The Flavoprotein-Catalyzed Reduction of Aliphatic Nitro-Compounds Represents a Biocatalytic Equivalent to the Nef-Reaction

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Electronic Supporting Information

General

TLC were run on silica (Merck silica gel 60, F_{254}) or alumina (Merck aluminum oxide 150, F_{254}). ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX spectrometer at 360 and 90 MHz, resp. Chemical shifts are reported in ppm [δ] and coupling constants are given in Hz. Phenylacetone (**1c**), 2-octanone (**2c**), nitrocyclohexane (**3a**), cyclohexanoneoxime (**3b**), cyclohexanone (**3c**), 1-nitrohexane (**4a**), 1-hexanal (**4c**), 2-butanone (**5c**), 2-phenylpropanal (**6d**) were from Aldrich. NADPH and $NADP^+$ were purchased from Biocatalytics (Order number 004642), NADH, NAD^+, glucose dehydrogenase (475 U/ml) were from Codexis and glucose-6-phosphate, glucose-6-phosphate-dehydrogenase were obtained from Jülich Chiral Solutions. PETN reductase from *Enterobacter cloaceae* PB2 (PETN-Red, 2.6 mg/ml), morphinone reductase from *Pseudomonas putida* M10 (Mor-Red, 1.9 mg/ml), *N*-ethyl maleimide reductase from *Escherichia coli* W2252 (NemA, 1.9 mg/ml) were provided by Neil C. Bruce (Department of Biology, University of York). *Lycopersicon esculentum* OPR3 and YqjM from *Bacillus subtilis* were expressed and purified as recently reported.[1] The open reading frame of *Lycopersicon esculentum* OPR1 was cloned into pET21a and expressed as a C-terminal hexahistidine tagged protein in *E. coli* BL21 cells and the expressed recombinant protein was purified on a Ni-NTA affinity column (Invitrogen) according to the manufacturer's protocol. *Zymomonas mobilis* reductase,[2] OYE1, OYE2, OYE3, [3] YcnD[4] and YhdA from *Bacillus subtilis*[5] and Lot6P from *Saccharomyces cerevisiae* were expressed and purified as previously reported.[6]

Synthesis of Substrates and Reference Material

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2-Nitro-1-phenylpropane (1a) and (*E/Z*)-1-phenyl-2-propanoneoxime (1b): 2-Nitro-1-phenyl-1-propene (1.4g, 8.78mmol) was dissolved in MeOH (5ml) and was hydrogenated at atmospheric pressure at room temperature in presence of 10% Pd/C (72 mg) as catalyst. After 40min, the mixture was filtered through Celite, evaporated to dryness and purified *via* flash chromatography on silica (ethyl acetate/petroleum ether 1:1) to yield **1a** (591.7mg, 3.63mmol, 41%) and **1b** (141mg**,** 0.864mmol, 10%).[7]

1-Phenyl-2-nitro propane (1a): TLC: R_f 0.76 (silica, ethyl acetate/petroleum ether 1:1; vanillin/sulfuric acid); GC-MS (EI): *m/z* 165, 118, 91, 77; ^{1}H-NMR (MeOD): δ 1.53 (3H, d, J = 6.62), 3.04-3.09 (1H, dd, J = 5.95 and 14), 3.21-3.32 (1H, dd, J = 8.2 and 14), 4.7 (1H, m), 7.19-7.32 (5H, m); ^{13}C-NMR (MeOD): δ 17.90, 40.58, 84.50, 126.82, 128.27, 128.68, 130.08. NMR data corresponded to literature.[8]

(*E/Z*)-1-Phenyl-2-propanoneoxime (1b): TLC: R_f 0.64 (silica, ethyl acetate/petroleum ether 1:1, vanillin/sulfuric acid); GC-MS (EI): *m/z* 149, 131, 116, 91; ^{1}H-NMR (CD_3COCD_3): δ 1.72 (3H, s), 3.46 (2H, s), 3.73 (2H, s), 7.21-7.33 (5H, m), 9.79 (N-OH, s, br); ^{13}C-NMR (CD_3COCD_3): δ 11.93, 18.70, 33.94, 41.57, 126.13, 126.42, 128.4, 128.83, 129.05, 137.66, 137.87, 154.73, 154.93. NMR data corresponded to literature.[9,10,11]

(*E/Z*)-Octan-2-oneoxime (2b): Method 1: Octane-2-amine (65µl, 0.387mmol) in CH_2Cl_2 (2ml) was added dropwise into a stirred solution of *m*-CPBA (240mg, 0.93mmol, 67%) in 9ml CH_2Cl_2. Within one hour a yellow colour change could be observed. $Na_2S_2O_5$ (177 mg, 0.93mmol) was added and stirring was continued for 30min. Afterwards the mixture was filtered through Celite, washed with $NaHCO_3$ (3 x 11ml) and evaporated to dryness yielding 83% of **2b** (83.1mg, 0.58mmol).[12] Method 2: To a mixture of 2-octanone (1g, 7.80mmol) and hydroxylamine hydrochloride (813mg, 11.7mmol) in ethanol (10ml), pyridine (0.5ml) was added. The mixture was refluxed for two hours and after completion of the reaction, ethanol was evaporated, the residue was extracted with EtOAc, washed several times with 3M HCl and water, dried over Na_2SO_4, evaporated and purified by flash chromatography on alumina (ethyl acetate/ petroleum ether 1:100) to yield 48% of **2b** (477mg, 3.3mmol).[13] TLC: R_f 0.22 (alumina, ethyl acetate/petroleum ether 1:10, $KMnO_4$); GC-MS (EI): *m/z* 143, 129, 114, 101, 86, 73; ^{1}H-NMR (CD_3COCD_3): δ 0.88 (3H, t, J = 6.64), 1.29 (6H, s), 1.47 (2H, m), 1.79 (3H, s), 2.17 (2H, t, J = 7.40 and 15.1), 9.34-9.38 (N-OH, s, br); ^{13}C-NMR (CD_3COCD_3): δ 12.09, 13.42, 18.89, 22.34, 25.33, 26.15, 27.89, 31.45, 35.32, 155.71, 155.79. The NMR data were corresponding to literature.[14]

2-Nitrooctane (2a): 2-Octanoneoxime (**2b**, 50mg, 0.35mmol) was dissolved in 3.19ml of glacial acetic acid in a dry three-necked round bottomed flask and immersed in an oil bath at a

temperature of 55° C. Solid sodium perborate tetrahydrate (271mg, 175mmol) was added in portions with vigorous stirring over a period of 30min. When addition was complete, the reaction mixture was stirred at the same temperature for 6h. After cooling, the reaction mixture was treated with an ice cold saturated solution of sodium bicarbonate (4 x 1ml), followed by extraction with diethyl ether (2 x 1ml), washed several times with cold water, dried over Na_2SO_4, evaporated and purified by flash chromatography on alumina (ethyl acetate/petroleum ether 1:10) to give **2a** as an orange liquid (17.8mg, 0.112mmol, 35.6%).[15] TLC: R_f 0.29 (alumina, ethyl acetate/petroleum ether 1:1, $KMnO_4$); ^{1}H-NMR ($CDCl_3$): δ 0.84-0.96 (3H, t, J = 6.27), 1.26-1.28 (6H, m), 1.53-1.57 (3H, d, J = 6.63), 2.09-2.17 (2H, m), 4.53-4.59 (1H, m); ^{13}C-NMR ($CDCl_3$): δ 13.95, 19.17, 22.44, 25.61, 28.83, 29.68, 35.17, 83.56.

2-Nitrobutane (5a): 2-Nitro-butane **(5a)** was synthesized according to the same procedure used for 2-nitrooctane to give **5a** as a liquid (115mg, 1.3mmol, 33%).[15] TLC R_f 0.51 (silica, ethyl acetate/petroleum ether 1:10, Et_3N, $KMnO_4$); ^{1}H-NMR ($CDCl_3$): δ 0.85-0.95 (3H, m), 1.20-1.27 (m, 2H), 1.7 (3H, d, J = 6.77), 3.4-3.5 (1H, m).

1-Nitro-2-phenylprop-1-ene (6a): To a stirred mixture of acetic anhydride (40 ml) and nitric acid (5.28 g, 65%) was added 2-phenylpropene (3.2 ml, 12.2 mmol) at 0 °C. After 20min, the solution was poured into water (180 ml) and stirred for additional 30min. The organic layer was washed with saturated aqueous $NaHCO_3$, water and then dried (Na_2SO_4). Removal of the solvent under reduced pressure gave an oily residue of crude 2-acetoxy-1-nitro-2-phenylpropane, that was purified *via* flash chromatography on silica (ethyl acetate/petroleum ether 1:30). A solution of the nitroacetate in triethylamine (15 ml) and chloroform (30 ml) was stirred for 3 h at room temperature. After the addition of HCl (2N, 30 ml), the mixture was extracted with dichloromethane and dried (Na_2SO_4). Evaporation of the solvent followed by flash chromatography on silica (ethyl acetate/petroleum ether 1:25) afforded 1-nitro-2-phenylprop-1-ene **6a** as yellow oil (788mg, 4,83mmol, 40%).[15] GC-MS (EI): *m/z* 39, 44, 51, 65, 77, 91, 115, 120, 130, 135, 145, 163; ^{1}H-NMR ($CDCl_3$): δ 2.66 (3H, d, J = 1.3); 7.32 (1H, d, J = 1.4); 7.46 (5H, s). NMR data corresponded to literature.[16]

1-Nitro-2-phenylpropane (6b): *trans*-β-Nitrostyrene (0.45 g, 3 mmol) in 20 ml dry Et_2O was added to methylmagnesium iodide (5 ml of a 3M solution, 15 mmol) in 40 ml of dry Et_2O at -20 °C. Within 10min, the solution was added to ice cold 5% aqueous HCl solution and stirred for 30min. The mixture was extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and the solvent was evaporated to give 1-nitro-2-phenylpropane **6b** (124 mg, 0.75 mmol, 25%).[15] ^{1}H-NMR ($CDCl_3$): δ 1.4 (3H, d, J = 7.0), 3.62-3.72 (1H, m), 4.47-4.60 (2H, m), 7.23-7.38 (5H,

m); ^{13}C-NMR ($CDCl_3$): δ 18.7, 38.6, 81.9, 126.9, 127.6, 129.0, 140.9. NMR data corresponded to literature.[16,17]

***(E/Z)*-2-Phenyl-propanaloxime (6c):** To a stirred solution of 2-phenylpropanal (200µL, 1.79mmol) in 5mL of ethanol, hydroxylamine hydrochloride (249mg, 3.58mmol) was added together with pyridine (435µL, 5.37mmol). After 24h the mixture was washed with water (20ml) and extracted twice with ethyl acetate. The combined organic layers were washed with brine and dried over Na_2SO_4 and evaporated. The product was purified by flash chromatography on silica (ethyl acetate /petroleum ether 10:50) to give **6c** as a liquid (116µl, 1.04mmol, 58%).[13] GC-MS (EI): *m/z* 39, 51, 63, 77, 91, 105, 117, 132, 149; ^{1}H-NMR ($CDCl_3$): δ 1.46-1.51 (3H, d, J = 6.48), 3.67-3.76 (1H, m), 4.45-4.52 (1H, m), 7.26-7.40 (5H, m), 7.55-7.58 (1H, d, J = 7.2), 8.21 (OH s, br); ^{13}C-NMR ($CDCl_3$): δ 18.31, 18.79, 35.01, 40.40, 126.78, 126.97, 127.23, 127.43, 128.73, 128.77, 141.87, 142.12, 141.87, 142.12, 154.75, 155.27. NMR data corresponded to literature.[18]

Analytical procedures

For all GC-MS measurements an Agilent 7890A GC system equipped with an Agilent 5975C mass-selective detector (EI 70 eV) with a (5%-phenyl)-methylpolysiloxane phase column (Agilent HP-5ms, 30 m, 250 µm, 0.25 µm) was used. Helium was used as carrier gas (flow 2 mL/min). GC-FID analyses were carried out on a Varian 3800 using H_2 as carrier gas (14.5 psi).

Determination of conversion

Conversion of substrates were analyzed by GC-FID using a 6% cyanopropyl-phenyl phase capillary column (Varian CB1701, 30m, 0.25mm, 0.25µm), detector temperature 240°C. Temperature program for **1a-1c**: 70°C hold 0min, 5°C/min to 140°C, hold 0min, 25°C/min to 170°C, hold 15min. Retention times: **1a** 17.01min, **1b** 16.18min and 16.31min, **1c** 11.36min. Temperature program for **2a-2c** and **5a, 5c:** 40°C hold 0min, 5°C/min to 140°C, hold 0min, 25°C/min to 170°C, hold 1min. Retention times: **2a** 12.66min, **2b** 17.03min and 17.17min, **2c** 6.86min, **5a** 5.99min, **5c** 2,73min. Temperature program for **3a, 3b, 3c:** 70°C hold 0min, 5°C/min to 140°C, hold 0min, 25°C/min to 170°C, hold 0min. Retention times: **3a** 11.22min, **3b** 10.12min and **3c** 5.47min. Temperature program for **4a** and **4b:** 40°C hold 0min, 5°C/min to 140°C, hold 0min, 25°C/min to 170°C, hold 15min. Retention time: **4a** 9.09min and **4b** 3.54min. Temperature program for **6a-6d**: 70°C hold 0min, 5°C/min to 140°C, hold 0min, 25°C/min to 170°C, hold 15min. Retention times: **6a** 14.84min and 15.44min, **6b** 13.69min, **6c** 12.48min and 13.06min**, 6d** 6.54min.

*Determination of Enantiomeric Excess and Absolute Configuration of **6b***

Enantiomeric excess of 1-nitro-2-phenylpropane **6b** was determined using a cyclodextrin capillary column (CP-Chirasil-DEX CB, 25 m, 0.32 mm, 0.25 μm film). Temperature program for **6b**: 105 °C hold 5min, 1 °C/min to 115 °C, hold 1min, 15 °C/min to 180 °C, hold 2min. Retention times: *(S)*-**6b** and *(R)*-**6b** 16.67min and 17.00min. The absolute configuration of **6b** was determined *via* co-injection with independently synthesized reference material.[19]

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