Assembling liposomes by means of an oligonucleotide tagged with a lipophilic unit

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ABSTRACT
We synthesized DNA–cholesterol conjugate molecules and introduced them into 1-palmitoyl-2-oleoyl-sn-3-phosphocholine (POPC) liposomes to create DNA–tagged liposomes. These liposomes aggregated selectively depending on the sequence of DNA tag and the resultant pattern of the aggregation was a wide-spreading network structure, which was observed under a phase contrast microscope. The structure was minutely analyzed by transmission electron microscopy (TEM) and confocal laser-scanning microscopy. As a result, a hierarchical structure of the amphiphilic molecules was constructed from the liposome to the network via clustered structures.

INTRODUCTION
Studies for synthesizing life has drawn attention in a past decade in terms of the origin of life [1-6]. Szostak et al. pointed out that the system that has self-replicating informational substances encapsulated in a self-replicating compartment would be a minimal cell subject to Darwinian evolution [7,8]. They consider that self-replicable RNA plays roles of information and catalysts. On the other hand, a spherical shell made of a lipid bilayer called vesicle or liposome is regarded as a suitable material to enclose informational components because it is similar to biological membranes. Therefore liposomes are often used in the field of an artificial life synthesis [2-5]. If a genuine artificial cell model is completed, the creation of an artificial multicellular life will be focused as the next target. The main feature of multicellular organisms is that cells assemble selectively depending on their shapes, functions, and so on. In this study, DNA–tagged liposomes that bear DNA–cholesterol conjugates [5] in their lipid bilayer were prepared to make liposomes assemble (Figure 1). Since DNA forms a selective and firm complementary strand, it is expected that the DNA–tagged liposomes are connected even in the presence of Brownian movements of the liposomes. Furthermore, although we aim at synthesizing an artificial cell model, it is interesting to build up a supramolecular system consisting of a lot of amphiphilic molecules from the viewpoint of material science.

![Figure 1. Schematic diagram of adhesion of the DNA–tagged liposomes. Each liposome contains complementary oligonucleotide.](https://academic.oup.com/nass/article-abstract/481/101/105/167)

RESULTS AND DISCUSSION
Three types of DNA–cholesterol conjugate molecules that

Scheme: Synthesis of a DNA–cholesterol conjugate.
a) Tosyl chloride, pyridine, r.t., 5 hr, 71%; b) cholesterol, t-BuOK, THF, 50°C, overnight; c) 80% AcOH, 50°C, 2 hr, 33% (2steps); d) tetraisopropyl-2-(cyanethyl)phosphorodiamidite, diisopropylammonium tetrazolide, CH2Cl2, r.t., overnight; e) the standard amidite procedure on the solid support. DNA: pentadecadeoxyribonucleotide, dye: fluorescein (green) or cyanine3 (red).
consist of DNA 15mer, tetraethylene glycol spacer, cholesterol and fluorescent dye were synthesized as shown in the scheme. Combinations of a DNA and a dye are (oligothymidine/fluorescein), (oligodeoxyadenosine/cyanine3), and (oligodeoxyctydine/cyanine3), respectively. POPC liposomes containing the conjugate molecules were prepared as solutions of 50 mM sodium phosphate buffer and named Lip.(T), Lip.(A) and Lip.(C), respectively. A diameter of the liposomes was reduced to 200 nm through the filtration using a polycarbonate filter. These solutions were transparent under a phase contrast microscope because the vesicles were too small to be observed by optical microscopy. When pairs of these solutions [Lip.(T)–Lip.(A), Lip.(T)–Lip.(C), and Lip.(A)–Lip.(C)] were mixed and observed under a phase contrast microscope, aggregates of over micrometer-size appeared in the case of Lip.(T)–Lip.(A) mixture immediately after mixing. After 3 hours, large aggregates were formed as shown in Figure 2. However, in other two cases, any aggregates could not be observed, namely the mixed solutions were still clear even after 3 hours. It was confirmed by fluorescent microscopic images that this network structure fluoresced in both green (fluorescein) and red (cyanine3). Furthermore, the liposome network “melted” at 55 °C and was reconstructed when the temperature was decreased to 25 °C. These results indicate that this network is mediated by the DNA duplex formation of the oligothymidine–tagged and the oligodeoxyadenosine–tagged liposomes. Freeze fracture TEM micrographs of the assembled aggregates shows a cluster structure that consists of several tens of liposomes. The size of the cluster is about 500nm × 1500nm (this is consistent with phase contrast micrographs). This liposomal cluster is thought to be a building block of the network structure.

Two-dimensional high-resolution images and three-dimensional structure were observed under a confocal laser-scanning microscope. It became clear that the network of liposomes was three-dimensional, and widely spread without a break over a centimeter scale. Based on the above analyses, the process of the network formation is considered as follows. In the initial stage of the aggregation, dozens of liposomes obeying Brownian dynamics adhere each other through DNA-cholesterol tags and form liposome clusters. After clusters are constructed, they assemble to construct larger clusters, which aggregate to form a branched network [9-11]. This hierarchical assembly of the network structure is visible by naked eye and it is interesting not only from the aspect of the artificial multicellular life synthesis but also in the field of supramolecular chemistry.

CONCLUSION
DNA-cholesterol conjugate induced assembly of liposomes, which are constructed by assembly of amphiphilic molecules. Aggregation selectively occurs according to the sequences of the DNA part of liposomes. This result would be a basis for the artificial multicellular life synthesis.

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REFERENCES