Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supporting Information

Direct Electron Transfer between Cyt c and Heme-Aβ relevant to Alzheimer's Disease†

*Chandradeep Ghosh, Soumya Mukherjee and Somdatta Ghosh Dey**

Department of Inorganic Chemistry, Indian Association for the Cultivation of Science, Kolkata, India, 700032

AUTHOR EMAIL ADDRESS: icsgd@iacs.res.in

Experimental details:

Materials:

All reagents were of the highest grade commercially available and were used without further purification. Amyloid beta (Aβ) peptide (1-16) (Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys) used in this study was purchased from GL Biochem (Shanghai) Ltd. with >95% purity. Hemin, Cytochrome C and the HEPES buffer were purchased from Sigma. Sodium dithionite was purchased from Merck.

Instrumentation:

All the absorption spectra were obtained by a UV-vis diode array spectrophotometer (Agilent 8453). EPR spectra were obtained by a Jeol (JES FA200) spectrophotometer at 77 K in a liquid nitrogen finger dewar. Parameters for the high-energy region: modulation width, 12 gauss; amplitude, 140; time const, 300 ms; power, 10 mW; frequency, 9.27 GHz. Resonance Raman data were collected using a Trivista 555 triple spectrograph (Princeton Instrument) and 413.1 nm excitation from Kr+ laser (Coherent, Sabre Innova SBRC-DBW-K). Gratings used in the three stages were 900, 900 and 1800 grooves/mm. The optics (plano-convex lens, mirror etc), used for the collection of Raman data were purchased from Sigma-Koki Japan. Power on the sample was ~10 to 15 mW. Raman shifts were calibrated with naphthalene and indene. Data acquisitions were done for 300 seconds. The wave number accuracy was ± 1 cm-1 for well defined peaks.

Sample Preparation and Spectral Characterization:

The peptide stock solutions were made of 0.5 mM strength in 100 mM pH 7 hepes buffer. Heme-Aβ sample was prepared by incubating 0.8 equivalent of heme to Aβ for 6 hrs. Reduced heme-Aβ was prepared by reducing the heme-Aβ sample using sodium dithionite. Cyt c solution of concentration 2-3 mM was prepared in 100 mM pH 7 hepes and the exact concentration was determined from its absorbance at 550 nm for the completely reduced species. All the sample solutions made for EPR and sesonance Raman were 0.5 mM and diluted to 0.0125 mM in pH 7 hepes buffer for absorption studies. For EPR and rR studies the pH of heme-Aβ samples were calibrated to pH 7.

The kinetics of the electron transfer reactions were followed spectrophotometrically at low temperature (2 ˚C). The rate constant for the reaction was determined by monitoring the changes in the absorption intensity at 550 nm. The reactions were carried out at two different pH (pH 7 and 10), three different buffer concentrations (10 mM, 100 mM and 1 M) at pH 7 and adding KCl as an external electrolyte to the buffer at pH 7.

PROS calculation:

For PROS calculation, a xylenol orange assay was performed as follows. A total of 4.9 mg of Mohr's salt and 3.9 mg of xylenol orange were dissolved in 5 mL, 250 mM H_2SO_4 and stirred for 10 min. A total of 200 µL of this solution was taken in 1.8 mL of nanopure water and a calibration curve for the quantitative estimation of H_2O_2 was obtained for 0.05, 0.1, 0.5, 1, 2.5, 5, and 10 µM concentrations of H_2O_2 by recording their absorbance at 560 nm (Figure S4). The calibration curve was expressed as absorbance at a fixed wavelength of 560 nm versus concentration of H_2O_2 in micromolar units for a 2 mL volume. For the detection of PROS of an unknown quantity, a blank was obtained in the UV-Vis spectrophotometer with 1.8 mL of nanopure water in a cuvette. A total of 200 µL of the xylenol orange solution was added to this cuvette and the absorbance was recorded. This served as the control. Heme-Aβ complex was reduced by dithionite under anaerobic conditions (observed by absorption spectroscopy), followed by reoxidation by O_2 (followed by absorption spectroscopy). A total of 200 µL of 0.025 mM reoxidized solution was added to the cuvette containing the control. Absorbance of this solution was recorded. The value of absorbance of the above solution (after subtracting the control) at 560 nm when plotted on the calibration curve yielded the corresponding H_2O_2 concentration.

Figures:

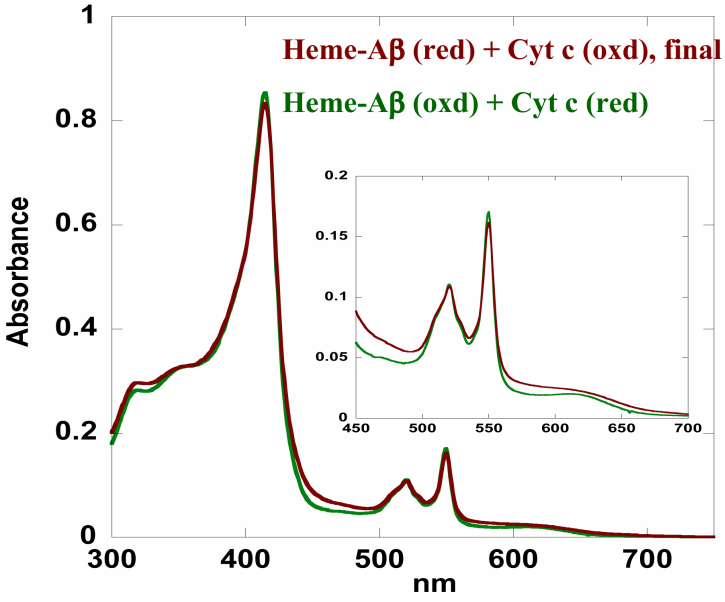

Figure S1. Final absorption spectra on reacting reduced heme-Aβ with equimolar oxidized Cyt c (brown) overlaid with oxidized heme-Aβ and reduced Cyt c mixture (green).

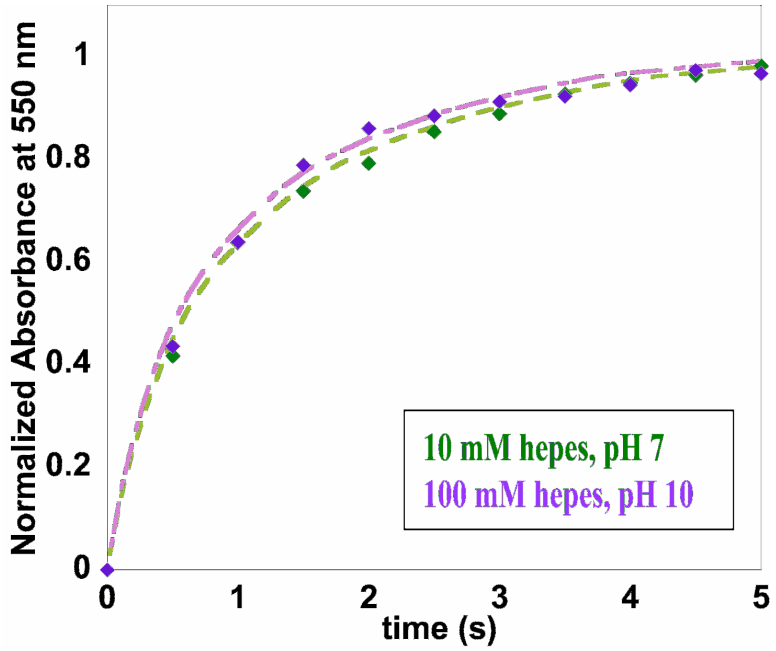

Figure S2. Kinetic traces of the increase in absorption intensity at 550 nm as a function of time (A vs. time plot) at different reaction conditions.

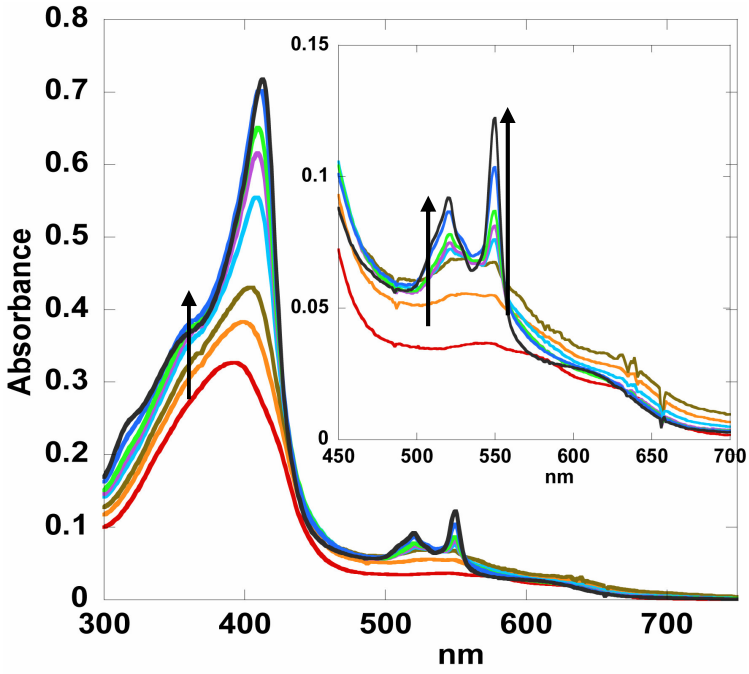

Figure S3. Change in absorption spectra of 1:1 mixture of reduced heme-Aβ and oxidized Cyt c in the presence of 1 M KCl in 100 mM hepes buffer at pH 7. The red spectrum is at 0 secs while the final black spectrum is recorded after 13 secs.

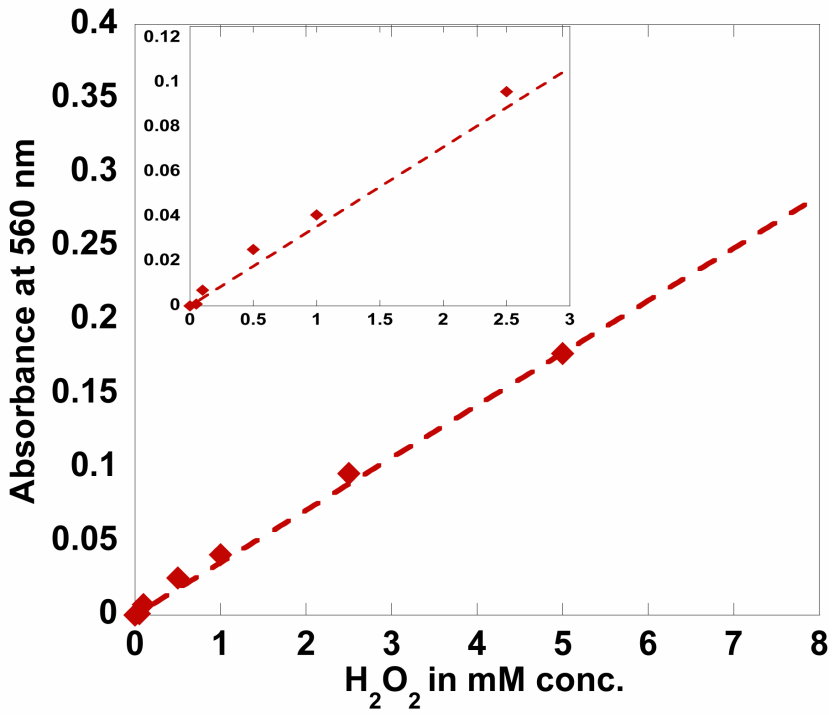

Figure S4. H_2O_2 detection by the Xylenol orange method.