Enhancement of cesium-ion-uptake function of membrane vesicles and establishment of ion recovery technology

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Research objective

Twelve years after the occurrence of the Fukushima Daiichi Nuclear Power Plant accident, the release of treated water, comprising the radionuclide-free portion of the ever-increasing amounts of contaminated water, into the ocean continues to be a focal issue. This is because in more than 70% of the stored "treated water," radioactive nuclides such as radioactive strontium (Sr) have not been removed to levels below the detection limits set for release into the ocean. In addition, there are problems such as the need to continually perform radionuclide removal processing to achieve the requisite levels [1)]. Thus, there is still a need for more effective and inexpensive techniques for radionuclide removal (particularly Sr and cesium (Cs)) from the contaminated water. Environmental purification technologies using microorganisms are inexpensive and can maintain a low ecological load [2)]. Meanwhile, the half-life of ^{137}Cs can be as long as 30 years, and long-term environmental pollution can thus become a significant problem. In addition, Cs has similar physicochemical properties to potassium (K) and diffuses throughout the body in the same way as K [3)]. Furthermore, Cs^+ in bacteria is taken up by the cells via the K^+ uptake system. On the other hand, as there is no Cs^+ excretion system and Cs^+ accumulates in the cells, Cs^+ is toxic to bacteria [4)]. Meanwhile, in our laboratory, *Microbacterium* sp. TS-1, which is resistant to high cesium concentrations, has been isolated from Jumping Spiders ground extract [5)]. Strain TS1 possesses a Cs^+/H^+ antiporter (CshA) capable of taking H^+ into cells and exporting Cs^+. This study aims to utilize CshA to construct a system for recovering Cs from pollutants.

Methods

Developing a simple membrane vesicle recovery system for practical applications is necessary as a technology for using inverted membrane vesicles containing the abovementioned CshA enzyme to recover and concentrate large amounts of Cs^+ from the environment. Practical applications cannot be realized unless a more efficient membrane vesicle collection system becomes available. In addition, an ultracentrifuge is required to collect the inverted membrane, which is practically unsuitable. Therefore, we attempted to optimize the recovery conditions. *Escherichia coli* inverted membrane vesicles expressing CshA were confined to a spherical gel with a diameter of approximately 2 mm using calcium alginate. The requirements for

creating a spherical gel of calcium alginate embedded with membrane vesicles expressing CshA were determined by trial and error, and the optimal conditions for the mixing ratio were thereby determined. In this experiment, we used the highly Cs-resistant *E. coli* strain ZX1 obtained by transforming *E. coli* Mach1 with CshA possessed by the TS-1 strain. In addition, an *E. coli* KNabc strain in which the major Na^+/H^+ antiporter was deleted was used as a negative control. First, high-pressure cell disruption was performed to prepare inverted membrane vesicles from the ZX-1 strain using a French press (Glen Mills). The prepared inverted membrane vesicles were wrapped in 1% sodium alginate aqueous solution using a double-tube nozzle and dropped into 100 mM calcium chloride aqueous solution to form a spherical gel (Figure 1). Twenty spherical gels were aliquoted into 15 ml centrifuge tubes, and 3 ml each tube was dispensed into a 30 mM Tris-HCl buffer (pH 8.5) mixture containing 30 mM CsCl and 2.5 mM succinic acid. The mixture was shaken for 1 h, and the supernatant was collected. The Cs^+ concentration in the supernatant was measured using a flame photometer (BWB Technologies).

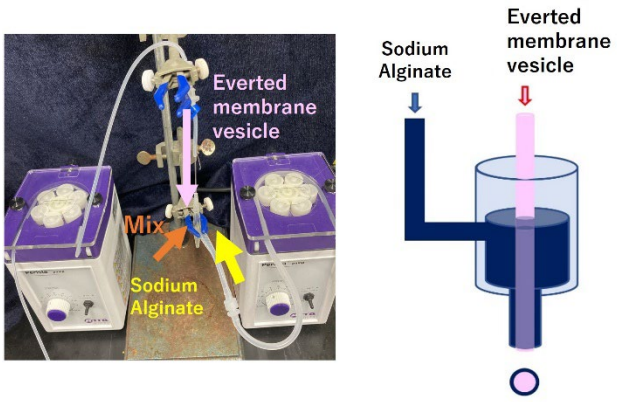

Figure 1. Apparatus for producing inverted membrane-embedded alginate gel using a double-tube nozzle and its schematic.

Results

The everted membrane vesicles from strains KNabc and ZX1 were prepared with concentrations of 10 mg/ml, 20 mg/ml, and 40 mg/ml, respectively, and alginate gels without everted membrane vesicles were mixed with 30 mM CsCl and 2.5 mM succinate. The vesicles were then incubated in 30 mM Tris-HCl buffer (pH 8.5) containing acid for 1 h with shaking. A decrease in Cs^+ concentration was confirmed in both the KNabc and ZX1 strains (Figure 2).

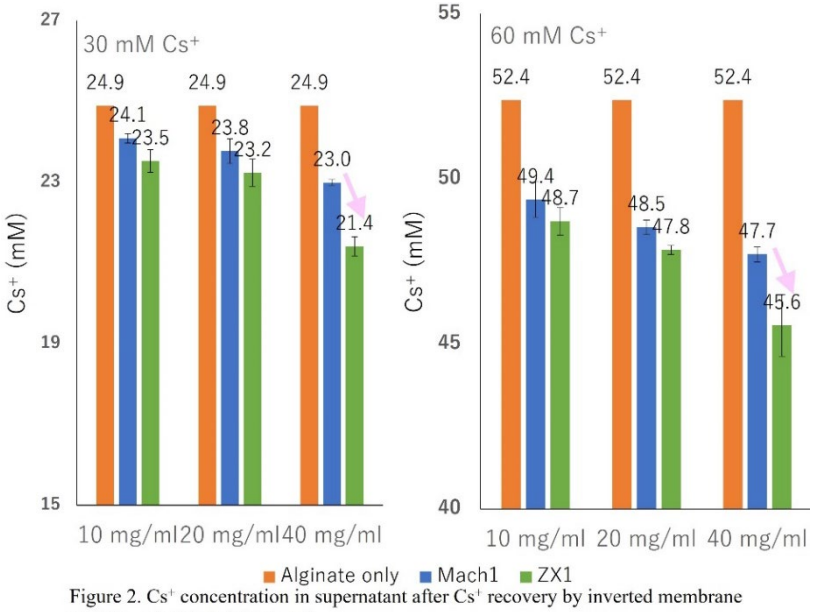

Figure 2. Cs^+ concentration in supernatant after Cs^+ recovery by inverted membrane vesicle-embedded alginate gel

Since the volume of the solution increases by adding 20 spherical gels, the Cs^+ concentration measured only with the alginate gel will also be lower than the Cs^+ concentration of the original solution.

In particular, the everted membrane vesicles of ZX1 recovered more Cs^+ than those of KNabc. In addition, the Cs^+ concentration in the gel alone, without the addition of everted membrane vesicles, was consistent with the decrease in Cs^+ concentration accompanied by an increase in volume when 20 gels were added. Therefore, we concluded that Cs^+ is not directly adsorbed onto the gel.

Conclusion

We previously confirmed that Cs^+ can be recovered using an everted membrane expressing CshA. However, when using the spherical gel prepared by mixing everted membrane vesicles with alginic acid, selective recovery of Cs^+ by CshA was not observed. Therefore, in this study, we attempted to recover Cs^+ using an alginate spherical gel embedded with everted membrane vesicles expressing CshA using a double-tube nozzle (Figure 1). In the alginate spherical gel embedded with everted membrane vesicles, the Cs^+ recovery rate increased as the concentration of the embedded everted membrane vesicles increased. Consequently, we have demonstrated that Cs^+ can be recovered in the form of a spherical gel with an approximate diameter of 2 mm, eliminating the need to retrieve the everted membrane vesicles using an ultracentrifuge. We believe increasing the number of everted membranes embedded in the alginate gel can further increase Cs^+ recovery. Various conditions are currently being considered to further improve the Cs^+ recovery rate.

References

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