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Research Article

Recovery of DNA Crown Cells (artificial cells) from Egg white with Albumin

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Abstract

DNA crown cells are artificial cells, in which the outside of the membrane were covered of DNA. DNA crown cells (artificial cells) may be prepared by incubating within egg white with sphingosine -DNA-adenosine mixtures. DNA crown cells are easy synthesized and multiplied within egg white. To date, many types of DNA crown cells have been synthesized using various types of DNA. However, it is unclear whether DNA Crown Cells have any practical value in applied fields, to investigate potential applications, it is necessary to collect pure artificial cells from egg white.

In the present experiments, I prepared DNA crown cells using DNA of *Streputomyces kanamyceticus* within egg white, and then examined whether DNA crown cells could be recovered from egg white containing these cells. I found that DNA crown cells could be recovered from egg white using egg albumin.

Keywords: DNA crown cells; Sphingosine-DNA *Streptomyces* k*anamyceticus*; Cell recover

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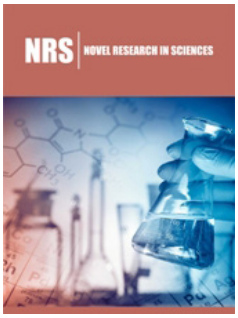

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Introduction

There has been significant progress in the generation of artificial cells since 1960s [1,2]. Recently, approaches for generating fully operational (self-replicating) artificial cells that are covered with DNA (known as DNA crown cells) have been reported [3-5] and these are primarily generated when they are incubated with egg white. By such methods [4], un-limited DNA crown cells could be theoretically prepared. and the several types of DNA crown cells have been synthesized [3-9]. Such cells have characteristic structures, large loupe of DNA that are similar to plasmids. Hence, it was expected that DNA crown cells would possess similar as plasmids.

To clarify such the properties of such cells, it is important to collect pure cells from egg white.

DNA crown cells which are generated within egg white are generally recovered as follows. Dulbecco' modified Eagles' medium containing 10% bovine serum (10ml) is added to egg white (2 ml) containing DNA crown cells, followed by incubation at 37 ℃ for 2 days. Then, DNA crown cells (precipitates) are collected. In a previous report (10), it was found that DNA crown cells using DNA from *Streptomyces griseus,* which produce several types of antibiotic, could be prepared and the antibiotic was produced in the co-cultures of egg white containing DNA crown cells and yeast (beer).

However, it remains unclear whether antibiotic can be produced using DNA crown cells alone, as DNA crown cells together with egg white used. To resolve such issues, experiments must be carried out using purified cells. Therefore, in the present experiments, methods to collect such cells were examined. The present experiments demonstrated that DNA crown cells precipitate with the treatment of albumin, and could be easy collected from egg white.

Materials and Methods

Materials

The following materials were used: Sphingosine (Sph) (Sigma, USA), DNA (extracted from *Streptomyces kanamyceticus*), adenosine (Sigma, USA and Wako, Japan), the lipids monolaurin (Tokyo Kasei, Japan) Edible white legwhorn eggs punched from a market.

A-M compound (synthesized with the mixtures of adenosine and monoraulin) [8].

albumin from egg, albumin from bovine serum, albumin from human serum (Wako, Japan, respectively).

Dulbecco' minimal Essential Eagle's Medium (MEM) (Sigma, USA), bovine serum (Sigma, USA)

Methods

Preparation of DNA (*S.kanamyceticus*) crown cells: DNA crown cells were prepared as described previously [8]. Briefly, 90 µL of Sph (10mM) and 40 µL of DNA (1,7 µg/µL) were mixed and the mixture was then heated. A-M compound (50 µL) was added, and the mixture was injected into the white (albumin) of an egg. After injection, Eggs were incubated at 37 ℃ for 7days and 2m L of egg white was added to MEM containing bovine serum (10%) (DMEM) of 10mL. After 2days of incubation at 37 ℃, the precipitates were observed. A drop of the precipitates was placed on the slide glass and covered, followed by observation under a microscope.

Recovery of DNA crown cells from egg white with Albumin and Imaging of DNA *(S.kanamyceticus*) Crown Cells: A total of 2ml of egg white which contained DNA (*Streptomyces kanamyceticus*) crown cells, and 2ml of egg white not contained crown cells were added to test tube. Distilled water was added and give a total volume of 9ml. After mixing, 1 ml of bovine serum albumin (10%) was added to the tube. After mixture, tube were left to stand for about 2 hours in room temperature.

A total of 2ml of egg white which containing DNA (*Streptomyces kanamyceticus*) crown cells was added to test tube. Distilled water added to give a total volume of 9ml. After mixing, 1ml of bovine serum albumin (10%), egg albumin (10%) and human serum albumin (10%), respectively, was added to test tube. After mixing, tubes were left to stand for about 2 hours at room temperature. Precipitates were then observed. A drop of the precipitates was then placed on the slide glass and covered, followed by observation under a microscopy.

Results and Discussion

Preparation of DNA (S. kanamyceticus) crown cells

Methods for preparing DNA crown cells (artificial cells) have been established using several types of DNA [3-9]. These procedures have been summarized in several reports [4,8]. In the use of DNA from *Streptomyces kanamyceticus,* Sph was mixed with DNA (*S. kanamyceticus*) and heated. A-M compounds were then added to Sph-DNA mixtures. which were then incubated in egg. After transplantation of 3~5 times (3~5 generations), egg white were removed and DNA (*S.kanamyceticus*) crown cells were recovered by incubation with D-MEM.. DNA (*S. kanamyceticus*) crown cells over three generations are shown in Figure 1. Cells of circular or modified shape (approximately 5~10µm in size) were noted. Cells had several characteristics, such as shape, in addition to the obvious characteristics of DNA crown cells (having a large loop of DNA and being able to replicate in egg white).

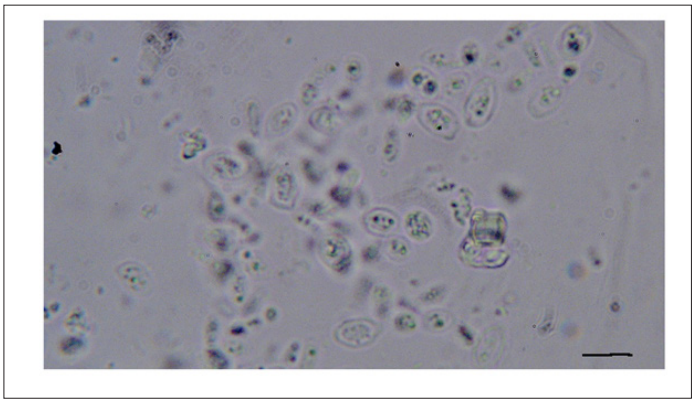

Figure 1: Preparation of DNA (S. kanamyceticus) Crown Cells.
Sphingosine (Sph) was added to DNA. After heating, adenosine-monolaurin (A-M) compound was added to Sph-DNA mixture, Mixtures containing DNA crown cells were incubated in egg white. Egg white was transferred into a new egg every 7 days. An aliquot of third generation egg white (2 mL) was cultivated in Dulbecco's Modified Eagle's Medium containing 10% bovine serum at 37°C for 2 days. Precipitates were observed using microscopy. Cells of circular or modified shape were observed. Cell size was approximately 5~10 µm. Scale bar = 10 µm.

Recovery with albumin

In general, DNA crown cells were obtained after incubation of DMEM and egg white contained cells. For example, when 2 mL of egg white were incubated with a total 10 mL of DMEM, precipitates (cells) were obtained. This method takes 2 days and needs large amount of DMEM to obtain cells. Therefore, I am investigating other methods to collect such cells, and focused on the albumin present in bovine serum. Specifically, bovine serum albumin solution was added to egg white which containing cells and after about 2 hours, the precipitates were observed (Figure 2a), while no precipitates were observed with addition of normal egg white (not containing cells) (Figure 2b)., Cells that were present in egg white were precipitated with bovine serum albumin. Next, it was examined whether precipitates were observed using several types of albumin.

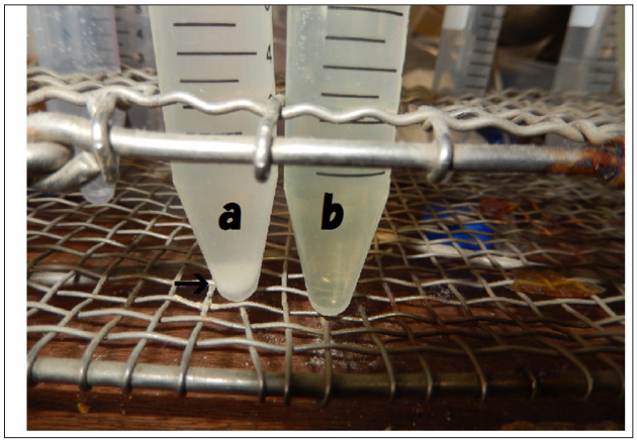

Figure 2: Recovery of DNA crown cells from egg white with bovine serum albumin.
Bovine serum albumin solution was added to egg white with DNA crown cells and mixed. Figure 2a shows an image taken at 2 hours after addition. Precipitates (arrow) were observed in egg white (containing DNA crown cells), but were not observed in normal egg white (not containing cells) (Figure 2b), showing that DNA crown cells were precipitated with bovine serum albumin.

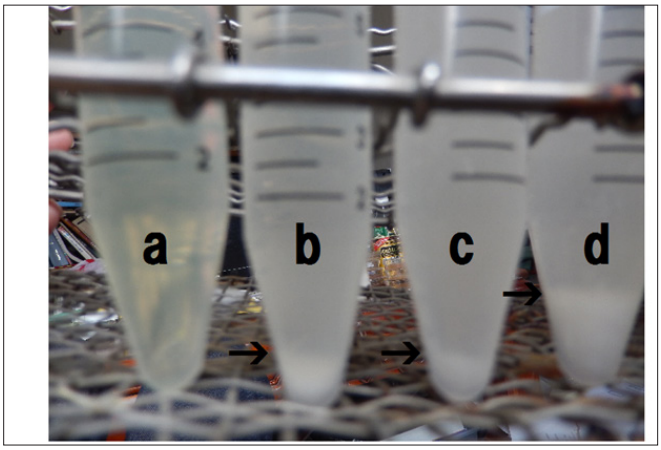

Figure 3: Recovery of DNA crown cells from egg white with several types of albumin.
Solutions of bovine serum albumin, human serum albumin and egg albumin, respectively, were added to egg white containing cells and mixed.

Figure 3 shows images obtained at 2 hours after addition.
Precipitates (arrow) were observed with all albumin samples tested.
Yield was best with egg albumin.
a. Egg albumin solution was added to normal egg white (not containing cells).
b. Human serum albumin c. Bovine serum albumin. d. Egg white albumin.

Figure 3 shows the precipitates when human serum albumin (b), bovine serum albumin (c), and egg albumin (d), respectively, were added to egg white containing DNA crown cells. No precipitates were observed in mixtures with non-treated egg white (not containing cells) or egg albumin (Figure 3a). Based on these results, egg albumin may be best for collecting artificial cells. as egg albumin is relatively inexpensive, and it may give larger yield when compared with other albumin.

Next, it was examined whether other DNA crown cells (Akoya DNA [7], bovine meat DNA [6], Human placenta DNA [9]) were collected with egg albumin. The precipitates were observed in all samples tested (Data not shown).

Cell suspensions were placed on glass slides and were observed using microscopy. Cells which were indicated in Figure 1 were observed (Data not shown). Thus, DNA crown cells that were present in egg white could be recovered with egg albumin. It is likely that this occurs based on the binding of cells and egg albumin. As in previous experiments, cells could also be recovered using DMEM. Therefore, cells may precipitate based on an association with components other than albumin. It is important to assess the characteristics of DNA crown cells in order to determine such components.

However, the present purpose was to establish a technique to collect DNA crown cells from egg white, and possible methods were herein described.

Conclusion

DNA (*S. kanamyticeus*) crown cells were prepared within egg white and could be recovered with egg albumin.

Thus, the present experiments showed a technique to collect DNA crown cells that were present in egg white.

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