Electronic Supplementary Information

Suppression of Amyloid-β Adsorption on Endoplasmic Reticulum Stress-Mimicking Membranes by α-Tocopherol and α-Tocotrienol

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Materials and Methods

Materials

The unsaturated lipid 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and saturated lipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). The fluorescent probe rhodamine B 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (Rhod-DHPE) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Rhod-DHPE mainly localizes to the DOPC-rich liquid-disordered phase. Two forms of vitamin E (VE), α-tocopherol (Toc) and α-tocotrienol (Toc3), were obtained from Tama Biochemical Co., Ltd. (Tokyo, Japan). Amyloid-β (human, 1–42; Aβ) was obtained from the Peptide Institute, Inc. (Osaka, Japan). Fluorescently-labeled (HiLyte Fluor^{TM} 488) Aβ (1–42) (Aβ-488) was obtained from AnaSpec, Inc. (Fremont, CA, USA). Ultrapure water (specific resistance ≥ 18 MΩ) was obtained using a Millipore Milli-Q purification system (Merck Millipore, Billerica, MA, USA). The chemical structures of DOPC, DPPC, Toc, and Toc3 are presented in Figure S1.

Preparation of liposomes for microscopic observation

Liposomes were prepared by the natural swelling method. DOPC, DPPC, Toc, and Toc3 were dissolved in chloroform/methanol (2:1 v/v) at a concentration of 2 mM. Rhod-DHPE was dissolved in chloroform at a concentration of 0.1 mM. These solutions were stored at -20℃. Lipids and VE were mixed at the desired composition to give a total volume of 20 μL, and 2 μL Rhod-DHPE was further added. We added 0–10% VE to the lipid membranes with a fixed ratio of DOPC:DPPC = 1:1. Therefore, the lipid compositions examined by microscopy were DOPC/DPPC/VE = 50:50:0, 48.5:48.5:3, 47.5:47.5:5, 46:46:8, and 45:45:10. The organic solvent was evaporated under a gentle N_2 gas flow and the lipids were further dried in a vacuum desiccator for more than 3 h to completely remove residual organic solvent. Lipid films were hydrated with 200 μL Milli-Q water at 37 °C for more than 3 h to prepare liposomes. The final concentration of lipids and Rhod-DHPE was 0.2 mM and 1 μM, respectively.

Microscopic observation at room temperature

The observation chamber was prepared using grounding silicon film with a hole on slide glass. The liposomal solution was added to the hole of the chamber and a cover glass was placed on the silicon film. We observed liposomes using an IX 71 fluorescence microscope (Olympus, Tokyo, Japan), and the observation time was restricted to 90 s to avoid photo-induced lipid oxidization. At room temperature, we counted more than 100 liposomes in each condition.

Miscibility temperature of phase-separated structures

To assess the thermal stability of phase-separated structures, we measured the miscibility temperature (T_{mix}), which was defined as the temperature at which the fraction of phase separation was 50%. Sample temperature was controlled by a temperature controller (MATS-555MORA-BU; Tokai Hit, Shizuoka, Japan). We observed samples using an IX 71 fluorescence microscope (Olympus) for 90 s after maintaining each temperature (±0.5 °C) for 5 min and counted more than 60 liposomes. To obtain T_{mix}, the resulting data were fitted by Boltzmann's sigmoid function as follows:

$$p = \frac{1}{1 - \exp[(T - T_{\mathrm{mix}})/dt]} \quad \text{(S1)}$$

where p is the fraction of phase-separated liposomes, T is the temperature, and dt is the slope of Boltzmann's sigmoid function.

Differential scanning calorimetry (DSC)

DPPC, Toc, and Toc3 were dissolved in chloroform/methanol (2:1 v/v) at a concentration of 300 mM. These solutions were stored at -20 ℃. VE was added to the DPPC solution to be 0–10% of the total volume, so that the final volume was 30 μL. The examined lipid compositions for DSC were DPPC/VE = 100:0, 97.5:2.5, 95:5, 92.5:7.5, and 90:10. The organic solvent was evaporated under a gentle N_2 gas flow and lipids were further dried in a vacuum desiccator for more than 3 h to completely remove residual organic solvent. Then, 60 μL Milli-Q water was added to the lipid film and the final lipid concentration was 150 mM. The lipid solution was mixed with a vortex mixer for several seconds and then sonicated at 60 °C for 1 h. A DSC-822e (Mettler Toledo International, Inc., Greifensee, Switzerland) was used for DSC. The sample solution (13 mg ± 0.2 mg) was placed in a 40-μL aluminum pan. A cell filled with Milli-Q water was used as a reference cell. The weight of Milli-Q water in the sample and reference cells was identical. For the measurements, heating/cooling cycles were repeated three times between 20 and 60 °C. Heating and cooling were conducted at 5 °C/min after holding at 20 ° C and 60 °C for 3 min, respectively. We analyzed the third heating process as representative data. The same measurements were performed at least three times to ensure reproducibility of the data.

The thermographs obtained from DPPC/VE binary mixtures were asymmetric. Peak deconvolution was performed with Multi Peak fit included in Origin Pro 2020b and expressed by two Gaussian functions (Figure S2).

Aβ protofibril preparation

Aβ protofibrils were prepared as described previously[1,2]. Aβ and Aβ-488 were dissolved in aqueous ammonia (0.02% NH_3 aq.) and stored at -80 °C. Aβ and Aβ-488 (0.02% NH_3 aq.) were mixed at a ratio of 2:1. Then, the Aβ mixture was diluted to 80 μM with 20 mM Tris-HCl (pH 7.4). To form Aβ protofibrils, the Aβ mixture was incubated at 37 °C for 12 h. Aβ protofibrils (80 μM) were added to the liposomal solution at a ratio of 1:15 to a final Aβ concentration of 5 μM and observed under an FV-1000 confocal laser scanning microscope (Olympus) at room temperature.

Supplementary figures

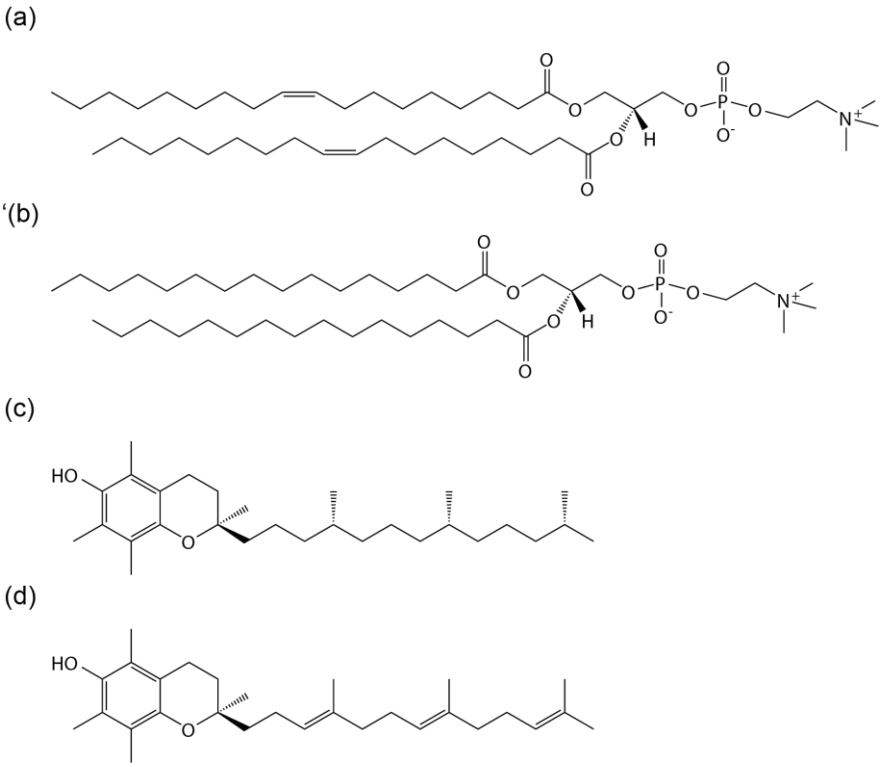

Figure S1. Chemical structures of (a) DOPC, (b) DPPC, (c) Toc, and (d) Toc3

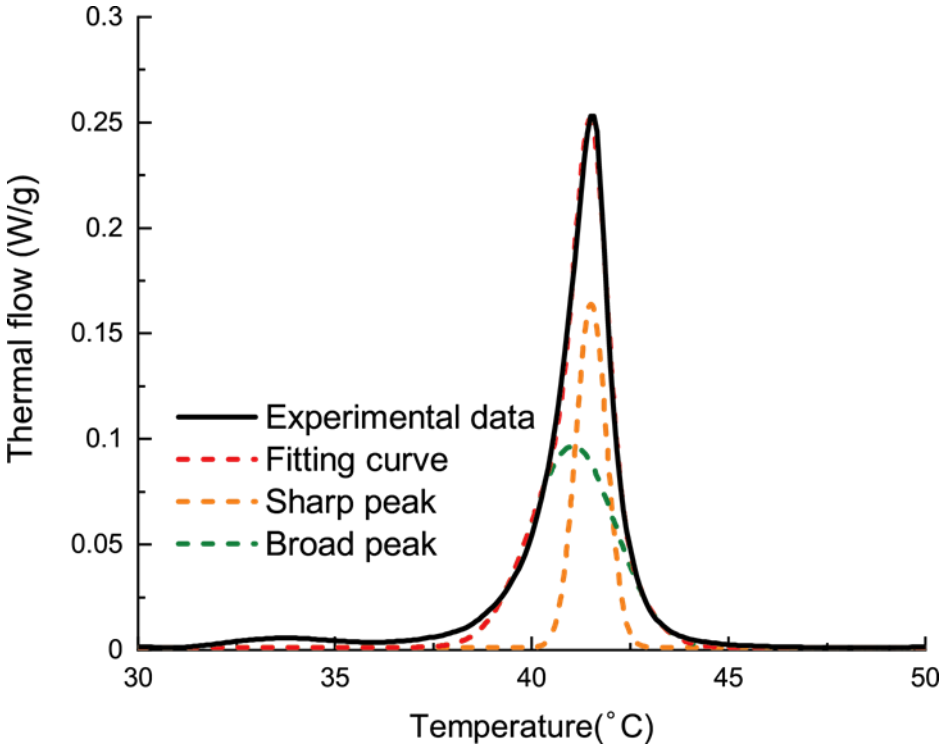

Figure S2. A representative example of peak deconvolution for DPPC/Toc = 97.5:2.5. Black line represents the experimental data. The experimental data were deconvoluted into two symmetric peaks that are indicated by orange and green dashed lines. The sum of these two lines, which is indicated by a red dashed line, was the fitting curve.

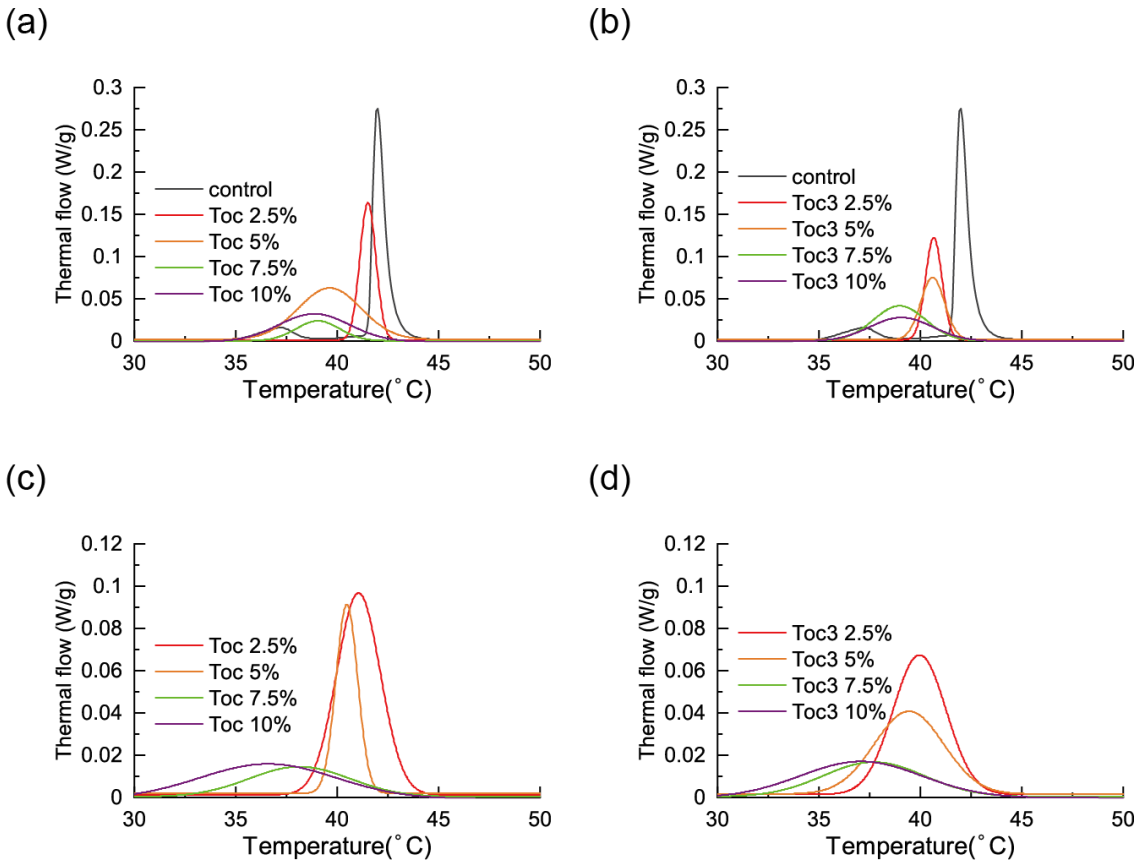

Figure S3. Sharp and broad peaks obtained from peak deconvolution in the DPPC/Toc and DPPC/Toc3 thermographs shown in Figure 2a, b. (a, b) Sharp peaks obtained from DPPC/Toc (a) and DPPC/Toc3 (b). (c, d) Broad peaks obtained from DPPC/Toc (c) and DPPC/Toc3 (d). Control in (a) and (b) indicates pure DPPC (Toc/Toc3 = 0%). Since only a symmetric sharp thermograph is obtained at the control composition, we did not perform peak deconvolution and show the experimental thermograph as a sharp peak. The molar ratios of Toc and Toc3 in DPPC are given in the figures.

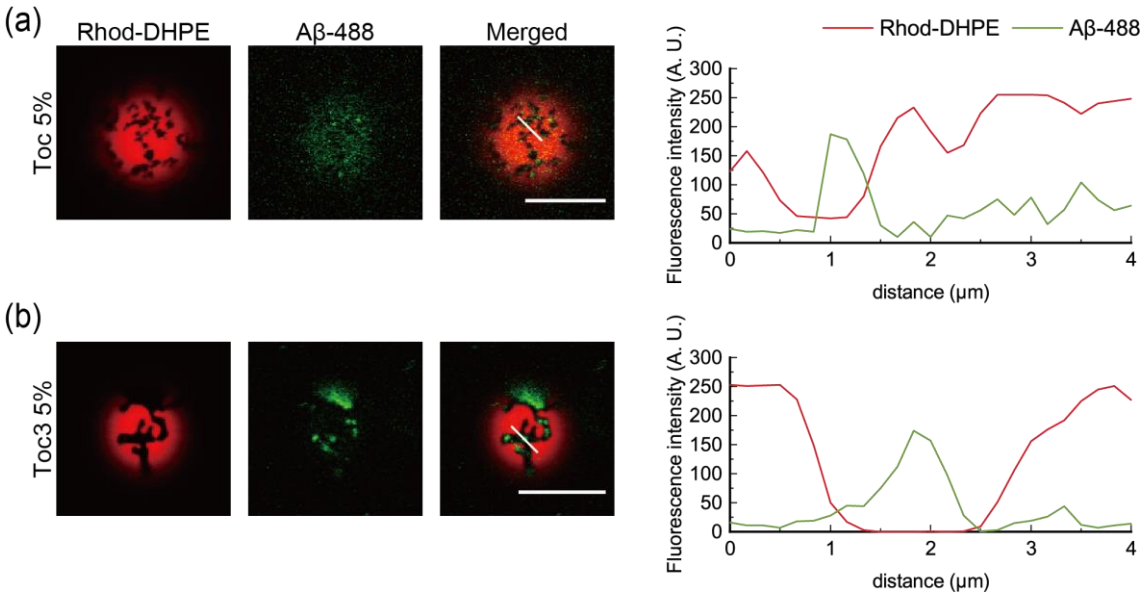

Figure S4. Microscopic images obtained from confocal laser scanning microscopy and fluorescence intensity profiles along the white lines in the merged images for 5% Toc (a) and 5% Toc3 (b). Red and green fluorescence represents Rhod-DHPE and Aβ-488, respectively. Red and green lines in the fluorescence intensity profiles along the white lines from left top to right bottom indicate the intensities of Rhod-DHPE and Aβ-488, respectively. Scale bars, 10 μm.

References

(1) Phan, H. T. T.; Vestergaard, M. C.; Baek, K.; Shimokawa, N.; Takagi, M. Localization of Amyloid Beta (Aβ1-42) Protofibrils in Membrane Lateral Compartments: Effect of Cholesterol and 7-Ketocholesterol. *FEBS Letters* **2014**, *588*, 3483–3490.

(2) Morita, M.; Hamada, T.; Tendo, Y.; Hata, T.; Vestergaard, M. C.; Takagi, M. Selective Localization of Alzheimer's Amyloid Beta in Membrane Lateral Compartments. *Soft Matter* **2012**, *8*, 2816–2819.