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Control by osmolarity and electric field strength of electro-induced gene transfer and protein release in fission yeast cells

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Abstract

A high electric pulse was applied to the uptake of DNA into cells, the sterilization of cells, and the release of protein from cells. These applications to fission yeast showed a strong dependence on both the osmolarity of pulsing sorbitol solution and the intensity of the electric pulse. In electroporation, high transformation efficiency was obtained with a wide range of sorbitol (0.6–1.6 M) at 10.0 kV/cm for about 5 ms. Furthermore, the highest efficiency was achieved in 1.5 M sorbitol at a higher strength, 12.5 kV/cm, although the cell survival rate dropped. The release of protein generally increased with increasing electric field strength, due mainly to leakage from dead cells under hypotonic conditions. However, protein was released significantly in 1.5 M sorbitol at a lower strength, 7.5 kV/cm, although a high survival rate was maintained. Thus, the application of the high electric pulse to fission yeast under hypertonic conditions increased the uptake and release of macromolecules controlled by the electric field strength.
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1. Introduction

The application of a high electric field to cells increases membrane permeability, making possible the exchange between intracellular and extracellular macromolecules [1,2]. Although the mechanism of electro-permeabilization remains unclear, the membrane's dielectric breakdown caused by the high electric field is known to provoke reversible or irreversible changes in membrane structure [3]. These alterations depend strongly on the intensity of electric pulse. In transformation, electroporation (also known as electro-transformation or electroinjection) is currently the most common form of reversible electro-permeabilization in practical use, by virtue of its ability to introduce foreign DNA into intact yeast cells while maintaining a high survival rate [4–8]. Transformation is the genetic alteration of a cell resulting from the expression of introduced foreign DNA. In protein release, on the other hand, electroextraction—a form of irreversible electro-permeabilization beyond repair in the membrane breakdown caused by excessive electric pulse intensity—can release cytoplasmic enzyme from the cell, thereby disrupting the membrane and killing the cell [9–13].

Yeasts are widely used as hosts for the industrial production of protein. Especially, the fission yeast *Schizosaccharomyces pombe* resembles higher eukaryotes in the structure and function of genes and proteins [14]. Thus, *S. pombe* has high potential as a host for expression systems for foreign genes derived from higher animals. In electroporation experiments of *S. pombe*, osmolarity treatments before and after the electric pulse improve the transformation efficiency. These experiments included pretreatment with dithiothreitol (DTT) and isosmotic 0.6 M sorbitol [15], hyperosmotic preincubation with 2.0 M sorbitol or 1.5 M NaCl [16], and hyperosmotic post-incubation with 2.0 M sorbitol and pH buffer [17]. On the other hand, the effectiveness of the use of a slight hypertonic solution of 1.0−1.2 M sorbitol during an applied electric pulse is unsubstantiated; instead, it is generally used for intact *S. pombe* cells [7,8]. For other yeast protocols, 0.27 M sucrose is used [5]. In contrast, for

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mammalian cells a hypotonic condition (i.e., a low osmotic solution such as pH buffer) during the electric pulse is optimal to obtain high transformation efficiency [18]. In the electroextraction procedure of yeast cells, post-pulse incubation media with 0.25 M KCl or 0.24 M glycerol contributed to higher enzyme activity of released protein [10,12,13]. Although these adjustments in osmolarity are important factors with regard to both electro-transformation and electroextraction, the effects of changes in osmolarity on electro-permeabilization during an electric pulse in yeast cells have not been intimately examined until now.

In this paper, we describe the effects of osmolarity during an electric pulse on cell electro-permeabilization, such as DNA uptake and protein release in fission yeast cells. This membrane permeability was controlled by both the pulsing osmolarity and the electric field strength.

2. Materials and methods

2.1. Yeast strain and culture

The *S. pombe* strain ATCC38399 (*h*$^{-}$ *leu1-32*) was grown in synthetic defined (SD) medium (0.67% Bacto yeast nitrogen base w/o amino acids, 2% glucose) with 150 μg/ml of leucine to a density of approximately 1×10^7 cells/ml in the late-log phase at 30 °C. Cells were collected by centrifugation at 1600*g* for 5 min, and the resulting pellet was washed three times with ice-cold sterilized ultrapure water (18 MΩ cm). The pellet was then resuspended in 0−2.0 M sorbitol to give 10^8–10^9 cells/ml, and was placed on ice until use. The sorbitol solutions were adjusted with ultrapure water to avoid arcing during an electric application.

2.2. Electropulsation

The cell suspension was transferred to a chilled cuvette made of parallel flat aluminum electrodes and having a 0.2-cm electrode gap (Bio-Rad Laboratories). An exponentially decaying capacitive discharge pulse (0.5–2.5 kV) was applied using the Gene Pulser II with Pulse Controller Plus (Bio-Rad Laboratories). The time constant setting was held at 25 μF and 200 Ω, which yielded approximately 5 ms.

2.3. Transformation by electroporation

The electroporation procedure was based on a previously described method [19]. Fifty microliters of the cell suspension, at a concentration of 1×10^9 cells/ml, was mixed with 0.5–5 ng of plasmid DNA. The plasmid pAL7 (carrying the yeast *LEU2* gene as a selectable marker, 6.5 kb) was used and purified using the Qiagen Plasmid Mini Kit (Qiagen) [20]. The pulsed cells were immediately diluted with the same osmotic sorbitol solution and spread on minimal selection plates (0.67% Bacto yeast nitrogen base w/o amino acids, 2% glucose, and 2% agar). Transformant (i.e., cell transformed with plasmid DNA) colonies appeared in 4–6 days at 30 °C. Transformation efficiency was assessed based on the amount of plasmid used and the number of colonies grown on the plates.

2.4. Cell survival

The survival of cells following the electric pulse was determined when 100 μl of the cell suspension at 1×10^8 cells/ml was pulsed and immediately diluted with the same osmotic sorbitol solution and spread on yeast extract–peptone–dextrose (YPD) agar plates (1% Bacto yeast extract, 2% Bacto peptone, 2% glucose, and 2% agar). Colonies appeared in 3 days at 30 °C.

2.5. Protein release

A 200 μl volume of the pulsed-cell suspension at 1×10^9 cells/ml was centrifuged at 1600*g* for 5 min, and the protein content in the supernatant was determined according to the method of Bradford with bovine serum albumin as a standard [21].

3. Results and discussion

3.1. Effects of osmolarity during electric pulse and electric field strength on gene transfer

In the electroporation of yeast, sorbitol is a suitable substance for a pulsing solution due to its nonelectrolyte, nonfermentative sugar and to its osmotic stabilizer. However, the effects of osmolarity of a pulsing sorbitol solution on the gene transfer, survival, and release of protein in yeast were not sufficiently reported until now.

Fig. 1 shows the effects of sorbitol concentration during an electric pulse on transformation efficiency. The electric field strength of 10.0 kV/cm was applied within the range of 0–2.0 M sorbitol. The best efficiency was obtained in 1.0 M sorbitol at 10.0 kV/cm. This result agreed with the finding that the optimal electric field strength is 10 kV/cm when intact *S. pombe* cells are suspended in 1.0 M sorbitol [22]. The general use of 1.0–1.2 M sorbitol for the electroporation of intact yeast is attributable merely to its use as an osmotic stabilizer for yeast spheroplast to avoid disrupture [23,24]. Above 10^6 transformants per μg of DNA, the efficiencies obtained were, surprisingly, high enough for the use of electro-transformation at the wide range of 0.6–1.6 M sorbitol. A marked drop in efficiency was observed below 0.4 M sorbitol of hypotonic solution. Thus, the optimized transformation was obtained with 10.0 kV/cm when the osmolarity of the pulsing solution was balanced with, or greater than that of, the isotonic solution (0.6 M sorbitol).

In electroporation experiments, the important parameters affecting the transformation efficiency are electric field strength and pulse duration. In this report, relationships between the osmolarity of the pulsing sorbitol solution and the electric field strength were also investigated, as

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