Effect of Cholesterol on the (−)-Epigallocatechin Gallate-Induced Burst of PC-GUVs

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Tea catechins such as (−)-epigallocatechin gallate (EGCg) have been considered to have antibacterial activity and antioxidant. Several investigations indicated that lipid membranes are one of the targets of catechins for their activities. However, the detail interaction of catechins with lipid membranes remained unclear. In our previous report [1], we investigated the interaction of EGCg with single giant unilamellar vesicles (GUVs) of egg phosphatidylcholine (egg PC) using the single GUV method[1]. We found that low concentrations of EGCg (≥ 30 μM) induced a rapid leakage of a fluorescent probe, calcein, from the inside of a single egg PC-GUV, which changed into a small lump of lipids after the leakage. Phase-contrast microscopic images revealed the detailed process of the EGCg-induced burst of GUVs, the decrease in their diameter, and their transformation into small lumps. The strong correlation between the leakage and the burst of GUVs indicated that the leakage of calcein occurred as a result of the burst of the GUV. In contrast, human cells have an activity to protect the EGCg-induced bursting of cells. Thereby it is important to elucidate a factor of the protection. In this report, we investigated the effect of cholesterol (chol) on the EGCg-induced bursting of PC-GUVs.

At first, we investigated effect of EGCg on DOPC-GUVs using phase-contrast microscopy. Low concentrations of EGCg (≥ 30 μM) induced bursting of GUVs, which is similar to that of egg PC-GUVs. On the other hand, much higher concentrations of EGCg were required for the burst of DOPC/chol(6/4; molar ratio)-GUV(Fig. 1 (A,B)); at a concentration < 100 μM, no bursting was observed, and the fraction of burst GUV was 0.5 at 500 μM EGCg (cf. for DOPC-GUV, 50 μM EGCg) (Fig. 1 (C)). We also determined the partition coefficient of EGCg from aqueous solution into membranes, K_p. K_p for DOPC/chol (6/4) membranes ($=4\times10^4$) was larger than that for DOPC membranes ($=1\times10^4$). Next, we investigated the dependence of spacing of the PC-MLV on the EGCg concentration, using small-angle X-ray scattering. The spacing of DOPC/chol(6/4)-MLV decreased with increasing EGCg concentration, and above the critical molar ratio of EGCg to lipid the spacing nearly remained constant (5.3 nm). This result suggests that neighboring membranes came in close contact with each other. The critical molar ratio on DOPC/chol(6/4) membranes(=0.2) was smaller than that on DOPC membranes(=0.3).

The molar ratio of the EGCg bound to the lipid membrane to the lipid in the external monolayer of DOPC/chol(6/4)-GUVs were larger than that of DOPC-GUVs as a same EGCg concentration in the aqueous solution. However, higher concentrations of EGCg were required for burst of DOPC/chol(6/4) -GUVs compared with DOPC-GUVs. These results indicate that cholesterol increases the stability of PC-GUV against the binding of EGCg to the membrane. We will discuss the mechanism of the inhibition of the burst of GUVs by cholesterol.

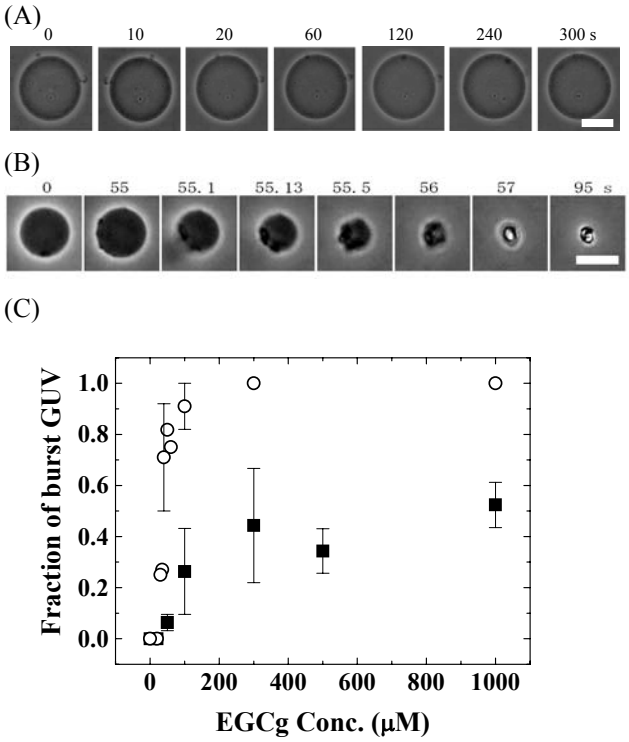

Fig. 1: Phase contrast images of single DOPC /chol(6/4)-GUVs in the interaction of (A) 100 μM EGCg and (B) 500 μM EGCg. The numbers above each image show the time after the addition of EGCg. The bars corresponds to 20 μm. (C) Dose-response of EGCg for the fraction of burst GUV among all the examined single PC-GUVs at t = 5 min after the addition of EGCg. (○) DOPC and (■) DOPC/chol (6/4).

References

[1] Tamba, Y., Ohba, S., Kubota, M., Yoshioka, H., Yoshioka, H. and Yamazaki, M. 2007. Single GUV Method Reveals Interaction of Tea Catechin (−)-Epigallocatechin Gallate with Lipid Membranes. *Biophys. J.* 92:3178-3194.