Zoological Studies 34, Supplement I (1995)

Zoological Studies

The DNA Uptake Mechanism of Transfection Mediated by Cationic Liposomes

Sek-Wen Hui and Ya-Li Zhao

Membrane Biophysics Laboratory, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

Cationic liposomes have become a popular transfection vector for gene delivery in vitro as well as in vivo. Because of their efficiency and ease of production, handling, and quality control, these liposomes provide an excellent alternative mode to viral vectors. Aminolipids such as 1, 2-bis (oleoyloxy)-3-(trimethylamonio)propane (DOTAP) have been used as cationic components of these liposomes. Yet, in spite of wide use of these liposomes, results have not been consistent, mainly because the mechanism of their cellular uptake is not entirely clear, and the factors affecting the uptake efficiency are not known.

In most cases, DOTAP and other cationic lipids work better as a delivery vector when mixed with a non-bilayer-preferring phospholipid such as dioleoloyl phosphatidylethanolamine (DOPE). These liposomes form complexes with negatively charged DNA; the residue positive charges in these complexes facilitate their adhesion to the cell surface. The subsequent fate of these complexes is not well understood. Either the entire complex is phagocytized by the cell, or its lipids fuse with the plasma membrane of the cell, thereby delivering the DNA to the cytoplasm.

We studied the factors affecting the DOTAP-mediated transfection of CHO cells. DOTAP:DOPE (1:1 molar ratio) liposomes were mixed, at a charge ratio of 1:2, with pCMV-β-gal, a plasmid carrying the reporter gene that codes for the enzyme β-galactosidase. 5 mol% of a fluorescent tracer, rhodamine-PE, was added to the lipids to form liposomes used for microscopy experiments. Ethidium bromide was used to label "exposed" DNA in double-labelled experiments. The complexes, when initially formed, were typically 50 nm in size, as measured by freeze fracture electron microscopy and by quasi-elastic light scattering. When added to cell culture media, the complexes aggregated because of polyvalent anions in the media. The fate of DNA-liposome complexes was determined by fluorescence confocal microscopy at various times after adding to CHO cells in different media. We found that the size and formation rate of aggregates were closely related to the uptake of DNA, and therefore the efficiency of transfection.

DNA-liposome complexes aggregated to >1μm almost immediately upon adding to phosphate buffered saline (PBS) or to normal F-10 culture medium containing $NaHCO_3$ (BRL/GIBCO). When added to a special F-10 medium without HCO_3^+ anions, the complex aggregation was much slower, and the terminal aggregate size was much reduced (~380 nm). There was no aggregation when the complexes were added to isotonic sucrose medium containing no anions. The transfection efficiency was measured by counting the number of clones expressing the β-gal gene, using X-gal staining. The subsequent transfection efficiency correlates closely with the size and rate of complex aggregation.

When observed by conventional and confocal fluorescence microscopes, visible aggregates formed immediately after the addition of liposome-DNA complexes to cells in normal F-10 culture medium containing bicarbonates. Most of these aggregates were in suspension and some were adsorbed on the cell surface upon sedimentation (Fig. 1). After 1 hr, many of the aggregated complexes were internalized, and the plasma and cytoplasmic membranes became bright, presumably from fusion with labelled liposome membranes. Figures 2 to 4 represent XY optical sections of a typical cell at 9, 2-5 and 1 μm from the supporting substrate. The XZ section of the same cell is shown in Fig. 5. Aggregated complexes are clearly seen inside the cell. By 3 and 4 hrs (Figs. 6 and 7 representing one out of a series of optical sections of

Fig. 1. An optical section 8-10 μm from the substrate of a cell in normal F-10 medium, immediately after liposome/DNA addition.

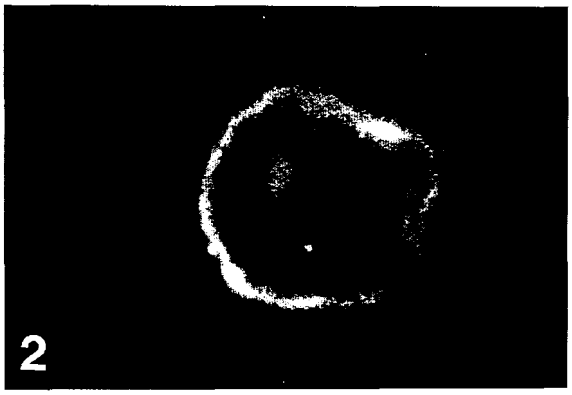

Fig. 2. An optical section 9 μm from the substrate of a cell in normal F-10 medium, 1 hour after liposome/DNA addition.

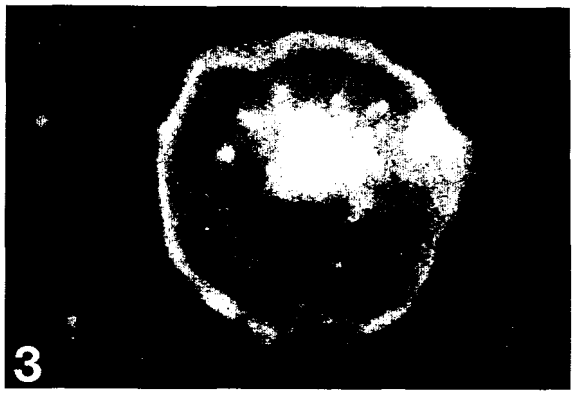

Fig. 3. An optical section 2-5 μm from the substrate, of the same cell in fig. 2.

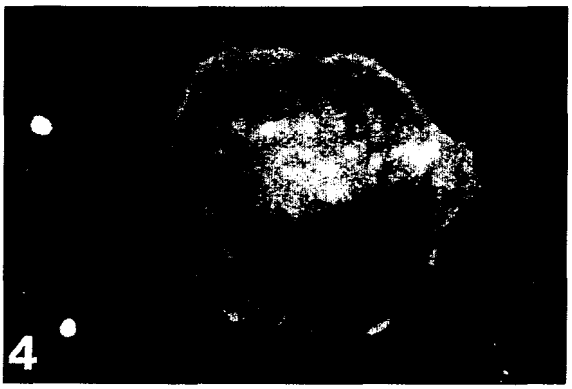

Fig. 4. An optical section 1 μm from the substrate, of the same cell in fig. 2.

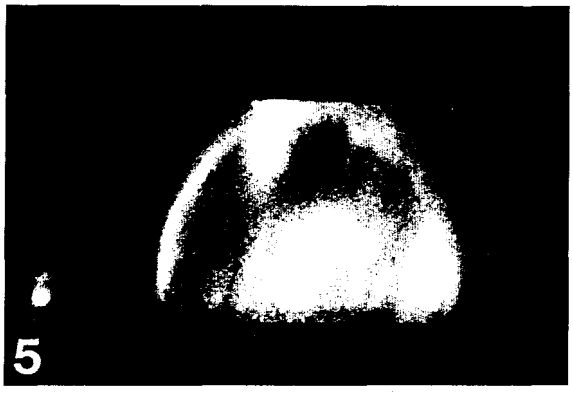

Fig. 5. An XZ optical section of the same cell in fig. 2.

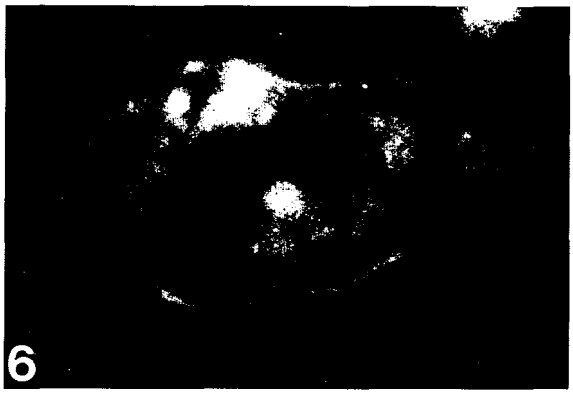

Fig. 6. An optical section 4 μm from the substrate of a cell in normal F-10 medium, 3 hours after liposome/DNA addition.

each cell, respectively), bright aggregates were mostly internalized. The plasma membrane and some cytoplasmic membranes became fluorescent at the same time. On those cells with fluorescent labelled liposome aggregates adhered to their surfaces, these aggregates were often swept to a cap if cells were kept at 37° for over an hour. In contrast, only a few small aggregates were formed an hour after the addition of liposome-DNA complexes to cells in special F-10 media without added bicar-

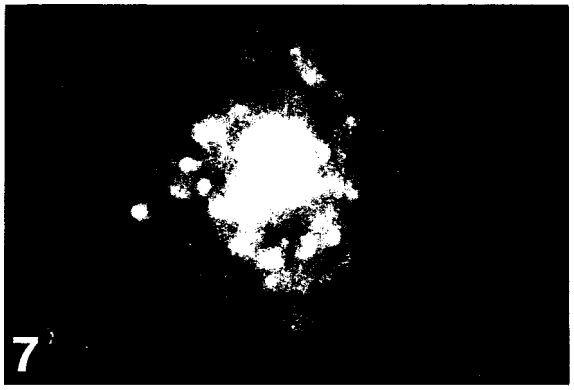

Fig. 7. An optical section 3-5 μm from the substrate of a cell in normal F-10 medium, 4 hours after liposome/DNA addition.

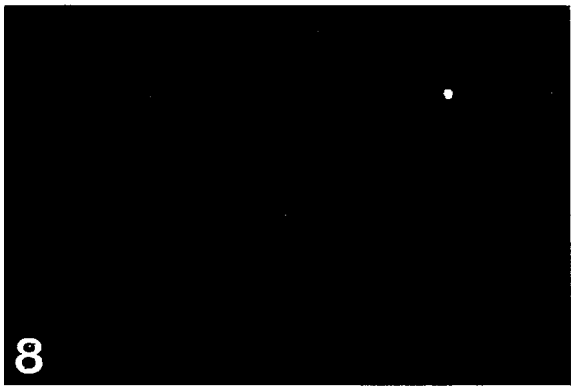

Fig. 8. An optical section 7 μm from the substrate of a cell in bicarbonate-free F-10 medium, 3 hours after liposome/DNA addition.

bonate or other anions, and few are internalized (Fig. 8). Cell membranes were not fluorescent, indicating no significant fusion between liposome and cell membranes. The transfection of cells in media with few aggregates remained at the background level, whereas the transfection levels in cells in media containing large aggregates were high (Table 1).

Our results indicate that the intake mechanism of liposome/DNA is activated by cationic lipid aggregates over a certain size. Aggregates over a certain critical size is needed to trigger phagocytosis and fusion. Without induced aggregation of liposome/DNA complexes by anions in media, fusion and internalization of complexes with cells are limited, hence the transfection efficiency is poor. The mechanism is not unlike that of calcium phosphate induced transfection. This mechanism may explain certain reported transfection inconsistencies when the physical structure of the cationic liposome/DNA complexes was not well defined. From time-dependent, 3-D confocal microscopy studies, we have shown that internalization, and to some degree fusion of fluorescent liposome/DNA complexes to cell membranes is the mechanism of DNA intake, and that the amount of intake is determined by the aggregation of these complexes by anions in the transfection media.

Table 1. Transfection of CHO cells

Transfection medium	Averaged aggregate size (μm)	Number of transfected clones
Normal F-10	1880	155
Sucrose + PBS	1120	50
Sucrose	47	13
HCO_3-free F-10	380	46

REFERENCES

Murray EJ, ed. 1991. Gene Transfer and Expression Protocols, Humana Press, Clifton, New Jersey.

Felgner PL, TR Gadek, M Holm, R Roman, HW Chan, M Wenz, JP Northrop, GM Ringold, MDanielsen, 1987. Proc. Natl. Acad. Sci. USA **84:** 7413.

Alton EWFW, PG Middleton, NJ Caplen, SN Smith, DM Steel, FM Munkonge, PK Jeffery, DM Geddes, SL Hart, R Williamson, KI Fasold, AD Miller, P Dickinson, BJ Stevenson, G McLachlan, JR Dorin, DJ Porteous. 1993. Nature Genetics **5:** 135-142.