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The Effect of Physico-Chemical Characteristics of Synthetic Cationic Amphiphiles on DNA Transfection

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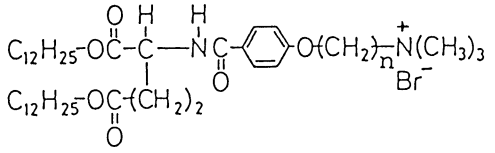
Bilayer membranes of double-chain ammoium amphiphiles were utilized for DNA transfection into eukaryotic cells. The efficiency of the DNA transfection was much higher when fluid, vesicler bilayers were used than when rigid, helical bilayers were used.

In DNA transfection mediated by cationic liposomes, the liposomes interact with DNA spontaneously, fuse with tissue culture cells, and facilitate the delivery of functional DNA into cells. This method requires no special equipment, is very simple, reproducible and efficient, and can be used to transfect wide variety of culture cells.[1,2] We have shown that one of dialkyl ammonium amphiphiles 1, which can form stable bilayer membranes, has high transfection ability.[3]

In this paper we describe the correlation between the efficiency of synthetic amphiphiles 1 in DNA transfection and their physico-chemical characteristics, such as aggregate morphologies and membrane fluidity.



1 (n = 2, 4, 6, 8)

Transfection experiments were carried out as described by Felgner et al.[1,2] with some modifications.[3] COS-1, simian kidney cell line transformed with simian virus 40 (SV40), was incubated with membrane/DNA

mixture containing amphiphiles 1 and plasmid DNA(pCH110), which has β-galactosidase gene(lac Z) and SV40 early promoter, in Dulbecco-modified Eagle Medium (DMEM) supplemented with 10% inactivated fetal calf serum. After the cells were cultured in the same medium in an atmosphere of 10 % CO_2 at 37 °C for 48 h, the transient expression of β-galactosidase gene incorporated into the cells was monitored both by the number of cells expressing the enzyme and the total enzyme activity expressed by the cells using 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside(X-gal)[4] and o-nitrophenyl-β-D-galactopyranoside[5] as the substrate, respectively. Chiral dialkylammonium amphiphiles 1 were synthesized as described by Kunitake et al.[6] These amphiphiles are comprised of two dodecyl tails, trifunctional glutamate connector, methylene spacer (the length of "spacer", n in 1) and cationic head.

The efficiency of amphiphiles 1 in the expression of β-galactosidase was measured by counting the number of the cells visualized by histochemical staining of the enzyme activity and assaying of the total enzyme activity in the extract of the cultured cells. As shown in Table 1, in both measurements of the expression of β-galactosidase, 1(n=2) was found to exhibit the highest DNA-transfection ability among the compounds examined. A good correlation between the two measurements suggests that all the cells incorporating plasmid DNA can express the gene and produce the enzyme protein at almost the same extent. Table 1 also shows that the increase in spacer length in amphiphiles 1 causes drastic decrease in the efficiency in the expression of β-galactosidase. Especially, although the difference in the spacer length between amphiphile 1(n=2) and 1(n=4) is only two CH_2 units, the former provided more than two fold higher expression than the latter. These results suggest that there is some correlation between the efficiency of the amphiphiles 1 in gene transfection and their physicochemical properties.

Table 1. Transfection efficiency of amphiphiles 1

Amphiphiles	β-Galactosidase expressed[a]	
	% cells stained with X-gal.	Activity of cell extracts nmol/min/2.2 cm dish, (%)
1(n=2)	11.6	3.80 (100)
1(n=4)	6.7	2.06 (54.3)
1(n=6)	0.6	0.13 (3.5)
1(n=8)	0.1	0.01 (0.2)

a) Cells were transfected with ammphiphiles /DNA complexes (13 nmol of amphiphiles 1 and 2 ug of plasmid DNA per 2.2 cm dish) and cultured at 37 °C for 48 h.

Amphiphiles 1 suspended in water were sonicated by Tomy Sonifier for several minutes to give clear solutions(10 mM). The solutions were stained negatively by uranyl acetate and subjected to electron microscopic examination.[7] Aggregate morphologies of 1 are quite different among the amphiphiles as shown in Fig. 1. 1(n=2) and 1(n=4) produced well-developed vesicles with diameter of 300-600 Å (Fig. 1a) and 500-3 000 Å (Fig. 1b), respectively. On the other hand, 1(n=6) gives fragmentary lamellae as in Fig. 1c, and 1(n=8) gives helical filaments[8] with length of 1-3 μm (Fig. 1d). The phase-transition temperature T_c (peak top temperature in the DSC scan),[9] as well as the aggregation behavior of 1 are summarized in Table 2. Transfection experiments and the morphological observation suggest that only the amphiphiles forming small stable vesicles, 1(n=2) and 1(n=4), have transfection ability. Since the transfection experiment was carried out at 37 °C, 1(n=2) and 1(n=4) would be in the fluid liquid crystalline state, whereas 1(n=6) and 1(n=8) in the gel state, suggesting that the membrane fluidity is also one of the important factors controlling the efficiency in DNA-transfection.

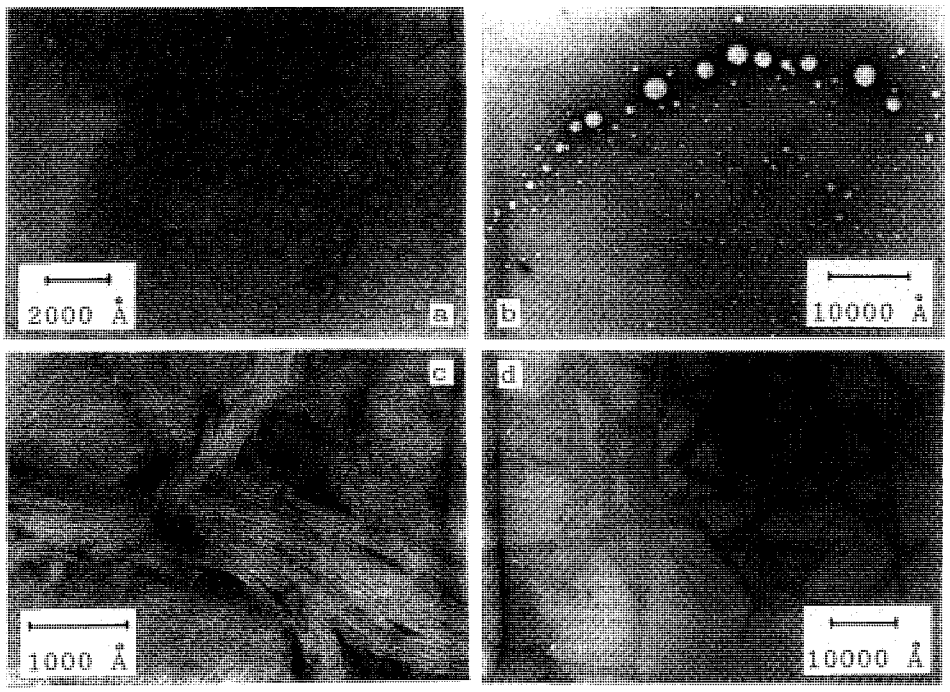


Fig. 1. Electron micrographs: 10 mM solution, stained by uranyl acetate.
a. 1(n=2), magnification, X 50000
b. 1(n=4), magnification, X 12000
c. 1(n=6), magnification, X 150000
d. 1(n=8), magnification, X 10000

Table 2. Influence of spacer length on the aggregation of amphiphiles 1

Amphiphile	Electron micrograph[a)]	T_c[b)](DSC peak)/ °C
1(n=2)	vesicle	24.8
1(n=4)	vesicle	29.4
1(n=6)	lamella	39.4
1(n=8)	filament	39.9

a) Sample, 10 mM in water.
b) Sample, 20 mM in water. Temperature was raised from 5 °C at a rate of 1 °C/min.

In conclusion, we found that a good correlation between the efficiency of the synthetic amphiphiles 1 in gene transfection and their physico-chemical characteristics, such as aggregate morphologies and membrane fluidity. Amphiphiles that form small vesicles in the fluid state are suitable for DNA-transfection. The results give us a guide to design more efficient compounds for the delivery system for DNA, RNA, and even proteins into eukaryotic cells.

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