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Is the Ferric Hydroperoxy Species Responsible for Sulphur Oxidation in Cytochrome P450s?

Max J. Cryle and James J. De Voss

Experimental Section

General Methods and Instrumentation. Thin layer chromatography (TLC) was performed on Merck 60 F_{254} silica gel backed aluminum plates. Melting point determination was performed on a Büchi melting point apparatus and values are reported without correction. NMR spectra were obtained using either a Bruker DX400 spectrometer or a Bruker DRX500 spectrometer using $CDCl_3$ as the solvent. Chemical shifts are given in ppm, referenced to the residual solvent peak (δ = 7.24 for $CDCl_3$). Coupling constants (*J*) are given in Hertz (Hz). The terms s, d, t, q, quint, m refer to singlet, doublet, triplet, quartet, quintet, multiplet; br implies the signal is broad. Elemental analyses were performed by the Microanalytical Service, Department of Chemistry, at the University of Queensland. GC/MS analyses were performed on a Shimadzu GC-17A chromatograph fitted with a DB-5 column (J&W Scientific, 30 m, 0.25 mm internal diameter) connected to a Shimadzu QP-5000 mass spectrometer (70 eV). HPLC analyses were performed either on a Shimadzu LC-10AT chromatograph connected to a Shimadzu SPD-M10AVP PDA detector or a Waters 600 chromatograph connected to a Waters 2487 dual absorbance UV detector; Sulfoxides were analysed with a Chiracel OB-H column (Daicel Chemical Industries, 25 cm, 0.46 cm diameter).

Enzymatic Turnover of 1-4 with Wildtype and T268A Mutant $P450_{BM3}$. Turnover of **1-4** was performed using the following protocol: $P450_{BM3}$ (Final Concentration 2 μM), substrate (Final Concentration 2.0 mM) and catalase (Final Concentration 1 μM) were combined in a 1.5 mL Eppendorf tube in 100mM phosphate buffer (pH 7.4). NADPH (Final Concentration 1.35 mM) was added to the solution (such that the final volume of solution was 500 μL) and this was left to incubate for 30 min at 37 °C. Phenylacetic acid (Final Concentration 125 μM) was then added as an internal standard. The reaction was then loaded onto solid phase extraction (SPE) cartridges (Phenomenex, strata-X reverse phase absorbant, 30 mg/mL capacity) that had been washed with methanol (1 mL) and equilibrated with distilled water (1 mL). The samples were washed with distilled water (0.5 mL) before being dried under vacuum for 30 minutes. The samples were then eluted with ethyl acetate (0.5 mL) and treated with diazomethane. The samples were concentrated before being analysed by GCMS and the sulfoxides by enantioselective HPLC (Chiralcel OB-H column). Quantification of absolute amount of product and therefore the coupling of the substrate was performed via comparison to the internal standard referring to a standard curve (vide infra).

Enantioselective HPLC Analysis of Standards 5-6 and Products from $P450_{BM3}$ Turnover of 3-4. HPLC Program for thiafatty acid turnover analysis and standard identification: Column (Chiralcel OB-H) Flow 0.5 mL $minute^{-1}$; 15% isopropanol in hexanes; PDA-UV detector (205 nm). Retention times: *S*-**6** 24.8 min, *R*-**6** 27.3 min; *S*-**5** 29.7 min, *R*-**5** 31.3 min).

Rate of NADPH consumption. The initial rate of reaction was measured by observing the disappearance of NADPH (extinction coefficient at 340 nm, ε = 6.22 cm^{-1} mM^{-1}) and calculating the slope of the first 5 seconds of the reaction. Two identical solutions (450 μL of 2 μM $P450_{BM3}$, 100 mM potassium phosphate buffer pH 7.4, 500 μM **1-4**, 1 μM catalase) were prepared, with 50 μL of pure water added to the reference solution. Initiation of the reaction occurred upon addition of 50 μL of NADPH solution (concentration such that the final solution concentration was 300 μM) to the other solution and the absorption change at 340 nm followed over a total of 30 seconds. Four experiments were performed per substrate for both the wildtype and T268A mutant of $P450_{BM3}$.

GC Analysis of Thiafatty Acid Turnovers and Standards. GC Program for analysis of the methyl esters formed after turnover of **3-4** and subsequent diazomethane treatment plus methyl ester standards on DB-5 column: Splitless mode; Column Flow 2.5 mL $minute^{-1}$; Total Flow 46.4 mL $minute^{-1}$; Injector 250°C; Detector 250°C; Oven 100°C (1.0 min equilibration) hold for 2.0 min, ramp 16°C $minute^{-1}$ to 250°C and hold for 15.0 min (total program time 26.38 min).

Thiafatty Acid Standard and Turnover GC Retention Times. (Methyl Esters). Dimethyl undecanedioate 9.59 min, **3** 9.52 min, **5** 11.23 min (Broad Peak), Dimethyl tridecanedioate 12.33 min, **4** 12.52 min, **6** 12.10 min (Broad Peak).

Relative GCMS Response of Standards. Electron Impact, 70 eV. Quantification performed through comparison to an internal standard (phenyl acetic acid). Relative responses of Thiafatty Acid : Diacid : Sulfoxide –1.0:1.0:1.9.

Characterisation of 3-6.

12-Thiatetradecanoic acid (3). White solid (MP: 53.0-54.0 °C; Lit. MP: 58.0-61.0 °C).[1]
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (3H, t, J_1 = 7.4 Hz), 1.25-1.40 (12H, m), 1.55 (2H, quintet, J_1 = 7.5 Hz), 1.59 (2H, quintet, J_1 = 7.2 Hz), 2.32 (2H, t, J_1 = 7.5 Hz), 2.50 (4H, m), 8.00 (1H, br s).
^{13}C NMR (100 MHz, $CDCl_3$) δ 14.8, 24.6, 25.9, 28.9, 29.0, 29.17, 29.19, 29.3, 29.4, 29.6, 31.7, 34.0, 179.7.
GC/MS (methyl ester): 260 ($M^{+\cdot}$, 6.3), 199 (34.0), 149 (4.3), 101 (7.3), 87 (26.1), 75 (42.2), 55 (83.3), 41 (100).
Anal. Calcd. For $C_{13}H_{26}O_2S$: C, 63.37; H, 10.63; S, 13.01. Found C, 62.96; H, 10.84; S, 13.35.
14-Thiahexadecanoic acid (4). Cream solid (MP 55.5-56.0 °C).
^{1}H NMR (500 MHz, $CDCl_3$) δ 1.21 (3H, t, J_1 = 7.5 Hz), 1.25 (16H, m), 1.55 (2H, quintet, J_1 = 7.5 Hz), 1.61 (2H, quintet, J_1 = 7.5 Hz), 2.32 (2H, quintet, J_1 = 7.5 Hz), 2.49 (2H, q, J_1 = 7.5 Hz), 2.52 (2H, t, J_1 = 7.5 Hz), 6.53 (1H, br s).
^{13}C NMR (125 MHz, $CDCl_3$) δ 14.8, 24.7, 25.9, 28.9, 29.0, 29.21, 29.24, 29.39, 29.49, 29.53 (2C), 29.67, 31.7, 34.0, 179.5.
GC/MS (methyl ester): 288 ($M^{+\cdot}$, 9.3), 227 (69.2), 97 (13.0), 87 (35.3), 75 (53.0), 55 (97.2), 43 (56.6), 41 (100).
Anal. Calcd. For $C_{15}H_{30}O_2S$: C, 65.64; H, 11.02; S, 11.69. Found C, 65.60; H, 11.00; S, 11.62.
Methyl 11-*R*-(ethylsulfinyl)undecanoate (5). White solid (MP: 48.0-50.0 °C); 60% *ee R*-isomer by DAG route;[2, 3] 45% *ee R*-isomer by Kagan oxidation route.[4-7]
^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22-1.35 (12H, m), 1.37 (3H, t, J_1 = 6.9 Hz), 1.44 (2H, m), 1.59 (2H, quint, J_1 = 7.2 Hz), 1.78 (2H, m), 2.28 (2H, t, J_1 = 7.5 Hz), 2.70 (1H, m), 2.80 (1H, m), 2.90 (2H, m), 3.64 (3H, s).
^{13}C NMR (125 MHz, $CDCl_3$) δ 7.1, 22.7, 24.90, 24.93, 28.8, 29.07, 29.10, 29.11, 29.15, 29.22, 29.26, 34.1, 51.5, 174.3.
GC/MS: 166 (2.4), 149 (2.8), 136 (2.3), 124 (9.9), 122 (4.1), 96 (22.7), 87 (35.5), 74 (64.3), 55 (100), 41 (66.2).
Anal. Calcd. For $C_{14}H_{28}O_3S$: C, 60.83; H, 10.21. Found C, 60.92; H, 10.49.
Methyl 13-*R*-(ethylsulfinyl)tridecanoic acid (6). Cream solid (MP 56.0-57.0 °C); 60% *ee R*-isomer by DAG route;[2, 3] 15% *ee R*-isomer by Kagan oxidation route.[4-7]
^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25 (14H, multiplet), 1.32 (3H, t, J_1 = 7.5 Hz), 1.44 (2H, m), 1.60 (2H, quintet, J_1 = 7.5 Hz), 1.74 (2H, m), 2.28 (2H, quintet, J_1 = 7.5 Hz), 2.64 (4H, m), 3.65 (3H, s).
^{13}C NMR (125 MHz, $CDCl_3$) δ 6.7, 22.6, 24.9, 28.9, 29.12, 29.19, 29.21, 29.32, 29.38, 29.47, 29.48, 34.1, 45.6, 51.4, 51.8, 174.2.
GC/MS: 152 (3.4), 123 (3.0), 96 (11.4), 87 (23.1), 74 (43.5), 69 (26.1), 59 (21.1), 55 (78.4), 43 (57.4), 41 (100).
Anal. Calcd. For $C_{16}H_{32}O_3S$: C, 63.11; H, 10.59; S, 10.53. Found C, 63.14; H, 10.92; S, 10.59.

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