Heme-Aβ Compound 0: A Common Intermediate in ROS Generation and Peroxidase Activity

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Supporting Information

Experimental details

Materials and methods

General methods

All reagents were of the highest grade, commercially available and were used without further purification. Aβ(1-16) was purchased from Shanghai Yaxian Chemical Co., Ltd., China with >95% purity. Hemin, Phosphate buffer, m-CPBA, H_2O_2, dopamine and D_2O were purchased from Sigma. Dimethylformamide (DMF) were purchased from Merck.

Sample preparation

Peptide and heme stock solution were prepared in DMF. The peptide stock solution was prepared at a concentration of 1 mM. Heme stock solution was prepared at a concentration of 10 mM. The peptide and heme solutions were mixed in a 1 : 1 ratio and kept for ~4 hours at room temperature for the formation of heme–Aβ(1–16).

Stopped-Flow Analysis

Stopped-flow analyses of heme-Aβ complexes with m-CPBA or a 1:1 H_2O_2–NEt_3 mixture were conducted on an SFM 4000 stopped-flow absorption spectrophotometer (Xe lamp). Reactions were initiated by mixing heme–Aβ complexes (75 μM) with m-CPBA (0.75 mM) or a 1:1 H_2O_2–NEt_3 mixture in a 1:10 concentration ratio in DMF at room temperature.

Dopamine Oxidation Kinetics

Kinetic experiments for dopamine oxidation by heme–Aβ(1–16) were performed in DMF and 100 mM phosphate buffer (pH 7) at room temperature. The molar ratios used were:

In DMF:

Heme–Aβ : m-CPBA : dopamine = 1 : 2000 : 800

Heme–Aβ : H_2O_2 : dopamine = 1 : 2000 : 800 (H_2O_2 used as a 1:1 mixture of $NEt_3 \cdot H_2O_2$).

In Phosphate Buffer (pH 7):

Heme–Aβ : H_2O_2 : dopamine = 1 : 2000 : 800.

Kinetic traces were recorded by monitoring the absorbance increase at 475 nm over time. Control experiments were conducted under identical conditions with final concentrations in the cuvette of 0.25 μM free heme, 0.25 μM Aβ, 0.5 mM oxidant (H_2O_2/m-CPBA), and 0.2 mM dopamine. Each kinetic trace represents the average of three independent runs.

EPR

EPR spectra were recorded on a JEOL FA200 spectrometer. Samples, prepared at a concentration of 1 mM heme-Aβ complex, were treated with 10 equivalents of m-CPBA (10 mM) or 0.5 mM heme-Aβ complex, were treated with 10 equivalents 1:1 H_2O_2–NEt_3 (5 mM) mixture at -55°C. Following the addition, the samples were frozen in liquid nitrogen. Spectra were acquired at 77 K using a liquid nitrogen finger dewar. The EPR settings were: frequency ~9.13 GHz, power ~2 mW, modulation width 14 Gauss, amplitude 40.00, time constant 0.03 sec, and sweep time 30 sec.

Resonance Raman

RR data were collected using a Trivista 555 spectrograph (Princeton Instruments) with 413.1 nm excitation from a Kr^+ laser (Coherent, Sabre Innova SBRC-DBW-K). Optical components, including the plano-convex lens and mirror, were obtained from Sigma-Koki, Japan. The laser power on the samples was set at approximately 2 mW and 10 mW. Samples, prepared at a concentration of 1 mM heme-Aβ complex, were treated with 10 equivalents of m-CPBA (10 mM) or 0.5 mM heme-Aβ complex, were treated with 10 equivalents 1:1 H_2O_2–NEt_3 (5 mM) mixture at -55°C. Spectra were acquired at 77 K using a liquid nitrogen finger dewar.

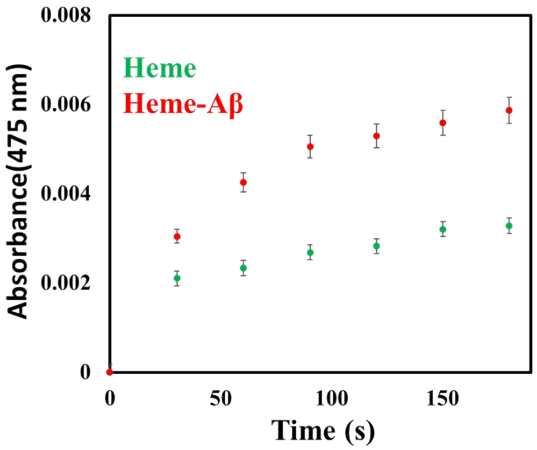

Figure S1: *Kinetic traces of dopamine oxidation (at 475 nm) in DMF: high-spin heme–Aβ(1–16) and H_2O_2, red; free heme and H_2O_2, green.*

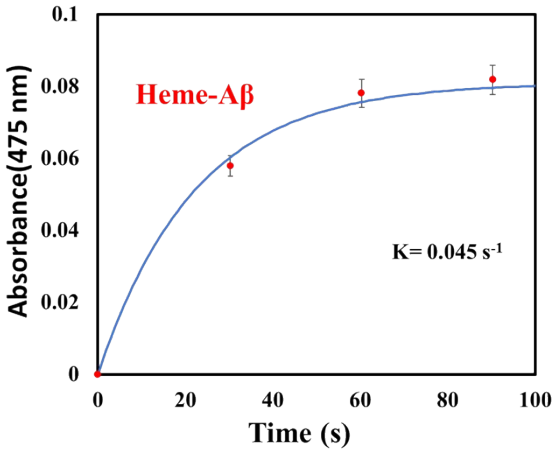

Figure S2: *Fit of kinetic traces for dopamine oxidation at 475 nm in DMF with high-spin heme–Aβ(1–16) and m-CPBA as the oxidant.*

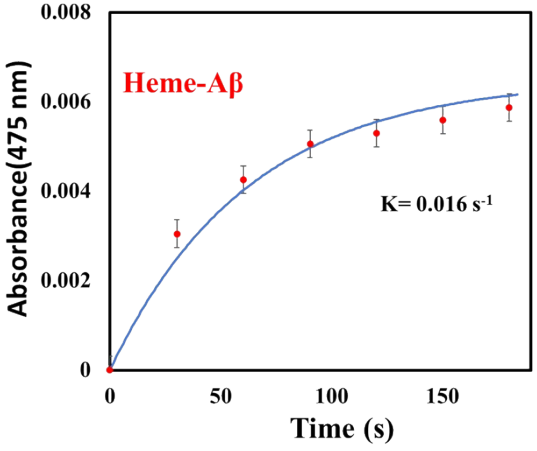

Figure S3: *Fit of kinetic traces for dopamine oxidation at 475 nm in DMF with high-spin heme–Aβ(1–16) and H_2O_2 (as a 1:1 mixture of $NEt_3 \cdot H_2O_2$) as the oxidant.*

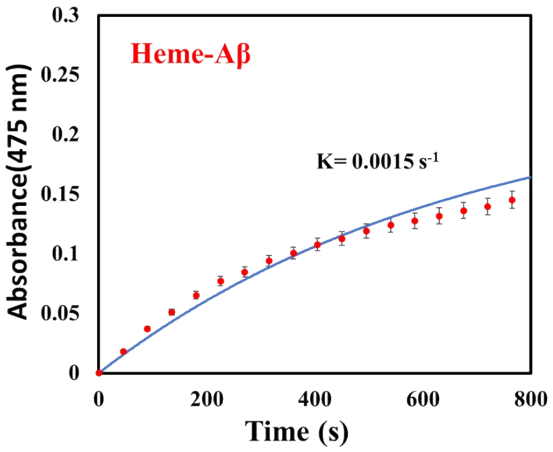

Figure S4: *fit of Kinetic traces of dopamine oxidation (at 475 nm) in phosphate buffer: high-spin heme–Aβ(1–16) and H_2O_2.*

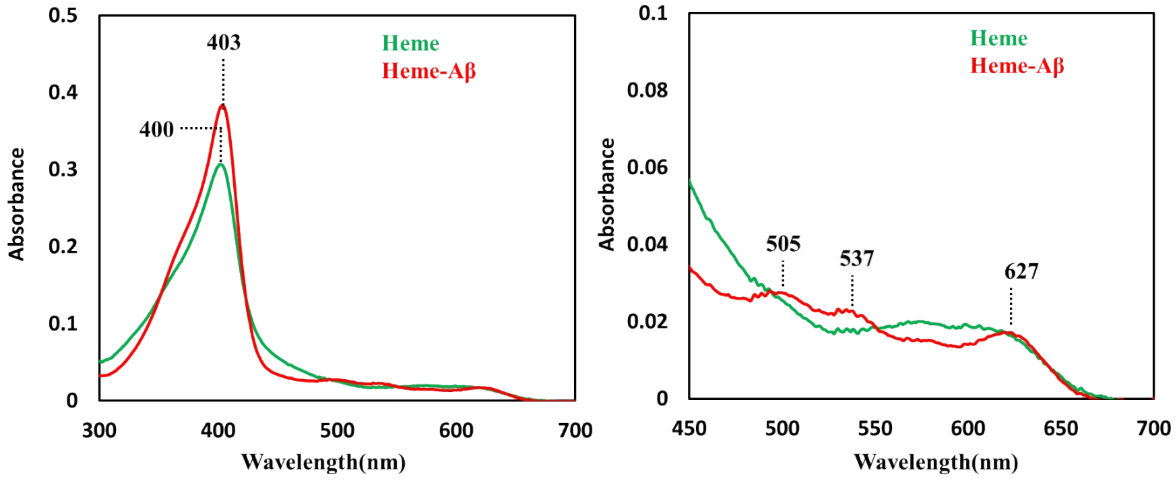

Figure S5: A. *Absorption spectra* ***B.*** *enlarged Q region of free heme, green; heme bound Aβ(1–16), red in DMF medium.*

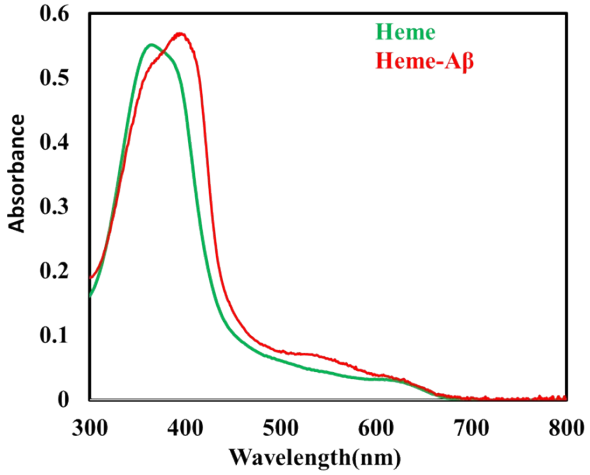

Figure S6: *Absorption spectra of free heme, green; heme bound Aβ(1–16), red in phosphate buffer at pH 7.*

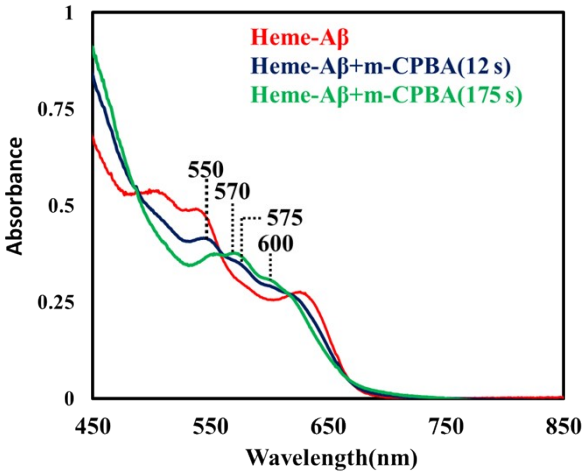

Figure S7: *Q-band region of the visible absorption spectrum showing changes when high-spin heme–Aβ(1–16) reacts with m-CPBA.*

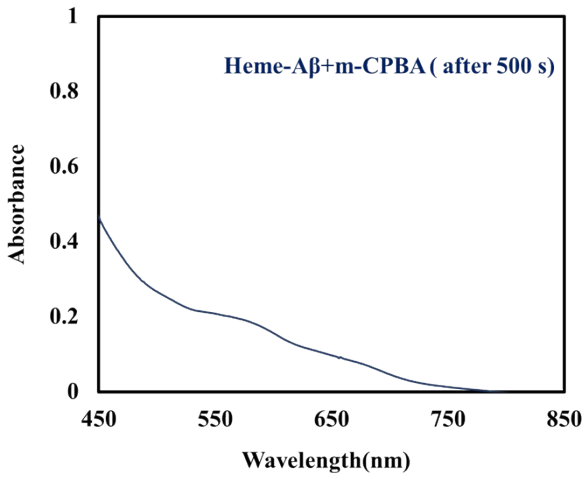

Figure S8: *The Q-band region of the visible absorption spectrum showing changes observed when high-spin heme–Aβ(1–16) reacts with m-CPBA in DMF over an extended period.*

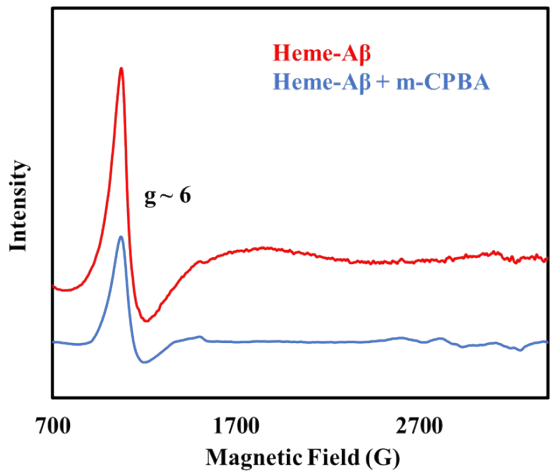

Figure S9: *EPR spectra of high spin heme–Aβ(1-16) and the reaction mixture of high-spin heme–Aβ(1-16) and m-CPBA in DMF at 77 K.*

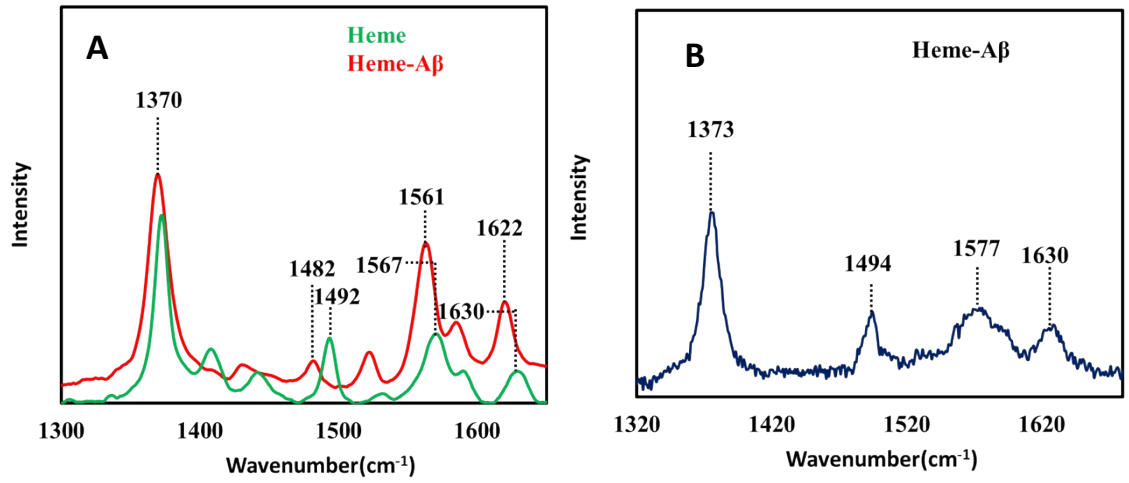

Figure S10: (A) *Resonance raman spectra of free heme, green; heme bound Aβ(1–16), red in DMF. (B) Resonance raman spectra of heme bound Aβ(1–16), blue in phosphate buffer pH 7.*

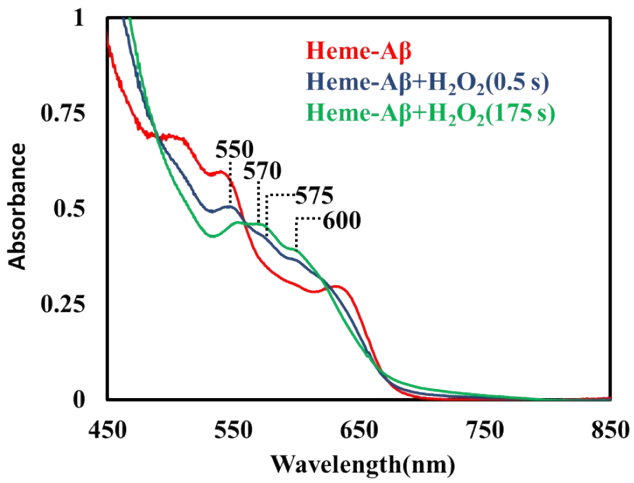

Figure S11: *Q-band region of the visible absorption spectrum showing changes when high-spin heme–Aβ(1–16) reacts with H_2O_2 in DMF.*

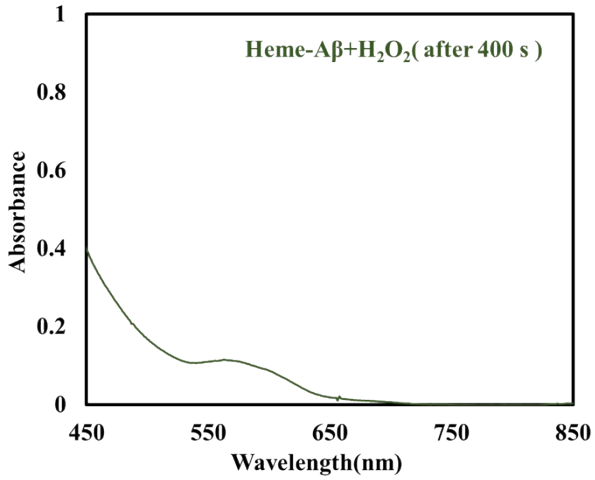

Figure S12: *The Q-band region of the visible absorption spectrum showing changes observed when high-spin heme–Aβ(1–16) reacts with H_2O_2 in DMF over an extended period.*

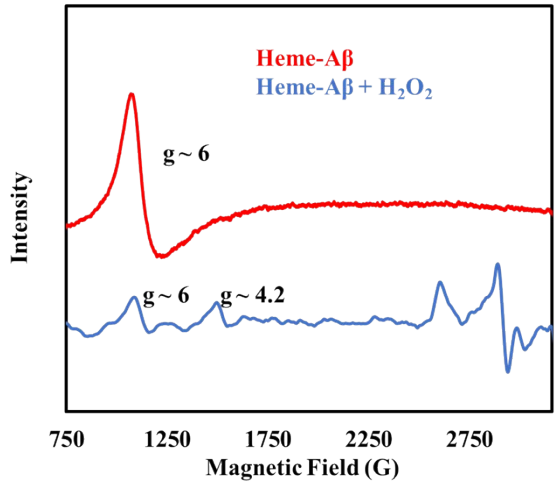

Figure S13: *EPR spectra of high spin heme–Aβ(1-16) and the reaction mixture of high-spin heme–Aβ and H_2O_2 in DMF at 77 K.*

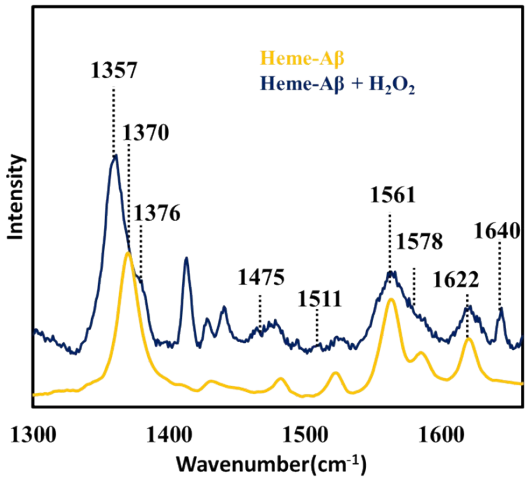

Figure S14: *High-frequency region of the resonance Raman spectrum of heme–Aβ(1–16), yellow; for the reaction of heme–Aβ(1–16) + H_2O_2, blue in DMF at 77 K.*

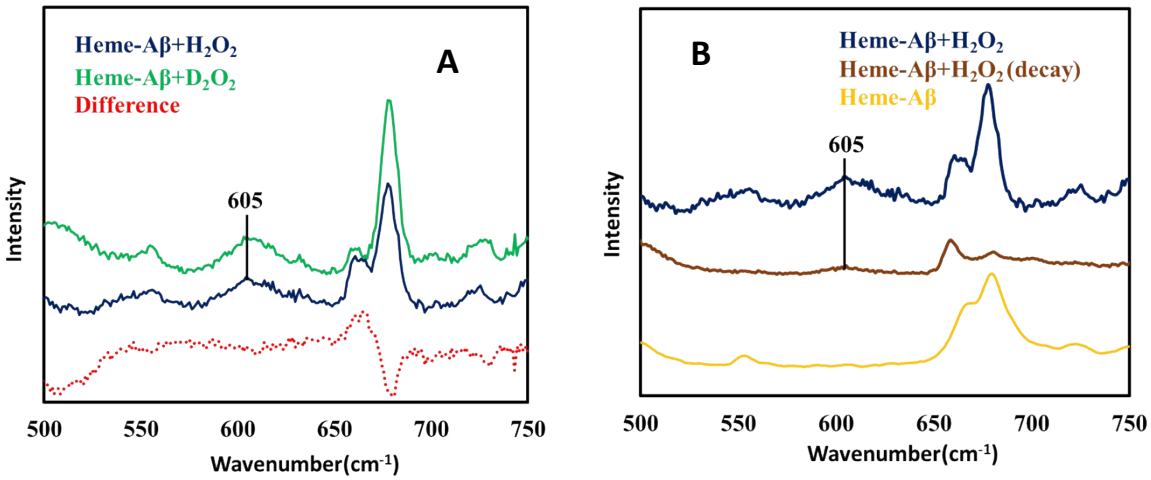

Figure S15: *(A) Low-frequency region highlighting the tentative Fe–O stretching mode in the reaction of Heme-Aβ with H_2O_2 (blue) and D_2O_2 (green) in DMF. The red spectrum represents the difference spectrum. (B) Low-frequency region showing the Fe–O stretching mode in the reaction of Heme-Aβ with H_2O_2 (blue), the decay product of Heme-Aβ and H_2O_2 (brown), and Heme-Aβ alone (yellow) in DMF.*