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ARTICLE

Reaction Mediated Artificial Cell Termination: Control of Vesicle Viability using Rh(I)-Catalyzed Hydrogenation

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Methods for artificial cell control by applying catalytic processes are receiving increasing attention as a basis for artificial control of cellular functions. Here we have developed a Rh(I)-based catalytic hydrogenation reaction of unsaturated bonds of lipids composing vesicles contained in aqueous media. The reduction reaction was applied to vesicles revealing that oleate vesicles collapse following catalytic reduction with H_2 and Rh(I) catalyst, while the distribution of EggPC liposomes was increased following reaction. Proliferation and size of the vesicles could be thus controlled by catalysis based on variations in vesicle membrane fluidity. This process is applicable for use in artificial cell and / or even living cellular system.

Introduction

Methods for artificial chemical control and operation of living cells have been widely studied and include application of organic functional molecules such as photoresponsive or "caged" compounds,[1-4] introduction of a substrate,[5-7] as well as interactions with carbon materials (e.g., graphene and fullerenes),[8-12] inorganic materials,[13-16] biohybrid materials[17-18] and more recently by catalysts and promoters.[19-22] From the viewpoint of cell control, artificial cells (i.e., vesicles) can be considered as a simplify model of living cells.

The operation of living systems depends on three aspects, namely, information, catalysis and membrane structure. Considering living systems in simple terms, information storage and expression involves interactions of proteins with nucleic acids (DNA, RNA), catalysis is enzyme-based (i.e. proteins), and membranes are largely composed of phospholipids.[23-26] Recently, development of artificial cell models has been investigated using artificial nucleic acids,[27-28] catalysts,[29-32] and membranes.[33-35] In this work, we have focused on how catalysis and membranes determine cellular structure and functions.

Oleate[36] and phospholipids (POPC, EggPC etc.)[37] are well known for their use in artificial cell membranes and some derivatives of these form large vesicles (often referred as giant vesicles). Furthermore, with respect to artificial catalysis, enzymes have been investigated and lipophilic acid catalysts have been used as simple models in Luisi's vesicle growth model[36] and Sugawara's artificial cell model.[38] However, there are only a few examples of the development of artificial catalysts for use in artificial model cells. On the other hand, control of living cell function using artificial catalysts and promoters for activation of biological molecules has been recently developed[19-22], being often referred to as 'bioparallel chemistry'.[22] We assumed that by applying an artificial catalyst to an artificial model cell, smaller-sized and 'smarter' functional artificial catalytic cells could be developed, and might make the basis for controlling the functions of living cells as well as those of artificial cells.

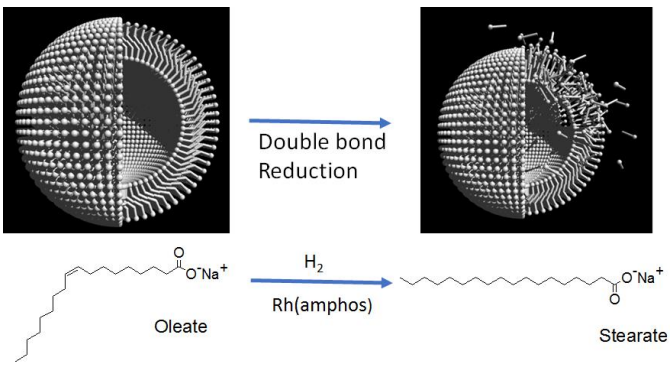

Fig.1 Schematic diagram of hydrogenation of unsaturated lipids composing vesicles.

Natural cell membranes are largely composed of phospholipids which have unsaturated fatty acids incorporated in their structures. Components of artificial cell model membranes such as POPC, EggPC and oleate also possess unsaturated bonds, which affect the fluidity of membranes in which they are contained. Considering cell membranes as important structural and functional elements of living systems, Vigh et.al. have presented significant work on cell membranes under reductive treatment with a Pd-catalyst.[39]

Here we have focused on the reduction of the unsaturated bonds in artificial structured membranes (i.e., vesicles, liposomes) induced by artificial catalysis. Initially, we monitored the reduction of oleate in vesicles by using optical microscopy (OM), dynamic light scattering (DLS) and NMR. Additionally, EggPC liposomes, which contain a

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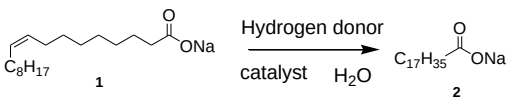
phospholipid used in drug delivery systems, were subjected to catalytic reduction.

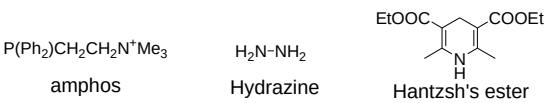
Results and Discussions

The model reaction of aqueous-phase catalytic hydrogenation of oleate to stearate was screened by applying Rh(amphos) catalyst, which is known to catalyze reduction of hexene to hexane.[40] Aqueous phase reduction was accomplished in quantitative yield after four hours reaction time. (Entry 1 in Table 1). Of the hydrogen donors used apart from H_2 gas (Entry 2-5 in Table 1), hydrazine gave a moderate yield at a reaction temperature of 60 ^{0}C. However, Rh(amphos) did not effectively catalyze reduction involving hydrazine. Oleate could be reduced using Rh(amphos)-catalyzed hydrogenation.

Table 1. Reduction of Sodium Oleate in water



Entry	Hydrogen donor	catalyst	condition	yield
1	H_2	Rh(amphos)	4h rt	quant.
2	Hydrazine	none	1d 60^{0}C	55%
3	Hantzsch's ester	Rh(amphos)	16h rt	N.R.
4	cyclohexadiene	Rh(amphos)	16h rt	N.R.
5	Hydrazine Ts	Rh(amphos)	16h rt	10%



Subsequently, the model reaction using vesicles was examined. Oleate vesicles are conventional cell models (giant vesicles) and were synthesized by stirring sodium oleate in aqueous buffer (pH 8.5, 0.22 M bicine, see Figure S1, Supporting Information). The double bonds contained in the vesicles were then reduced by introducing Rh(amphos) catalyst and H_2(gas), and the reaction was monitored using OM, NMR and DLS. Following double bond reduction in vesicle with Rh(amphos) and H_2, the number of large vesicles significantly diminished after 8h reduction as indicated by OM (Figure 2). The yield of the reaction of oleate to stearate was 13% according to NMR measurements (Figure S2a, see Supporting Information).

The variation of vesicle size distribution occurring during reduction was monitored by using DLS (Figure 4 (A)(B)). The population of small vesicles (~ 1 μm) was decreased by addition of 5 mol% Rh(amphos), and this may be related to variation in ionic strength. Following reduction, the population of small vesicles was further decreased. Additionally, the distribution of large vesicles was affected by the lower fluidity after reduction. Thus, it is considered that 1) 13% reduction of oleate causes large vesicles to collapse, 2) reaction is slower than in homogeneous systems, 3) smaller vesicles react faster, and 4) fluidity of the membrane is decreased by reduction. Oleate vesicles collapsed after reduction permitting control over vesicular destruction.

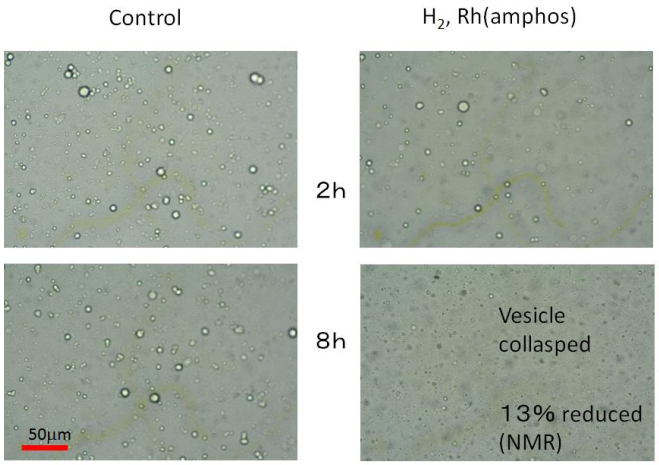


Fig.2 Reduction of oleate in vesicular form using Rh(amphos) with H_2. Increasing presence of stearate destabilizes large vesicles by reducing membrane fluidity. (Control: simple stirring without catalytic reduction). Scale bar is 50 μm and applies to all images.

We further investigated the effect of reduction by mixing oleate and stearate and observing the resulting assembled structures by OM. By addition and mixing of 20 mol% stearate for 2h, lamellae of stearate could be observed and vesicle structure was hardly affected. In this case, vesicle collapse was slow because of the low solubility of stearate. Conversely, by adding 50 mol% stearate and stirring for 6h, vesicles were fully decomposed. Variations in vesicle stability caused by lipid mixing were slow in contrast to the effect of the reduction process (Figure 3.)

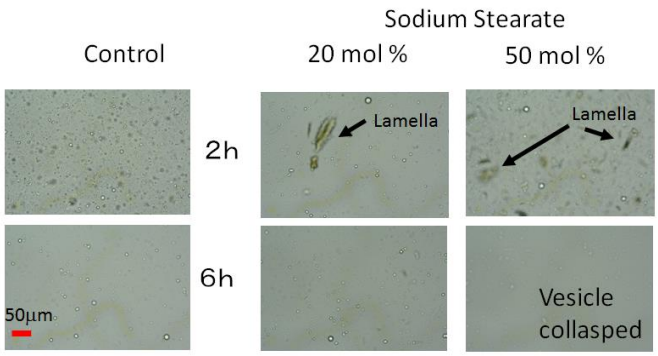


Fig.3 Mixing oleate vesicles with sodium stearate. Sodium oleate vesicles were mixed with 20–50 mol % sodium stearate and stirred for 2h and 6h. Scale bar is 50 μm and applies to all images.

The double-bond reduction reaction was also applied to EggPC liposomes. EggPC contains unsaturated bonds and has been investigated as a potential drug delivery system involving vesicles (liposomes). Catalyst (Rh(amphos)) was added to the EggPC liposome and catalytic reduction (sample-H_2) applied with stirring for 2h with reaction monitoring by NMR, followed

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by analysis using DLS (Figure 4C). A sample containing only catalyst was treated in the same way (sample-cat).

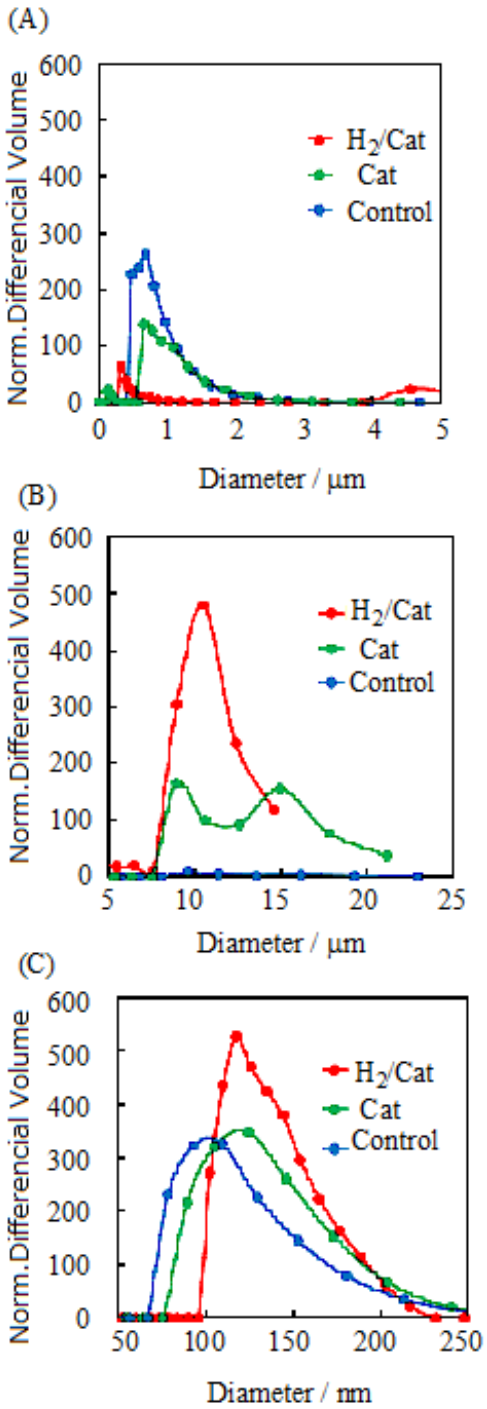

Fig.4 Diameter distribution of vesicles (differential volume). H_2/Cat: after catalytic hydrogenation (red line); Cat: control with catalyst only (green line); Control: oleate vesicles with stirring only. (Stirred for 2h). (blue line) (A) Oleate vesicles, 0 to 5 μm, (B) oleate vesicles, 5 to 25μm. (C) EggPC liposomes, 50 to 250 nm.

In the sample subjected to catalytic reduction, the distribution of liposome diameter increased to lie in the range 100–220 nm (with a shoulder at 140nm) over the control samples whose diameters were in the range 70–250 nm. NMR analyses indicate that yield of reduction was 18% (see Figure S2b in Supporting Information). The changes observed are due to the lower fluidity of the membrane components or a decrease in prevalence of smaller vesicles caused by the reduction reaction,[39] Thus, reduction could be successfully applied for vesicle size control permitting control of cell permeability in DDS.

Conclusions

In this work, we have developed the aqueous-phase reduction of the double bond of oleate using a Rh(amphos) catalyst and hydrogen. Double bond of oleate vesicles, which are frequently used as conventional model cells, could be reduced after 8h catalytic treatment, and vesicle collapse was observed. EggPC liposomes, which have been used in DDS research, were reduced after 2h catalytic treatment with a concurrent increase in the size of vesicles. We believe that this Rh(amphos) reduction process can be applied to control the fluidity and sizes of artificial model cells, presenting a basic model for catalytic control of artificial vesicular materials or even living cellular systems.

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Notes and references

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