Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Supplementary information

Peroxidase activity enhancement of horse cytochrome *c* by dimerization

Zhonghua Wang,[a,b] Takashi Matsuo,[a] Satoshi Nagao[a] and Shun Hirota*[a]

[a] *Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan,* [b] *College of Chemistry and Chemical Engineering, China West Normal University, Nanchong, Sichuan 637002, China.*

Fax: (+81)-743-72-6119; Tel: (+81)-743-72-6110; E-mail: hirota@ms.naist.jp

Table S1 Formation rate constants of cyt *c* Compound I and the oxidation rates of cyt *c*-catalyzed oxidation of guaiacol in the presence of *m*CPBA

Cyt *c*	Compound I formation rate (ms^{-1}) [a]	Guaiacol oxidation rate [b]
Monomer	0.0032 ± 0.0002	0.91 ± 0.02
Dimer	0.019 ± 0.001	5.5 ± 0.1

[a] Pseudo first-order rate constants of Compound I formation were obtained by fitting the absorbance increase at 595 nm to single exponential functions. Reaction conditions: 50 μM protein (heme unit); 2.5 mM *m*CPBA.

[b] Conditions: 2 μM protein (heme unit); 2.5 mM *m*CPBA; 100 μM guaiacol. The unit is μmol product/(μmol heme·sec).

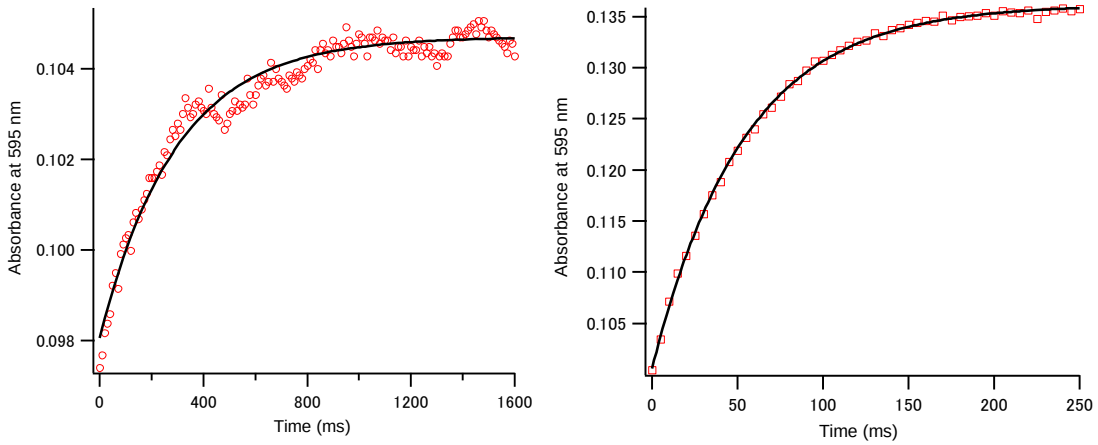

Fig. S1 Time-dependent absorbance changes at 595 nm of monomeric (left) and dimeric (right) cyt *c* by reaction with *m*CPBA. The pseudo first-order rate constants of Compound I formation are obtained by least-square fitting the data to single exponential functions (solid lines). Conditions: 50 μM cyt *c* protein (heme unit); 2.5 mM *m*CPBA; 50 mM potassium phosphate buffer, pH 7.0; 25°C.

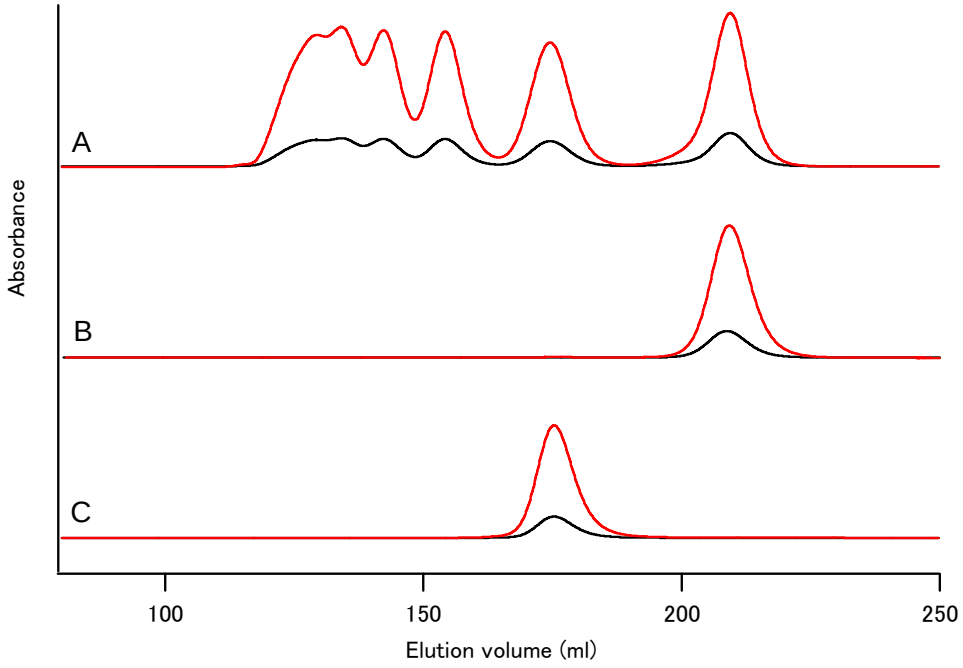

Fig. S2 Elution curves of horse cyt *c*. (A) Elution curve before separation. (B) Elution curve of monomeric cyt *c*. (C) Elution curve of dimeric cyt *c*. Absorbance at 408 nm (red) and 208 nm (black). Conditions: column: Hiload 26/60 Superdex 75; flow rate: 0.8 ml/min; solvent: 50 mM potassium phosphate buffer, pH 7.0; temperature, 4°C.

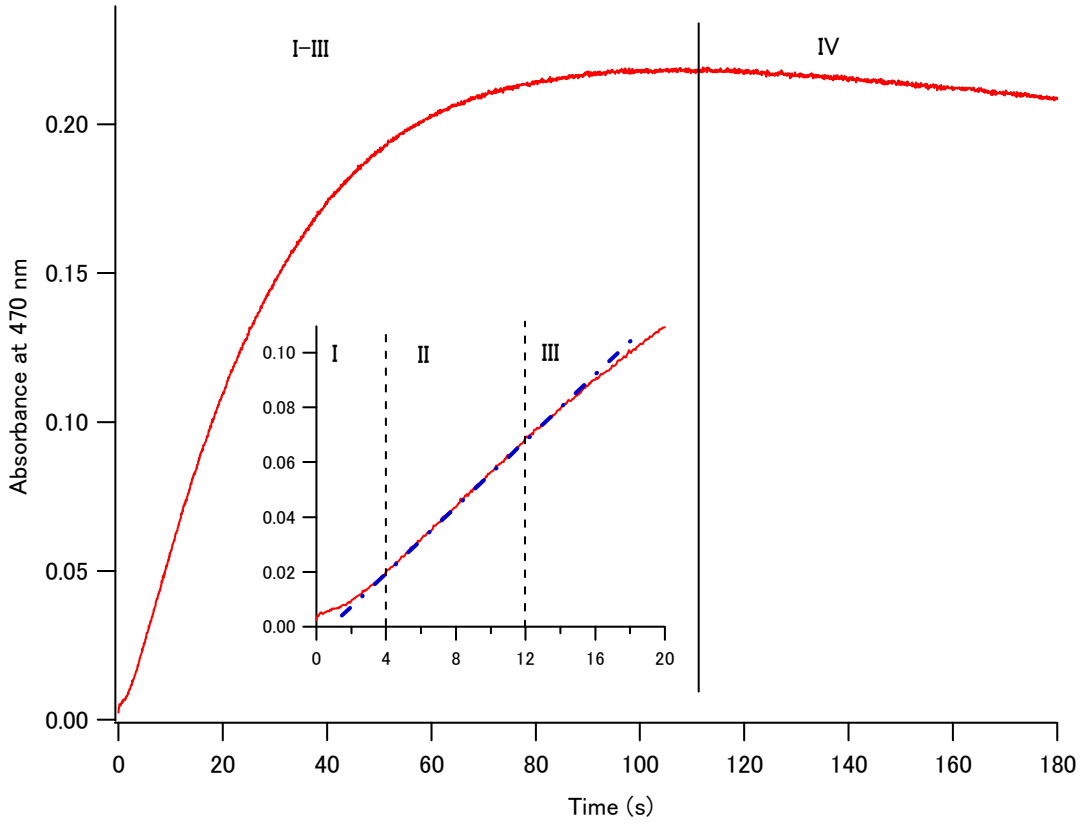

Fig. S3 Formation curve of guaiacol oxidation product catalyzed by monomeric cyt *c*. Absorbance change was monitored at 470 nm. Conditions: 2 μM monomeric horse cyt *c*; 100 μM guaiacol; 50 mM H_2O_2; 50 mM potassium phosphate buffer, pH 7.0; 25°C. Inset: The first 20-second curve of the product formation. The four phases labeled I–IV are explained in the text.