Proton-Coupled-Electron Transfer Enhances the Electrocatalytic Reduction of Nitrite to NO in a Bioinspired Copper Complex

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**Scheme 1.** The synthetic route to obtain complexes [**3**-H2O]2+ and [**4**-H2O]+.

EXPERIMENTAL SECTION

**General Experimental Remarks:** Tetrabutylammonium hexafluorophosphate (TBA-PF6 >98%) was supplied by TCI. Bis(2-pyridylmethyl)amine (97%), benzoic acid (99.5%), Cu(ClO4)2·6H2O (98%), sodium nitrite (min. 99.0%) and tetrabutylammonium nitrite were purchased from Sigma Aldrich. All chemical reagents and solvents were used as purchased. Electronic spectra were collected in quartz cuvettes on a JASCO V-670 spectrophotometer. Solution-phase IR spectra were collected with a Shimadzu FTIR-8400S and solid-state IR spectra were obtained on a Thermo Scientific Nicolet iS5. X-band EPR spectra were recorded on a Bruker ELEXSYS E500 spectrometer, and simulations performed with Bruker’s Xsophe software package.[[1]](#endnote-1) All 1H and 13C NMR spectra were recorded on a Bruker AV 400 instrument, at a constant temperature of 300 K. Chemical shifts are reported in parts per million from low to high field. Coupling constants (*J*) are reported in hertz (Hz). Standard abbreviations indicating multiplicity were used as follows: m = multiplet, t = triplet, d = doublet, s = singlet. CHN analyses were collected by the services facility at the School of Chemistry, University of Glasgow, as were LM-MS mass spectra (ESI, positive mode, Bruker micrOTOF-Q machine).

**Compound 1:** To a solution of 6-chloromethylpyridine-2-carboxylic acid methyl ester[[2]](#endnote-2) (270 mg, 1.45 mmol, 1 equiv.) in CH3CN (14 mL) was added 848 mg of Na2CO3 (8 mmol, 5.5 equiv.), giving a yellowish suspension. To this mixture, a solution of bis(2-pyridylmethyl)amine (319 mg, 1.6 mmol, 1.1 equiv.) in CH3CN (8 mL) was added, and the resulting mixture heated to reflux for 24 h. No change of color was seen when the amine was added, but as the reaction mixture was heated up it turned orange. After 24 h reflux, the mixture was allowed to cool to room temperature, and then filtered to remove the Na2CO3. The filtrate was then concentrated *in vacuo*, affording crude compound **1** as a tan solid. This solid was then triturated with ethyl acetate/hexane (4:1), yielding an off-white solid and an orange liquid. Isolation of the solid and a further round of trituration in ethyl acetate/hexane (4:1) then yielded pure compound **1** (380 mg, 1.09 mmol, 75%). 1H NMR (MeOD, 400 MHz), δ = 8.44 – 8.42 (m, 2H, Ha), 7.98 (dd, *J1* = 8, *J2* = 1, 1H, Hg or Hi), 7.92 (t, *J* = 8, 1H, Hh), 7.86 (dd, *J1* = 8, *J2* = 1, 1H, Hg or Hi), 7.78 (dt, *J1* = 8, *J2* = 2, 2H, Hb or Hc), 7.68 (d, *J* = 8, 2H, Hd), 7.29 – 7.24 (m, 2H, Hb or Hc), 3.96 (s, 3H, Hj), 3.94 (s, 2H, Hf), 3.88 (s, 4H, He). Letter codes correspond to those shown on the 1H NMR spectrum of this compound in the Supporting Information to this paper (Figure S1). 13C NMR (MeOD, 400 MHz), δ = 166.7, 161.0, 159.8, 149.5, 148.0, 139.1, 138.5, 127.9, 124.8, 124.6, 123.7, 61.1, 60.6, 53.0. ESI-LMMS (methanol): *m*/*z* = 371.1464 [M+Na]+ (calcd. for C20H20N4NaO2; 371.1484).

**Compound 2:** 498 mg (1.43 mmol) of compound **1** were dissolved in 5 mL of ethanol with the aid of ultrasonication. To this solution, 20 mL 0.5 M NaOH was then added and the mixture heated to reflux for 20 h. After this time, the reaction mixture was allowed to cool to room temperature and concentrated *in vacuo* to remove the ethanol. The resulting reaction mixture was then acidified to pH 4 with aqueous HCl and then extracted with CHCl3 (2 × 100 mL). The combined organic extracts were then dried over MgSO4 and concentrated under reduced pressure to yield compound **2** as a yellow oil (200 mg, 0.598 mmol, 42%). The compound gave spectra in agreement with those previously reported for this compound.[[3]](#endnote-3)

**Complex 3-H2O:** 95 mg (0.273 mmol, 1 equiv.) of compound **1** was dissolved in 3.5 mL of ethanol, giving a bright yellow solution. This solution was then added (with stirring) to a solution of 104 mg (0.273 mmol, 1 equiv.) of Cu(ClO4)2·6H2O in 3.5 mL EtOH, leading to the immediate formation of a dark blue precipitate. The reaction mixture was stirred for a further 10 minutes, after which it was filtered, yielding complex **3**-H2O as a dark blue solid (160 mg, 0.254 mmol, 93%). Anal. calcd. for C20H22Cl2CuN4O11: C, 38.20; H, 3.53; N, 8.91. Found: C, 38.13; H, 3.16; N, 8.89. ESI-LMMS (methanol): *m*/*z* = 411.0517 [M-H2O]+ (calcd. for C20H20CuN4O2; 411.0882).

**Complex 4-H2O:** 82 mg (0.24 mmol, 1.1 equiv.) of Cu(ClO4)2·6H2O were dissolved in the 3 mL of EtOH, giving a light blue solution. Meanwhile, 74 mg (0.22 mmol, 1 equiv.) of compound **2** were dissolved 3 mL of ethanol, giving a dark yellow-orange solution, and this solution then added to the blue copper solution. A greenish-blue precipitate immediately formed, which was filtered off and dried, giving complex **4**-H2O(0.074 g, 0.144 mmol, 60%). Anal. calcd. for C19H19ClCuN4O7: C, 44.37; H, 3.72; N, 10.89. Found: C, 44.68; H, 3.37; N, 10.73. MS-FAB/NOVA (low resolution): *m*/*z* = 396 ([M]+–H2O); ESI-LMMS (methanol): 396.0624 ([M]+–H2O) (calcd. for C19H17CuN4O2; 396.0648).

**Crystallography:** Crystallographic data were collected at the University of Glasgow on a Bruker APEX-II CCD diffractometer for [**3**-CH3CN](ClO4)2, and using a Bruker D8Venture with PhotonII detector and dual Imus 3.0 micro-source for [**4**-NO2]. For [**3**-CH3CN]2+, a blue, block-shaped crystal of dimensions 0.42 × 0.4 × 0.31 mm was used for single crystal X-ray diffraction data collection. C22H23CuN5O2·2(ClO4) crystallized in the triclinic space group *P*-1(space group No. 2), with unit cell dimensions *a* = 8.4706(7), *b* = 8.8319(8), *c* = 17.982(2) Å, α = 95.471(2)°, β = 99.637(2)°, γ = 102.415(2)° and *V* = 1283.2(2) Å3, T = 100 K. 15864 reflections were measured by ω scans, 5798 independent reflections with *R*int = 0.068, θmax = 27.4°, θmin = 1.2° using Mo *Kα* radiation, λ = 0.71073 Å. The structure was solved using Superflip[[4]](#endnote-4)-[[5]](#endnote-5)[[6]](#endnote-6) and refined using SHELXL[[7]](#endnote-7) within OLEX2[[8]](#endnote-8) which was also used for molecular graphics and to prepare material for publication. CCDC entry 1588602 contains the supplementary crystallographic data for this compound.

For [**4**-NO2], a green block-shaped crystal of dimensions 0.39 × 0.33 × 0.16 mm was used for single crystal X-ray diffraction data collection. C19H17CuN5O4·0.5(C2H3N) crystallized in the orthorhombic space group *Pbcn*, with unit cell dimensions *a* = 14.817(8), *b* = 19.01(1), *c* = 15.569(9) Å and *V* = 4386(4) Å3, T = 298 K. 8296 reflections were measured by ω scans, 5403 independent reflections with *R*int = 0.037, θmax = 28.3°, θmin = 2.2° using Mo *Kα* radiation, λ = 0.71073 Å. The structure was solved using XT[[9]](#endnote-9) and refined using SHELXL[[10]](#endnote-10) within OLEX2, which was again used for molecular graphics and to prepare material for publication. The nitrite nitrogen atom is well-ordered; however, the oxygen atoms show disorder and were modelled as follows: O1N was modelled as fully-occupied and common to both partially-occupied orientations of O2N and O3N, each of which was modelled as 0.5-occupied. All oxygen atoms show large atomic displacements. Distance similarity restraints were applied to the N1N–O2N and N1N–O3N distances. CCDC entry 1588603 contains the supplementary crystallographic data for this compound. More details on the crystallographic data and its collection can be found in the Supporting Information.

**Electrochemical Methods:** Electrochemical studies were performed in a three-electrode configuration using CH Instruments CHI760D potentiostats. For cyclic voltammetry, a Pt wire was used as the counter electrode, along with an Ag/AgNO3 pseudo reference electrode (CH Instruments). Potentials are reported relative to the ferrocenium/ferrocene couple, the position of which was judged by adding ferrocene to the samples analyzed. Working electrodes were washed with acetone and deionized water prior to use. Cyclic voltammograms were collected at room temperature under an atmosphere of Ar at a scan rate of 100 mV s–1, unless otherwise noted. A glassy carbon button electrode (area = 0.071 cm2, CH Instruments) was used as the working electrode for cyclic voltammetry. The supporting electrolyte was 0.1 M TBA-PF6 in acetonitrile, unless otherwise noted. Measurements were conducted without stirring and with *i*R compensation enabled. The *i*R test function available on the CH potentiostats uses the general method developed by He and Faulkner.[[11]](#endnote-11) Bulk electrolyses were carried out in 0.2 M TBA-PF6 in acetonitrile (15 mL) in a sealed single-chamber cell (headspace volume = 97 mL), using an Ag/AgNO3 pseudo reference electrode, a Pt wire counter electrode and a large area glassy carbon (Carbon-Vitreous 3000C (C) foil, 1.0 mm thickness, GoodFellow) foil electrode of area 4.6 cm2. Solutions were stirred during bulk electrolyses, which were conducted without considering any resistive losses; solution resistances were measured using the *i*R test function (as for cyclic voltammetry) and were found to be on the order of 10–30 Ω. At the currents typically passing in these experiments, the voltage drops caused by this uncompensated resistance were thus generally under 1 mV, and could be neglected.

**Colorimetric NO determination:** TheNO generated during bulk electrolysis was quantified in an airtight cell (headspace volume = 97 mL) in 0.2 M TBA-PF6 in acetonitrile (15 mL) using an Ag/AgNO3 pseudo reference electrode, a Pt wire counter electrode and a large area glassy carbon foil working electrode (area 4.6 cm2). The concentration of compound **3** or compound **4** employed was 7 × 10–6 M. The electrolyte also contained 120 equivalents of benzoic acid and 120 equivalents of TBA-NO2 relative to compound **3** or **4**. A control reaction was also performed containing these amounts of benzoic acid and TBA-NO2 but in the absence of any catalyst. A 14 mL vial was glued to the internal wall of the airtight cell above the level of the electrolyte solution, into which was placed 5 mL of an 8 × 10–5 M Co-TPP solution in dichloromethane, the concentration of which was cross-checked using the molar extinction coefficient reported by Berry and co-workers.[[12]](#endnote-12) The vial was left open at the top, so that NO in the headspace could diffuse into the Co-TPP solution, but the Co-TPP solution and the electrolyte could not mix. The electrolyte and headspace of the cell were thoroughly degassed with Ar for 30 minutes before initiation of electrolysis. Bulk electrolyses were then conducted at –0.91 V (*vs.* ferrocenium/ferrocene) with stirring. At the end of electrolysis, the solution continued to be stirred and the cell was left sealed for a further two hours, in order to allow time for NO in the headspace to diffuse fully into the Co-TPP solution. An aliquot of the Co-TPP solution was then withdrawn from the 14 mL vial, and its electronic spectrum measured. Given a Henry’s Law coefficient for the solubility of NO in CH3CN at room temperature of 1.35 × 108 Pa,[[13]](#endnote-13) the amount of NO dissolved in solution was calculated to be negligible under these conditions and was thus ignored.

A calibration curve equating the shift in the position of the Co-TPP absorbance band at λmax ≈ 530 nm was constructed (see Figures S10 and S11), using a similar sealed cell configuration to that described above with a dichloromethane/Co-TPP solution in an open vial attached to the inner wall of the cell, but where the Co-TPP solution was not in contact with the liquid in the main cell. The main body of the cell was then purged with Ar and filled with 20 mL of a 50 mM solution of ascorbic acid in glacial acetic acid/H2O (respectively 18 mL/2 mL), which is known to generate NO stoichiometrically from nitrite.[[14]](#endnote-14) Known aliquots of sodium nitrite (as a solution in water) were then added to this solution, which was then stirred in the sealed cell for 2 hours. After this time, an aliquot of the Co-TPP solution was withdrawn from the 14 mL vial, and its electronic spectrum measured. From this, a graph of shift in λmax *vs.* amount of NO generated was constructed.

**Quantification of nitric oxide release by chemiluminescence:** Bulk electrolysis was conducted as for the colorimetric tests for NO in identical sealed cells. NO release measurements by chemiluminescence were then performed using a Sievers NOA 280i chemiluminescence nitric oxide analyzer. The instrument was calibrated by passing air through a zero filter (Sievers, <1 ppb NO) and 89.8 ppm NO gas (BOC, balance nitrogen). The flow rate was set to 200 mL min–1 with a cell pressure of 6.5 Torr and an oxygen pressure of 6.1 psig. To measure NO production, nitrogen gas was flushed through the electrochemical cell, the resultant gas directed into the analyzer, and the concentration of NO recorded. The limit of detection of this analyzer is 0.5 ppb.

**Calculations.** The program package ORCA was used for all calculations.[[15]](#endnote-15) The input geometry for all molecules were generated using ArgusLab. The geometries of all molecules were fully optimized by a spin-unrestricted DFT method employing the BP86 functional with acetonitrile as solvent.[[16]](#endnote-16),[[17]](#endnote-17) Split-valence basis sets with one set of polarization functions (def2-SVP) were used for all atoms.[[18]](#endnote-18),[[19]](#endnote-19) A scalar relativistic correction was applied using the zeroth-order regular approximation (ZORA) method.[[20]](#endnote-20)-[[21]](#endnote-21)[[22]](#endnote-22) The RIJCOSX approximation combined with the appropriate Ahlrichs auxiliary basis set was used to speed up the calculations.[[23]](#endnote-23)-[[24]](#endnote-24)[[25]](#endnote-25) The conductor like screening model (COSMO) was used for all calculations.[[26]](#endnote-26) The self-consistent field calculations were tightly converged (1 × 10–8 *E*h in energy, 1 × 10–7 *E*h in the charge density, and 1 × 10–7 in the maximum element of the DIIS[[27]](#endnote-27),[[28]](#endnote-28) error vector). The geometry was converged with the following convergence criteria: change in energy <10–5 *E*h, average force <5 × 10–4 *E*h Bohr–1, and the maximum force 10–4 *E*h Bohr–1. The geometry search for all complexes was carried out in redundant internal coordinates without imposing geometry constraints. The stability of all solutions was checked by performing frequency calculations: no negative frequencies were observed. Single point calculations were performed on optimized coordinates using the PBE0 functional[[29]](#endnote-29),[[30]](#endnote-30) and triple-ζ-quality basis sets with one set of polarization functions (def2-TZVP) for all atoms.29

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