Interaction between Cell Membrane and Liposome Membrane under Heat Stress Condition

It has been reported that the liposome addition and heat treatment could enhance the production and release of chitosanase from *S. griseus* [1]. In this study, heat induced the interaction between the cell membrane and liposome membrane such as binding, fusion and internalization was investigated by the direct observation using fluorescence probe labeled-liposome and also aqueous two-phase partitioning method.

[Experimental]

0.025 mM POPC liposome (100 nm) containing 1 mol% Rh-PE as final concentration was co-incubated with intact and spheroplast *S. griseus* cell suspension (10⁶ CFU) for 1 h at various temperatures tested here. For inhibition of internalization of liposomes, intact and spheroplast *S. griseus* cells were incubated with 20 mM o-phenanthroline as final concentration for 1 h at room temperature prior to use. The treated cells were washed several times by Tris.HCl buffer in order to remove free binding labeled liposomes. The amount of liposome internalization to intact and spheroplast *S. griseus* cells was estimated as the increment of reduction of fluorescence intensity of labeled liposome suspension at the beginning and after 1 h of treatment with intact and spheroplast *S. griseus* cells. Fluorescence micrograph was utilized to observe direct interaction of labeled rhodamine-PE liposome with cell membrane of *S. griseus* under various heat conditions.

[Results and Discussion]

The liposomes labeled rhodamine were widely utilized to study the internalization of liposomes to cell [2,3]. The effects of heat treatment on the internalization of POPC liposome to *S. griseus* cell pretreated with and without liposomes were shown in Fig.1. The treatment of heat stress (41°C) significantly enhanced amount of liposome internalization to cell pretreated with inhibitor from 8% to 18%. This observation implies that heat stress (41°C) is more effective to induce internalization of liposome to cell than that at 37°C. Furthermore, the fluorescence micrograph of direct interaction of labeled liposome with cell membrane shown in Fig. 2 indicates that liposome internalization is heat dependence. Results also show that liposomes could interact with cell membrane of *S. griseus* cell pretreated with inhibitor under heat stress condition. This observation might relate to the modes of liposome internalization to cell by non-endocytosis. Sunamoto et al. have proved that PEO-liposomes could directly fuse and internalize to cells by endocytosis [3]. Their conclusion was based on the effect of inhibitors used such as Cytochalasins B and D, which mainly inhibited the ATPase for endocytosis pathway of phagocytosis. In fact, o-phenanthroline used in this study has a function to inhibit the activity of phospholipase C (PLC) hydrolyzing to cleave phospholipids head group of phosphotidyl choline lipids. This may cause the membrane curvature to induce the internalization of POPC liposomes. Membrane curvature plays the important roles for the endocytosis and/or exocytosis of biomembrane. In this study, although the conventional zwitterionic POPC liposomes were used, the binding of these liposomes to cell membranes induced by the heat stress was clearly observed. Such binding of liposomes to cell membrane further proceeded to their endocytosis to *S. griseus* cells. It is, therefore, the conventional liposomes could be internalized by endocytosis to cells through the direct interaction with lipid membrane of the *S. griseus* under heat stress condition. The driving force of such interaction was also investigated. It was found that, the surface net hydrophobicity (HFS) of *S. griseus* cells was increased with the increasing temperature. The hydrophobic interaction of lipid membrane of *S. griseus* cells with liposomes could be predominant, far larger than electrostatic one.

[References]
2. Straubinger et al., Biochemistry, 29, 4929 (1990)

![Fig. 1 Effects of heat on internalization of POPC liposome to *S. griseus* cells pretreated with and without inhibitor](image)

![Fig. 2 Fluorescence micrograph of interaction of labeled liposome with *S. griseus* cell membrane](image)

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