Acta Pharmaceutica Sinica B 2012;2(2):102–106

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

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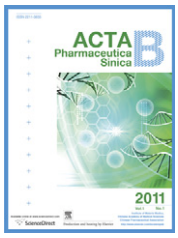

REVIEW

New insights into the role of cytochrome P450 reductase (POR) in microsomal redox biology

Todd D. Porter*

Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0596, USA

Received 14 January 2012; revised 7 February 2012; accepted 13 February 2012

KEY WORDS

Cytochrome P450;
Squalene monooxygenase;
Heme oxygenase;
Cytochrome b_5;
Microsomes;
7-Dehydrocholesterol reductase

Abstract Cytochrome P450 reductase (POR) is an essential electron transfer protein located on the endoplasmic reticulum of most cell types, and has long been appreciated for its role in cytochrome P450-mediated drug metabolism. Additional roles and electron acceptors for POR have been described, but it is largely with the recent availability of POR-null tissues that these supplemental roles for POR have been able to be explored. These studies have confirmed POR as the principal redox partner for the microsomal P450s responsible for drug and xenobiotic metabolism as well as cholesterol and bile acid synthesis, and for heme oxygenase, which catalyzes the initial step in the breakdown of heme. Surprisingly, these studies have revealed that squalene monooxygenase, an enzyme essential to cholesterol synthesis, has a second unknown redox partner in addition to POR, and that 7-dehydrocholesterol reductase, previously proposed to require POR as an electron donor, functions fully independently of POR. These studies have also helped define the role of cytochrome b_5 in P450 catalysis, and raise the question as to the extent to which POR contributes to b_5-dependent redox pathways.

*Tel.: +11 859 257 1137; fax: +11 859 257 7564.
E-mail address: tporter@uky.edu (Todd D. Porter)

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.
doi:10.1016/j.apsb.2012.02.002

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1. Early identification of redox partners for cytochrome P450 reductase

The early history of cytochrome P450 reductase (POR) is described in a comprehensive review by Masters and Okita[1]. Cytochrome P450 reductase was initially identified as a TPNH(NADPH)-dependent cytochrome c reductase present in liver extracts, but subsequent studies demonstrated that cytochrome c, a mitochondrial protein, was not this microsomal enzyme's natural redox partner. The redox partner for POR remained unknown for over a decade, until the early 1960s when it was suggested that POR might be involved in drug and steroid oxidations by the newly discovered cytochrome P450 system. The observation that POR activity was inducible by drugs that increased microsomal P450 expression was undertaken. These studies have greatly facilitated by antibody inhibition studies that clearly showed that antibody to POR inhibited microsomal drug oxidations. The reconstitution of cytochrome P450 activity by Lu and Coon in 1968[2] from solubilized components, including a POR-containing fraction, established P450 as the first redox partner for POR.

Antibody inhibition studies were used to identify additional redox partners for POR, and led to the identification of heme oxygenase[3], a microsomal enzyme that catalyzes the first step in heme degradation. Antibody inhibition was also used to show that steroid hydroxylations in the adrenal cortex[4], placenta[5], and testis[6] were dependent on POR, thus expanding the range of P450s that serve as POR partners to those involved in physiological biochemistry as well as drug and xenobiotic metabolism. Squalene monooxygenase (epoxidase) was identified as a redox partner for POR in 1977[7], making it the second non-P450 enzyme, after heme oxygenase, to use POR as an electron source; squalene monooxygenase acts in the cholesterol synthesis pathway. The ability of POR to donate electrons to cytochrome b_5[8] generated considerable interest as to the relevance of this pathway to P450 function, as b_5 was able to donate the second of the two required electrons to cytochrome P450 in isolated and reconstituted preparations. Moreover, cytochrome b_5 is part of the fatty acid desaturation and elongation pathways as well as the post-lanosterol sterol synthesis pathway, placing POR at the interface of drug and xenobiotic metabolism, steroid and cholesterol synthesis, and fatty acid metabolism. The last enzyme to be associated with POR is 7-dehydrocholesterol reductase, which catalyzes the final step in cholesterol synthesis; mutations in the gene for 7-dehydrocholesterol reductase are responsible for Smith-Lemli-Opitz syndrome[9]. Antibody inhibition studies again implicated POR as an electron donor to this enzyme, an idea further supported by reconstitution studies with partially purified enzyme preparations[10]. However, very recent studies from the author's laboratory have demonstrated that POR does not have a role in 7-dehydrocholesterol reductase function[11].

2. Studies with hepatic POR-null mice

The germline deletion of POR in mice results in embryonic lethality by day 14[12], making whole-animal knock-out studies unfeasible. The lethality appears due to the loss of cholesterol synthesis necessary for neuronal development and to the loss of sterol modification of proteins necessary for development[13,14]. To circumvent this problem the selective, tissue-specific deletion of POR expression was undertaken. These studies have greatly facilitated studies on the role of POR in drug metabolism, tissue and organ-specific toxicity of xenobiotics, and organismal development[15,16].

2.1. *Heme oxygenase*

In one of the two seminal studies on hepatic POR-null mice, Gu et al.[15] noted that the inducible form of heme oxygenase, HO-1, was induced 9-fold at the protein level in the livers of these mice, and yet heme oxygenase activity was essentially negligible in this tissue. The Wolf laboratory did not look at heme oxygenase protein or activity in their hepatic POR-null mice, but did note a modest increase (1.4 to 2-fold) in HO-1 mRNA levels in POR-null liver[17]. A microarray study from the Ding laboratory similarly found a 2.3-fold increase in HO-1 mRNA[18]. The expression of heme oxygenase-2, a constitutively expressed form present in normal liver, was not altered at the mRNA or protein level by the loss of POR expression. The lack of heme oxygenase activity in POR-null liver in the face of a significant increase in protein argues strongly that POR is the requisite and only electron transfer partner for this enzyme. Although there was some evidence for oxidative stress in the livers of these animals (evidenced by an increase in glutathione-*S*-transferase gene expression)[18] consistent with the increase in HO-1 expression, no direct evidence of heme toxicity was noted. Biliverdin and bilirubin levels were not measured in the livers or blood of these animals and the question of how heme turnover is handled in a hepatic POR-null liver remains to be determined.

2.2. *Squalene monooxygenase*

Squalene monooxygenase catalyzes the first oxidative step in the synthesis of sterols, yielding 2,3-oxidosqualene which is then cyclized by 2,3-oxidosqualene cyclase to the first sterol in the pathway, lanosterol. Squalene monooxygenase is an FAD-dependent monooxygenase which is unusual in its requirement for an additional electron transfer partner. Early purification studies demonstrated that activity could be reconstituted with POR[7], and it was not until hepatic POR-null microsomes became available that it could be shown that squalene monooxygenase retained partial activity in the absence of POR[19]. The second microsomal reductase, still unidentified, appears able to catalyze approximately 40% of the activity seen in POR-containing liver microsomes. Sterol analysis of the livers of hepatic POR-null mice revealed elevated levels of dihydrolanosterol, a metabolite of lanosterol formed by sterol 24-reductase, a downstream enzyme in cholesterol synthesis. This metabolite also accumulated in hepatocytes isolated from hepatic POR-null mice, demonstrating that squalene is converted to lanosterol *via* squalene monooxygenase and 2,3-oxidosqualene cyclase in the absence of POR. Studies with rat hepatoma cells in which POR expression is suppressed by RNA interference (RNAi) similarly demonstrated the accumulation of lanosterol and dihydrolanosterol[20], confirming that squalene monooxygenase activity was resilient to the loss of POR. Notably, sterol synthesis is blocked at lanosterol demethylase, resulting in the accumulation of lanosterol and dihydrolanosterol; lanosterol demethylase is a cytochrome