

SUPPORTING INFORMATION

Cytochrome c is an oxidative stress-activated plasmalogenase that cleaves plasmenylcholine and plasmenylethanolamine at the *sn*-1 vinyl-ether linkage

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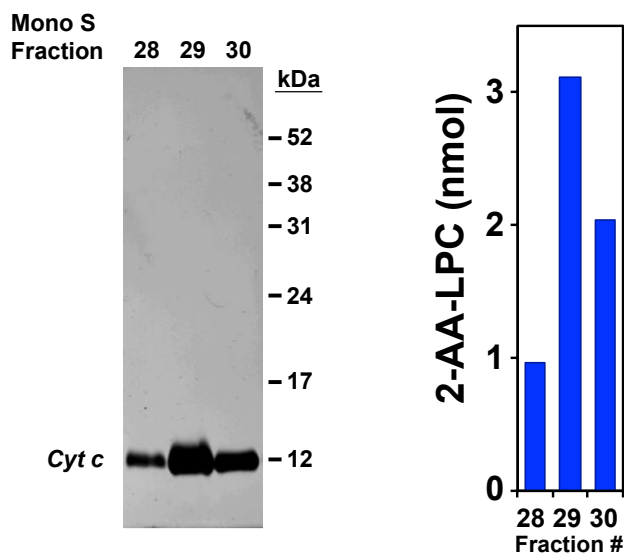


Figure S1: SDS-PAGE Silver Staining of Mono S FPLC Fractions of Cyt c Demonstrating Co-chromatography of cyt c mass with Plasmalogenase Activity. Cytochrome c (0.5 mg) was loaded onto a Mono S FPLC column equilibrated with 20 mM HEPES, pH 7.4 containing 0.1 mM DTPA followed by elution with a 0-500 mM linear gradient of NaCl. Fractions as indicated were collected and electrophoresed by SDS-PAGE and silver stained (*left panel*) and assayed for H₂O₂-dependent plasmalogenase activity (*right panel*) as described in *Experimental Procedures*.

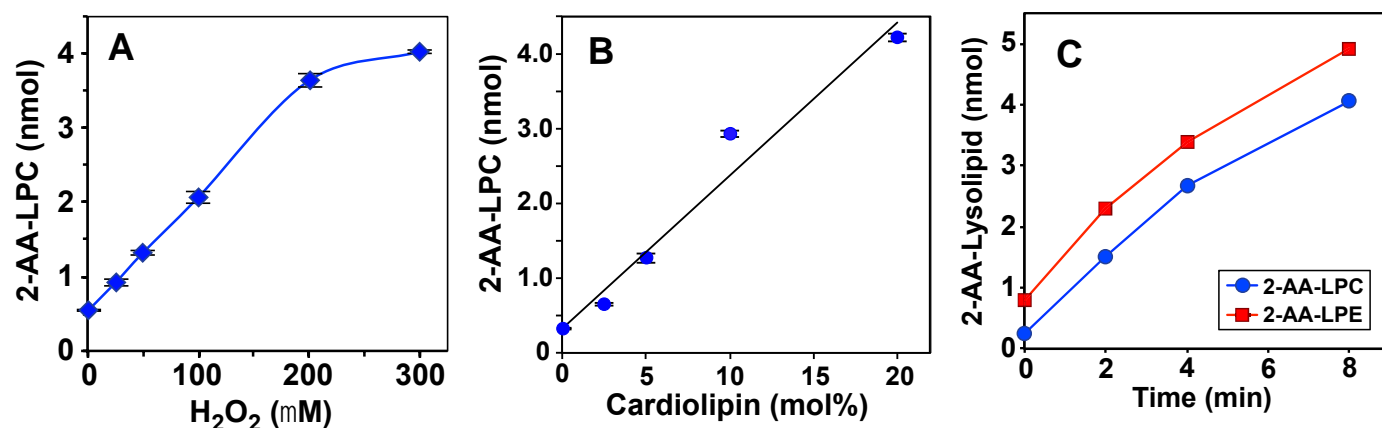


Figure S2: Dependence of Plasmalogenase Activity on H₂O₂, Cardiolipin, and Kinetic Analysis of Plasmenylcholine and Plasmenylethanolamine Vinyl Ether Cleavage by Cyt c. **A**, Hydrogen peroxide at the indicated concentrations was incubated with plasmenyl-SAPC (250 μ M)/CL (25 μ M) SUVs in the presence of cyt c (10 μ M) in 10 mM potassium phosphate, pH 7.0 containing 0.25 mM DTPA (200 μ L reaction volume) for 10 min at 37°C. **B**, Bovine heart cardiolipin (predominantly tetra-18:2 CL) as guest at the indicated mol% in host plasmenyl-SAPC (250 μ M) SUVs were incubated in the presence of cyt c (5 μ M) and H₂O₂ (200 μ M) for 5 min at 37°C. **C**, Kinetic analysis of the plasmalogenase reaction using plasmenyl-SAPC (250 μ M)/CL (25 μ M) SUVs or plasmenyl-SAPE (125 μ M)/POPC (125 μ M)/CL (25 μ M) SUVs in 20 mM HEPES, pH 7.0 containing 0.1 mM DTPA (200 μ L reaction volume) incubated at 37°C for 10 min. Following addition of internal standards (17:0-LPC and di-14:1-PC for plasmenyl-SAPC and 14:0-LPE and di-16:1-PE for plasmenyl-SAPE), reaction products were extracted and analyzed by ESI-MS as described in *Experimental Procedures*.

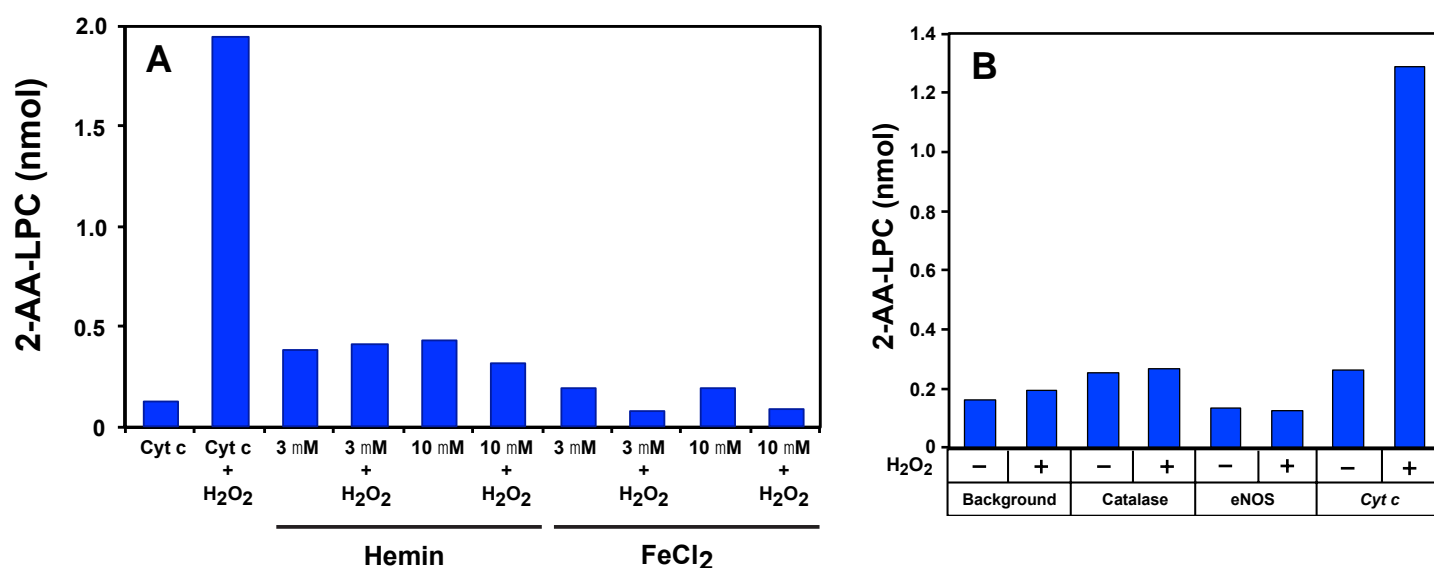


Figure S3: *Plasmalogenase Activity of Catalase, Nitric Oxide Synthase, Hemin or FeCl₂ Utilizing Plasmenyl-SAPC/CL SUVs Incubated in the Presence or Absence of Hydrogen Peroxide.* **A**, Hemin or FeCl₂ at the indicated concentrations were incubated with plasmenyl-SAPC (250 μM)/CL (25 μM) vesicles in the presence or absence of H₂O₂ (250 μM) in 10 mM potassium phosphate, pH 7.0 containing 0.25 mM DTPA (200 μL reaction volume) for 20 min at 37°C. **B**, Catalase (2.5 μM) or recombinant endothelial nitric oxide synthase (eNOS) (1 unit/reaction) were incubated with plasmenyl-SAPC (250 μM)/CL (25 μM) vesicles in the presence or absence of H₂O₂ (250 μM) in 10 mM potassium phosphate, pH 7.0 containing 0.25 mM DTPA for 5 min at 37°C. NADPH (1 mM), CaCl₂ (0.5 mM) and calmodulin (2.5 μg) were included in the reactions with eNOS. Following extraction (Bligh-Dyer) into chloroform, the amount of 2-AA-LPC was determined by ESI-MS using 17:0 LPC as internal standard as described in *Experimental Procedures*. Cyt c (10 μM) alone and cyt c + H₂O₂ served as negative and positive controls, respectively.

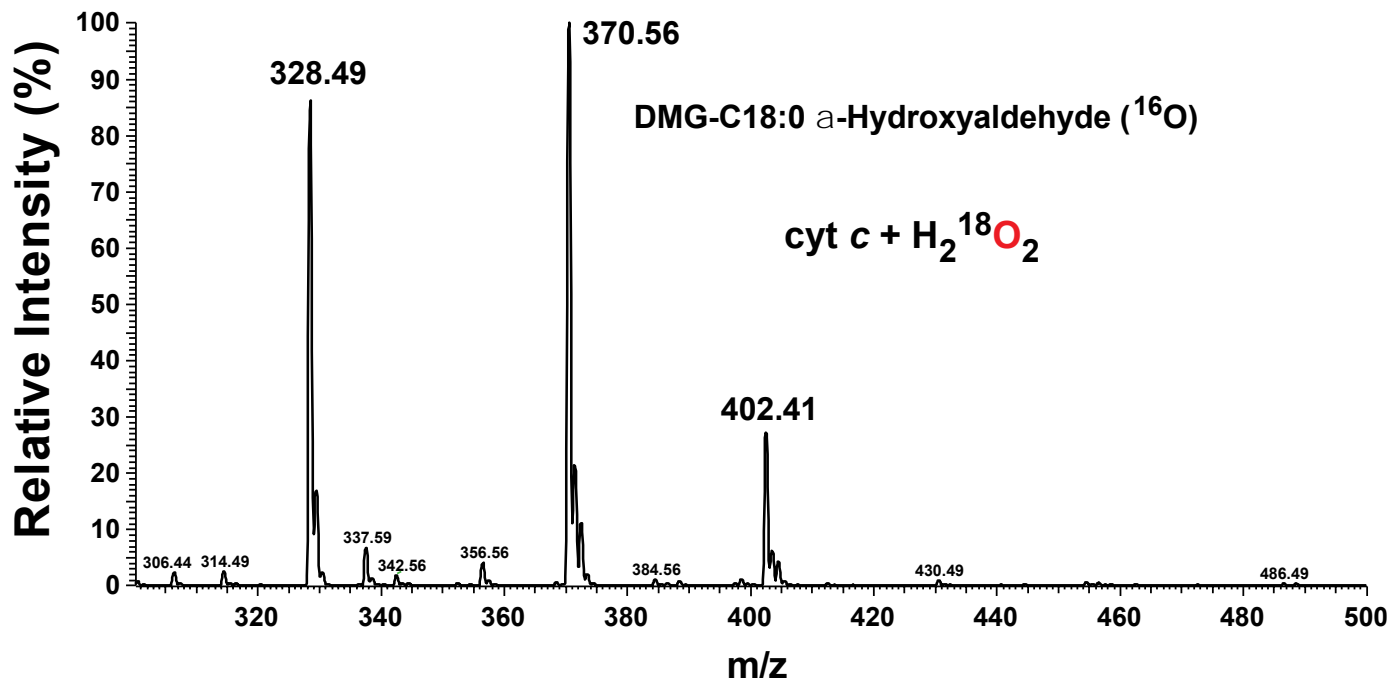


Figure S4: Oxygen in ^{18}O -Hydrogen Peroxide is Not Incorporated into the α -Hydroxyaldehyde Produced by the Plasmalogenase Activity of Cytochrome *c*. Plasmenyl-SAPC vesicles containing 10 mol% CL were incubated in the presence of $\text{H}_2^{18}\text{O}_2$ for 30 min at 37°C . Reaction products were extracted after addition of C16:0 alcohol as internal standard, derivatized with DMG and analyzed by mass spectrometry as described in *Experimental Procedures*. The ion peaks at $m/z = 370.56$ and 328.49 correspond to the C18:0 α -hydroxyaldehyde DMG derivative and C16:0 alcohol DMG derivative, respectively.