This Week's Citation Classic ®

Omura T & Sato R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* 239:2370-8, 1964.
[Institute for Protein Research, Osaka University, Japan]

This article reports spectrophotometric and other evidence that the CO-binding pigment, tentatively called "P-450," in liver microsomes is a protoheme-containing protein despite its anomalous spectral properties, the fact that it is autoxidizable, and that in microsomes this hemoprotein is reducible by NAD(P)H. [The *SCI*® indicates that this paper has been cited in more than 6,305 publications.]

Cytochrome P-450: An Inconspicuous Start

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In 1955, I spent seven months with Britton Chance at the University of Pennsylvania, where I became acquainted with Martin Klingenberg, who was working on cytochrome b_5 in liver microsomes. Then, while an assistant professor at the Institute for Protein Research of Osaka University, I became aware of Martin's 1958 paper,[1] which I read only because I knew him personally. It reported the occurrence in liver microsomes of a redox pigment, the reduced form of which binds CO and exhibits a difference spectrum having an intense peak at 450 nm. Martin could not explain its chemical nature because of its unprecedented spectral properties. A few months later, another paper on this pigment came out, again from Chance's laboratory,[2] but its nature still remained elusive. I was attracted by the pigment, but we did not have spectrophotometers that were powerful enough to measure difference spectra of such turbid samples as microsomal suspensions. In the meantime, our institute decided to purchase a Cary 14 recording spectrophotometer for joint use. So in April 1960, I was finally able to start to work on the pigment with Tsuneo Omura, who had just joined our group.

Our work was, however, tough sledding. We could show, for instance, that the pigment in liver microsomes is reducible by NADH and NAD(P)H and is highly autoxidizable, but no clues to its chemical nature came to light. One day I listened to a lecture by the late Kozo Kaziro and learned that ethyl isocyanide binding to hemoglobin causes a spectral change. I immediately decided to examine the effect of this compound on our system. When it was added to dithionite-reduced liver microsomes, this terribly bad-smelling compound induced a spectral change that was characteristic of a hemoprotein, though a minor anomaly still persisted. Furthermore, Tsuneo's competition experiments indicated that ethyl isocyanide and CO bind to the same entity. He also could show that anaerobic perturbation of liver microsomes with such agents as heated snake venom and deoxycholate converts the pigment to an alternate form, the reduced CO compound of which absorbs at 420 nm (instead of 450 nm), as does CO-hemoglobin. These observations convinced us of its hemoprotein nature.

In 1962, we published a preliminary report on this pigment,[3] in which it is provisionally called "P-450" (pigment absorbing at 450 nm) and is described as a new cytochrome. In 1964, we also published two papers reporting a full account of our work. One was the subject of this *Citation Classic*, and the other dealt with a half-attempt at solubilization and purification of the pigment.[4] My feeling at that time was that we had somehow managed to resolve a tiny problem in biochemistry, and I could not expect that the study on P-450 would flourish as extensively as it has.

When first published, our work was inconspicuous, but within a few years the interest in P-450 grew rapidly, probably because of the pioneering study of Ron Estabrook and colleagues[5] who reported the involvement of adrenocortical microsomal P-450 in steroid 21-hydroxylation. P-450 is the generic name for a large family of protoheme-containing proteins that are widely distributed in the biological kingdom and, as monooxygenases, play a crucial role in lipid and xenobiotic metabolism. In view of this, the term *cytochrome P-450* is clearly a misnomer, but it is still widely used—a fact for which I am responsible.

Finally, why has our paper been cited so many times? The reason is quite simple. Since our paper gives an extinction coefficient increment for P-450 in the CO-difference spectrum (91 $cm^{-1}mM^{-1}$ between 450 and 490 nm) and this value is essential for determination of P-450, most workers have to cite the paper. The increase in the number of citations to our paper simply reflects the increase in the number of publications in the field on P-450.

1. **Klingenberg M.** Pigments of rat liver microsomes. *Arch. Biochem. Biophys.* 75:376-86, 1958. (Cited 410 times.)
2. **Garfinkel D.** Studies on pig liver microsomes. I. Enzymatic and pigment composition of different microsomal fractions. *Arch. Biochem. Biophys.* 77:493-509, 1958. (Cited 295 times.)
3. **Omura T & Sato R.** A new cytochrome in liver microsomes. *J. Biol. Chem.* 237:1375-6, 1962. (Cited 195 times.)
4. ----------. The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. *J. Biol. Chem.* 239:2379-85, 1964. (Cited 1,895 times.)
5. **Estabrook R W, Cooper D Y & Rosenthal O.** The light reversible carbon monoxide inhibition of the steroid C21-hydroxylase system of the adrenal cortex. *Biochem. Z.* 338:741-55, 1963. (Cited 370 times.)

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