Role of Negatively-Charged Liposome on Gene Expression of GFP
~Negative Charged Liposome is Potent Inhibitor of Protein Folding in Post-Translation Process~
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Liposome is cited as successful application gene delivery and expression, drug delivery for treatment of cancer and disease cell by some other researchers. Liposome also has chaperone-like function[1][2]. Negative charged liposome can control the refolding of unfolded lysozyme as well as the activation of its enzymatic activity[3]. In a biological cell, apoptosis (cell suicide) has reported to be triggered by production of improperly unfolded or incomplete proteins[4]. When there is a large, rapid, and overwhelming accumulation of unfolded protein, a rapid apoptosis occurred; and then most cells will be died by apoptosis. Recently, it has been reported that the liposome can affect the gene expression in cell-free translation system[5]. In this study, the role of negatively-charged liposome on the in vitro gene expression was studied, resulting that the negatively-charged liposome inhibited the folding expressed polypeptides of green fluorescence protein (GFP) in post-translation process.

[Experimental]
Phospholipids molecules of POPC (1-palmitoyl, 2-oleyl-sn-glycero-3-phosphocholine) and POPG (1-palmitoyl-2-oleyl-sn-slycero-3-phosphoglycerol) at the ratio of (7/3) were used for making POPC/POPG liposome with a pore size of 100 nm. Rapid Translation system RTS 100 E.coli HY Kit (Roche Diagnostics, USA) was used for in vitro gene expression system of the green fluorescence protein (GFP) from the expression vector, pIVEX2.3d. The amounts of total GFP expressed were analyzed by SDS-PAGE and mature synthesized protein was identified by fluorescence intensity at 395 nm (excitation) and 509 nm (emission). Outer and inner membrane fluidity of liposome when interacted with GFP was identified by using TMA-DPH and DPH as fluorescence probes.

[Results and Discussion]
The effect of the addition of the negative charged POPC/POPG liposome on gene expression of GFP was carried out. The gene expression of mature GFP product was identified by detection of fluorescence intensity after 18 hours of expression. The result in Fig.1(a) indicated that, in the presence of liposome at 4 mM, the GFP fluorescence (synthesized and folded amounts GFP) was reduced up to 65% in comparison with control sample.

The total products of fold and unfold synthesized GFPs expressed in the presence and absence of POPC/POPG liposome were further analyzed by SDS-PAGE. Interestingly, the densitometer analysis with CBB method of the gel image showed that total expressed GFP products were the same when the GFP was expressed with and without liposome at the concentration of 1~4mM (Fig.1(b)). This result indicated that liposome only inhibit the folding of synthesized polypeptide GFP (mature protein - active protein) in post-translation but it did not inhibit gene translation of total synthesized protein product.

Interaction between liposome and mature expressed GFP was also investigated by ultrafiltration. The result indicated that the liposome also interacted strongly with mature synthesized GFP, resulting that the GFP interacted with the liposome could not pass through membrane filter (72% GFP was not filtrated because of its interaction with the liposome).

The effect of liposome on refolding of native standard GFP denatured by guanidine in refolding buffer was also carried out. The result showed that: liposome inhibited 55% refolding of native GFP denatured by guanidine in comparison with native GFP before denaturation. A changing of outer and inner membrane fluidity of liposome in the presence of GFP was carried out. The result indicated that POPC/POPG liposome strongly interacted with native standard GFP, resulted in reducing 10% outer membrane fluidity and 24% inner membrane fluidity.

The above findings on the negatively-charged liposome POPC/POPG during the GFP expression imply the future study on “silence” of gene product, such as toxic enzyme expressed in infective toxic bacteria.

[References]
5) B.T. Huong et al., Langmuir, accepted (2008)

Fig.1 GFP Expression in the Presence of Negatively-Charged Liposome (POPC/POPG, 0~4mM).
(a) GFP Fluorescence and (b) SDS-PAGE Image.

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