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Stability of Plasmid DNA during Liposome Encapsulation

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Liposome 봉입과정에서의 Plasmid DNA의 안정성

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Plasmids, YEpl3 and pMA56, were encapsulated into liposomes by two different procedures during which the plasmid DNAs were exposed ether to 60°C for 1.5 hr or to sonication for 2-5 min at 4°C. The encapsulated plasmids were then reextracted and their physical conformations and transformation abilities were examined. It was confirmed from the results that both plasmid DNAs were remained stable throughout the procedures of encapsulation into liposomes.

When phospholipids are dispersed in an aqueous solution, vesicles known as liposomes form, encapsulating water and solid materials inside their lipid membranes. A great variety of biologically active compounds and structures can be included within the liposomes. When the liposome membranes contact with membranes of protoplasts, fusions between the membranes may occur resulting in transfers of the biologically active materials into the protoplasts[1]. This may open a way of transfering genetic materials of relatively large sizes which are difficult to penetrate through protoplast membranes[2].

Large unilamellar vesicles (LUV) and reverse phase evaporation vesicles (REV) are known to have several advantages in encansulating plasmids over other types of liposomes[3,4,5]. In the process of encapsulating plasmid DNA into LUV, however, the mixture of plasmid DNA and lipid vesicles are usually exposed to 60°C for about 1.5 hr[6,7]. Instead of the heat treatment, the encapsulation of plasmids in REV requires sonication for 2-5 min at 4°C[5,8]. Such treatments may alter the genetic materials to be encapsulated either physically or biologically and consequently reduce the reliability of liposomes as vehicles. The purpose of this paper is to evaluate the stability of plasmids against the physical treatments involved in encapsulation processes.

MATERIALS AND METHODS

Strains and plasmids

Saccharomyces cerevisiae SHY3 which lacks synthetic ability for leucine and tryptophan[9,10] was used as recipient cells for transformation experiments. Plasmids of YEp13, containing *LEU2* gene[11], and pMA56, containing *TRP1* gene[12], were isolated through the protocols described by Maniatis et al.,[13] from strains C600[11] and JA221[14] of *Escherichia coli*, respectively.

Preparation of liposomes containing plasmids

LUV liposomes, containing either YEp13 or pMA56, were prepared by the method of ether injection described by various authors[3,4,6,7] with slight modifications. Thirty micromoles of phosphatidyl choline (PC) and three micromoles of phosphatidyl glycerol (PG) were dissolved in 5 ml of chloroform in a round bottom flask. The chloroform was

removed by evaporation under nitrogen gas. The thin film of lipid formed on inside-wall of the flask was dissolved in 10 ml of diethyl ether. The lipid solution was transferred into a 10 ml Gastight Hamilton syringe and injected through a 23-gauge needle at a rate of 0.17 ml/min into 2 ml of an aqueous. The aqueous solution, containing 20-40 ug of either YEp13 or pMA56, was maintained at 60°C. The liposome suspension was flushed with nitrogen gas to remove ether and to allow cooling at room temperature. Liposomes containing DNA were treated with 20-50 ug of DNase I per ml for 1.0 hr at 37°C to digest free DNA attached outside the liposomes. The suspension was then loaded on a 1.5 x 30 cm Sepharose CL-4B column and eluted by Hepes buffer (0.1M NaCl/KCl, 5 mM Hepes, pH 7.4) to purify the liposomes from the mixture of liposomes and DNA fragments.

REV liposomes containing plasmids were prepared by the method described by Szoka and Papahadjopoulos[5] and Fraley et al.[8] with the following modifications. Thirty micromoles of PC and three micromoles of PG were dissolved in 1 ml of chloroform in a screwcap test tube (13x100mm). The solvent was then removed by evaporation using nitrogen gas. The lipids were redissolved in 3 ml of diethyl ether. One milliliter of aqueous Hepes buffer containing 20-40μg of plasmid DNA was added and the two-phase system that resulted was sonicated briefly (2-5 min) in a bath-type sonicator (Cole-Parmer Model 8845-40) until the mixture becomes a clear one-phase dispersion that does not separate for at least 30 min after sonication. The sonication temperature was 4°C. The ether was fully evaporated by nitrogen gas stream. The steps for degrading free plasmid DNA by DNase I and liposome purification were the same as for LUV liposomes described previously.

The plasmid DNA was determined by the diphenylamine assay described by Burton[15]. For the determination of DNA concentration in liposomes, the encapsulated plasmids were reisolated from the liposomes by the phenol-chlorofom extraction followed by the ethanol precipitation[16].

Transformation of yeast protoplasts

Protoplasts of yeast cells were made according to van Solingen et al.[17] and Gunge and Tamaru[18]. The protoplasts were mixed with liposomes and transformation by the liposome-encapsulated plasmids was carried out following the procedures described by Hinnen et al.[19] and Sherman et al.[20]. Transformation occurred was detected by observing colonies appeared on the synthetic minimal medium[20] without containing leucine (for YEp13) or tryptophan (for pMA56).

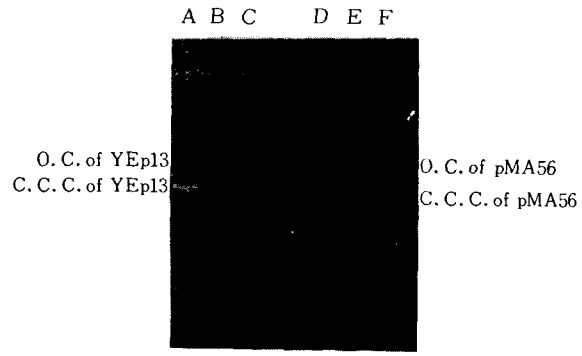

Fig. 1. Electrophoretic pattern of DNAs of YEp13 and pMA56 extracted before and after encapsulation into liposomes. Lane A, YEp13 extracted freshly from *E. coli* cells; Lane B, YEp13 reextracted from REV; Lane C, YEp13 reextracted from LUV; Lane D, pMA56 extracted freshly from *E. coli* cells; Lane E, pMA56 reextracted REV; Lane F, pMA56 reextracted from LUV.

Agarose gel electrophoresis

Electrophoresis was performed in a 0.7% agarose slab gel in TBE buffer containing 89 mM Tris-borate, 89 mM boric acid, and 2 mM EDTA, at pH 8.0. Electrophoresis was for 2 hr at 100 V. The gel was stained in ethidium bromide at 0.5 μg/ml for 45 min, rinsed with distilled water, and photographed with a Polaroid camera with type 084 film (ASA 3,000). Exposure was for 3 min with UV transilluminator.

RESULTS AND DISCUSSION

Physical stability of plasmids

In order to see physical changes on the shape of the plasmide which might be caused by either the heat treatment or the sonication applied during the preparation of LUV or REV, plasmids were reisolated from the liposomes and subjected to the electrophoretic analysis. The electrophoretic patterns of original plasmids, YEp13 and pMA56, were compared with that of the same plasmids experienced encapsulation processes in Fig. 1. The bands of reisolated plasmids have the positions same to that of the original plasmids. Apparently, the exposure of plasmid DNA at 60°C for about 1.5 hr during the process of encapsulation in LUV, or the sonication for 2 to 5 min at 4°C for REV encapsulation do not damage plasmid DNA significantly. However, the electrophoretic results do not eliminate possibilities of converting plasmids of covalently closed circular (CCC) form to open circular (OC) form, because a quantitative estimation for each

Table 1. Transformation activities of plasmids, YEp13 and pMA56, extracted before and after encapsulation in liposomes.

Plasmid DNA	Transformations/ μg DNA
YEp13 standard[a]	2.8×10^3
YEp13 reextracted from LUV[b]	2.5×10^3
YEp13 reextracted from REV	2.4×10^3
pMA56 standard	3.5×10^3
pMA56 reextracted from LUV	3.3×10^3
pMA56 reextracted from REV	3.4×10^3

a. Plasmids freshly isolated from *E. coli* cells.
b. Standard plasmids were encapsulated in liposomes and then reextracted the plasmids from the liposomes.

form of plasmid DNA is impossible by this electrophoretic analysis.

Biological stability of plasmids

Protoplasts of *S. cerevisiae* SHY3 were tried to be transformed by plasmids isolated before and after the encapsulating treatments and their transformation efficiencies were compared (Table 1). Both types of plasmids, YEp13 and pMA56, transferred into the yeast cells with same frequency without discriminating plasmids of freshly prepared or that of reisolated from the liposomes encapsulated them. From the results it may be concluded that neither YEp13 nor pMA56 lost or reduced their transformation abilities due to the heat treatment or sonication applied during the process of encapsulating the plasmid DNAs into liposomes.

요 약

플라스미드 YEp13과 pMA56을 두가지 다른 방법으로 liposome 속에 봉입시켰다. 이 두가지 방법에 따르면 플라스미드 DNA는 60℃에 1.5시간 동안 노출되거나, 4℃에서 2 - 5분 동안 sonication 처리를 받아야 한다. 일단 봉입된 플라스미드를 다시 뽑아낸 다음, 그들에 대한 물리적인 구조와 유전적 전환능력을 조사했다. 그 결과 이 두 플라스미드 DNA는 liposome 봉입과정을 통해 아무런 손상을 입지 않았음이 밝혀졌다.

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