The 2009 F. M. Becket Summer Research Fellowship — Summary Report

Electrochemical Evaluation of Cytochrome P450 27B1

by Steve Rhieu

Renal cytochrome P450 27B1 (CYP27B1) is a mitochondrial mono-oxygenase that selectively catalyzes the 1α-hydroxylation reaction of 25-hydroxycholecalciferol (25(OH)D_3) into 1α,25-dihydroxycholecalciferol (1α,25$(OH)_2D_3$), the biologically-active form of vitamin D_3.[1,2] *In vivo,* this process involves the uptake of two electrons from NADPH, which are used to incorporate one oxygen atom from O_2 into the 1α-site of 25(OH)D_3 and to reduce the second oxygen atom to water. Electron transfer from NADPH to the heme site of CYP27B1 involves the aid of other redox cofactors such as a FAD-containing NADPH-adrenodoxin reductase and an iron-sulfur protein known as adrenodoxin.[2] Recently, mouse CYP27B1 has been overexpressed in *E. coli* and its biochemical properties has been studied.[3] In this report, we describe the electrochemical properties of CYP27B1.

Heterogeneous electron transfer between the active site of P450 enzymes and an electrode is difficult to achieve because (1.) membrane-bound enzymes typically are unstable when solubilized, and (2.) the heme active-site often is buried within the protein. To overcome these issues, surfactant films have been used to immobilize membrane-bound enzymes onto an electrode in order to facilitate its direct electrochemistry.[4] Adopting this method, we first investigated the voltammetric properties of CYP27B1 immobilized on an edge-plane pyrolytic graphite (EPG) electrode coated with didodecyldimethylammonium bromide (DDAB). Using cyclic voltammetry (CV), the electrochemical properties of CYP27B1 were investigated in the absence of a mediator and under strictly anaerobic conditions (<1.5 ppm oxygen). Anaerobic conditions prevent the formation of an Fe^{II}-O_2 complex and a second electron transfer reaction.

Cyclic voltammetry reveals the electrochemical reversibility of CYP27B1 with an average midpoint potential of –185 ± 5 mV vs. Ag/AgCl. The relationship between peak currents and scan rates is linear up to 1 Vs^{-1}, indicating the presence of a diffusionless electroactive species confined to the surface of the electrode. Irreversible electrochemistry (*i.e.*, peak separation >200/n mV, taking n = 1) occurs when the scan rate exceeds 1.5 Vs^{-1} as shown by the Laviron plot (Fig. 1). Using Laviron's approach,[5] the electron transfer coefficient and the rate constant for heterogeneous electron transfer were determined to be 0.46 and 3.4 ± 0.5 s^{-1}, respectively. To demonstrate electrocatalysis, the CYP27B1/DDAB/EPG electrode was immersed in an oxygen-saturated solution containing 25(OH)D_3 with the potential poised at -600 mV vs. Ag/AgCl. However, no evidence of electrocatalytic product of 1α,25$(OH)_2D_3$ was found. As cited with other cytochrome P450s,[6,7] the surfactant film may block the access to the active site of the enzyme. Working from this hypothesis, we performed a reconstituted assay in the presence of DDAB to confirm enzyme activity. As demonstrated in Fig. 2, enzymatic product was not detectable in the presence of DDAB from HPLC analysis. This suggests that DDAB does interfere with product formation by changes around the active site of the protein either through conformational changes (i.e., denaturing the enzyme) or through physical impedance.

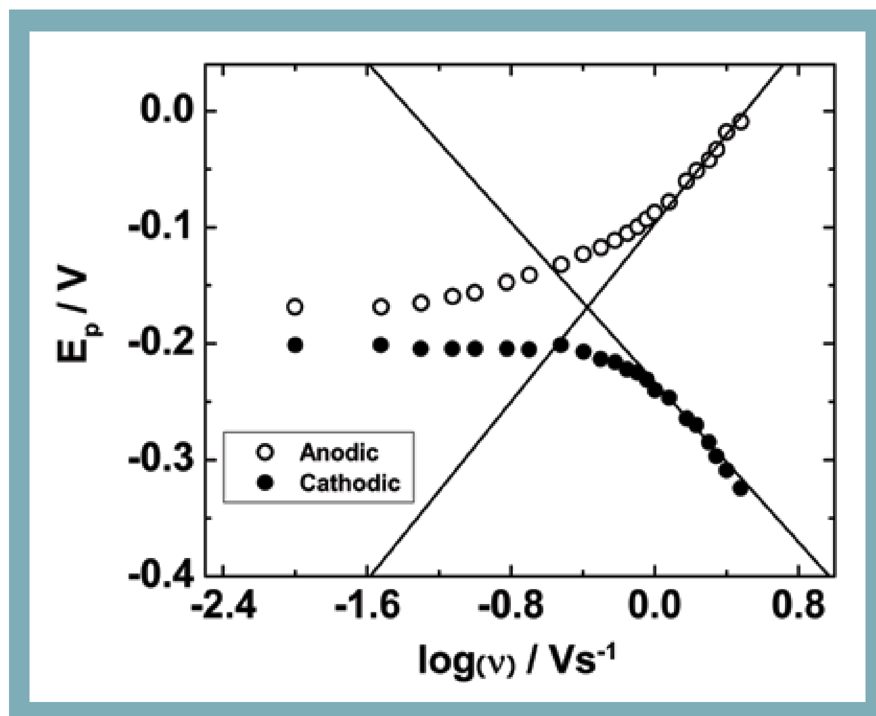

Fig. 1. *Lavion plot: dependence of the anodic (open circle) and cathodic (closed circle) peak potentials of a CYP27B1/DDAB/EPG electrode on the logarithm of the scan rate.*

From this study, we found that surfactant films assembled on carbon electrodes facilitates the direct electron transfer of CYP27B1. The results from this study form the basis of developing electrochemical-based vitamin D biosensors. Current efforts are focused on finding alternative methods of immobilization that will enable and optimize the electrocatalytic conversion of 25(OH)D_3 by CYP27B1.

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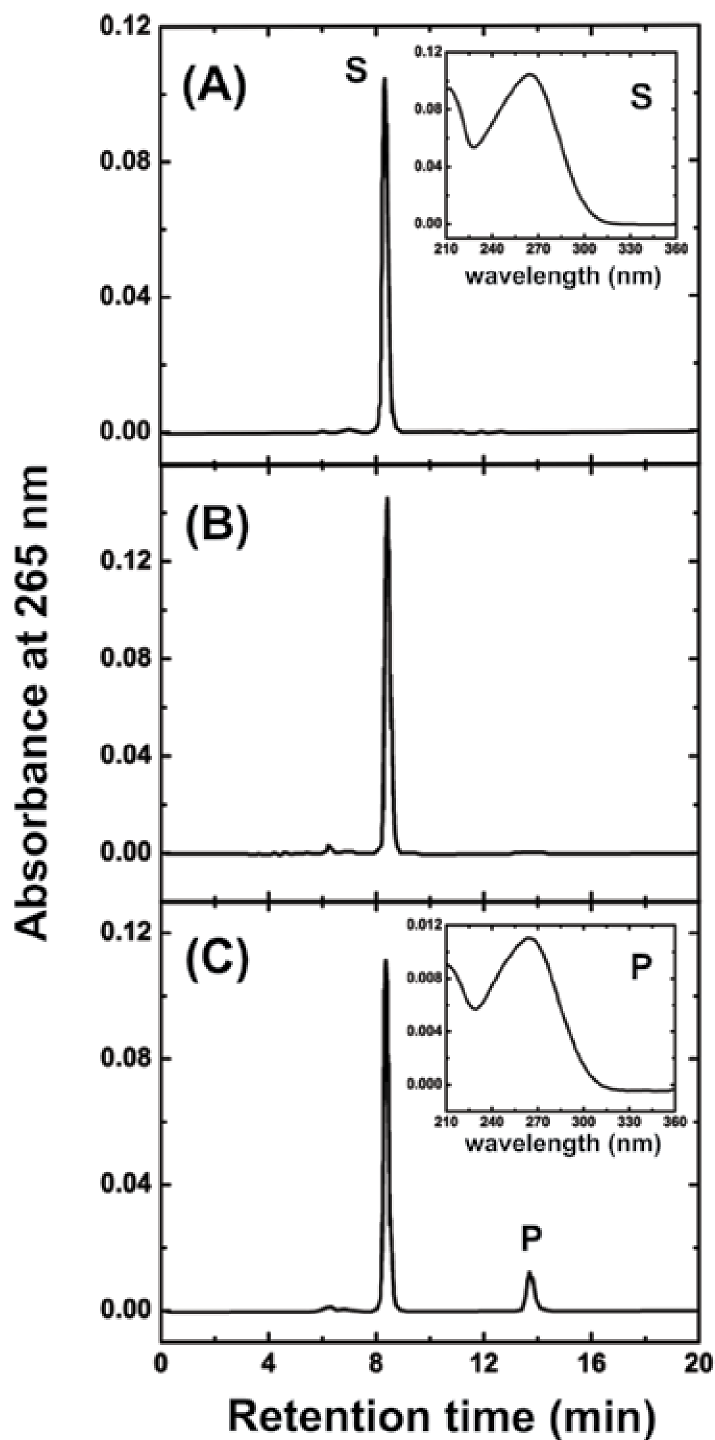

Fig. 2. *HPLC profiles for (a) synthetic standard of 25(OH)D_3, (b) extracted sample from a reconstituted assay in the presence of DDAB, (c) same conditions as (b) but without DDAB. Synthetic standard of 25(OH)D_3, S, eluted at ~8 min while enzymatic product, P, eluted at ~13.5 min. Insets show the UV spectrum of elutants, which demonstrates typical spectral characteristics of the vitamin D cis/triene chromophore.*

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