

These studies resulted in a series of publications on this reconstituted system.^{2,4} The heat-stable factor was later identified as phosphatidylcholine.⁵ In the 20 years following these studies, hundreds of cytochrome P-450s have been purified and characterized by this reconstituted system.^{6,7} I believe that this paper has been widely cited because it represents the first successful solubilization, resolution, and reconstitution of the liver microsomal cytochrome P-450 system, thus making it possible to study the many aspects of P-450.

I have often wondered whether I would have had the courage to take on this project had I had a thorough knowledge of the cytochrome P-450 literature, knowing that many investigators had unsuccessfully attempted to solubilize this membrane-bound enzyme system.

1. Peterson J A, Basu D & Coon M J. Enzymatic ω -oxidation. I. Electron carriers in fatty acid and hydrocarbon hydroxylation. *J. Biol. Chem.* 241:5162-4, 1966. (Cited 135 times.)
2. Lu A Y H, Junk K W & Coon M J. Resolution of the cytochrome P-450-containing ω -hydroxylation system of liver microsomes into three components. *J. Biol. Chem.* 244:3714-21, 1969. (Cited 370 times.)
3. Lu A Y H, Strobel H W & Coon M J. Hydroxylation of benzphetamine and other drugs by a solubilized form of cytochrome P-450 from liver microsomes: lipid requirement for drug demethylation. *Biochem. Biophys. Res. Commun.* 36:545-51, 1969. (Cited 135 times.)
4. ----- . Properties of a solubilized form of the cytochrome P-450-containing mixed-function oxidase of liver microsomes. *Mol. Pharmacol.* 6:213-20, 1970. (Cited 180 times.)
5. Strobel H W, Lu A Y H, Heidema J & Coon M J. Phosphatidylcholine requirement in the enzymatic reduction of hemoprotein P-450 and in fatty acid, hydrocarbon, and drug hydroxylation. *J. Biol. Chem.* 245:4851-4, 1970. (Cited 450 times.)
6. Lu A Y H & West S B. Multiplicity of mammalian microsomal cytochromes P-450. *Pharmacol. Rev.* 31:277-95, 1980. (Cited 755 times.)
7. Black S D & Coon M J. Comparative structures of P-450 cytochromes. (Ortiz de Montellano P. R. ed.) *Cytochrome P-450: structure, mechanism and biochemistry*. New York: Plenum Press, 1986. p. 161-216. (Cited 150 times.)

Received February 4, 1991