Roumanian Biotechnological Letters *Vol. 9, No. 1, 2004, pp 1541-1544*

Center for Research in Enzymology and Biotechnology
Roumanian Society of Biological Sciences

REVIEW

Secondary Effects of Electroporation with bipolar electric pulses: Electrostimulation

Received for publication, December 3, 2003
Published, January 20, 2004

TATIANA VASSU, D. FOLOGEA, ORTANSA CSUTAK, DIANA SMARANDACHE, ELENA SASARMAN, ILEANA STOICA, SIMONA SOARE
MICROGEN-Center for Research in Genetics, Microbiology and Biotechnology, University of Bucharest, Faculty of Biology, 1-3 Aleea Portocalilor, 772011-Bucharest, Romania
e-mail: tatiana@botanic.unibuc.ro

Abstract

In this paper, we have investigated the effect of bipolar electric fields on yeast division was investigated. Saccharomyces cerevisiae TS5 was subjected to an electric field as a succession of bipolar electric pulses, with amplitude in the range of 0-1.625 kV/cm and the pulses train length of 4 msec and 8 msec. The electrical parameters for a maximum electrostimulation effect was established.

Keywords: Electrostimulation, *S. cerevisiae*

Introduction

The interaction of a biological system with an external electric field is generally located at the cell membrane level, its electrical properties being very different from the external or internal medium. The primary result of such an interaction consists in an induced transmembrane potential, related to the intensity of electric field and the geometry of the cell.

For a spherical cell, having a radius R, the maximum of induced potential is given by [1]:

$$V = 1.5 * R * E$$

where E is the external electric field (kV/cm).

Inside the cell membrane, the electric field will be:

$$E_m = V / d = 1.5 * R * E / d$$

with d the membrane thickness.

For an average cell, with R=10 μM and d=0.01 μm, the external electric field will be increased inside the cell membrane about 10^3 times. So, it is natural to consider the cell membrane as the main level of the biological system interaction with the external electric fields. On the other hand, natural membranes are very complex, containing not only lipids but also a lot of proteins involved in transport phenomena or receptor proteins, most of them having electrical mechanisms of action. The activity of these proteins could also be affected by an external electric field amplified at the membrane level.

Experiments pointing to the stimulation effect of electric field were carried out using different parameters of electric field, such as the shape of the electric pulse and time of action [2-5].

Our idea of electrostimulation using bipolar electric pulses appeared after some experiments of gene transfer by electroporation in yeast strains. Usually, after electroporation, a single peak represents the maximum of transformation efficiency. Using a succession of bipolar electric pulses, with certain conditions regarding field intensity and duration, two separated peaks representing the maximum of transformation efficiency are obtained. Of course, these peaks have different values of amplitude, but their presence is suggesting that beside the cells death due to the electric field, another effect, a stimulation of the division must be present.

The aim of this paper is to study the stimulating effect of an electric field as a succession of bipolar electric pulses on yeast cells.

Material and Methods

Biological material

Saccharomyces cerevisiae TS5, grown in 10 ml liquid standard medium (YEPD)[6] at 30^0 C for 24 hr, are washed twice in 20% glycerol and then resuspended in 2 ml glycerol solution (20%). Following the electric treatment, the cells were inoculated in 2 ml YEPD medium, kept for 6 hr at a temperature of 30^0 C, diluted (10^{-4} diluting factor) and plated on standard solid medium. Two days later, the plating efficiency (UFC) was recorded.

Electrical treatment

Electroporation was performed using an electroporation device (home made) having an output as a succession of bipolar square electric pulses. In this experiment, 0.2 ml yeast suspension was exposed to electric field into electroporation cuvette (Al electrodes, 2 mm aperture, BioRAD, USA). The electric field varied in the range 0-1.625 kV/cm and the total length of the bipolar electric pulses train was 4 msec and 8 msec, respectively.

Results and Discussions

Results representing the stimulation effect of the electric field on yeast cells are plotted for 4 msec pulses train length (**Figure 1**) and 8 msec (**Figure 2**).

There are some differences in the experimental results, depending on the electrical conditions. First, the maximum value of the stimulation is higher using 8 msec pulses length than 4 msec. However, the amplitude of the electric field corresponding to maximum stimulation effect is different for the two cases, 1.25 kV/cm for 4 msec and 0.8 kV/cm for 8 msec. These differences could be explained taking into account the lethal effect of stronger electric field.

The position of the maximum effect depends both the intensity of the electric field and the pulse width. This is in agreement with other experimental results presenting a “response window”, where significant changes could be recorded [7]. In our case, this window must be defined at least by E*t, but maintaining reasonable values for E, in order to avoid cell lyses by disrupting the cell membrane (and of course, cell death). Therefore, we can assume the electric field has a stimulative effect and a lethal one, depending on the electrical parameters. We can also accept that each of these effects are operating only over specific thresholds [4]. Anyway, it is well known that, for a single cell, both electroporation and membrane rupture have this feature.

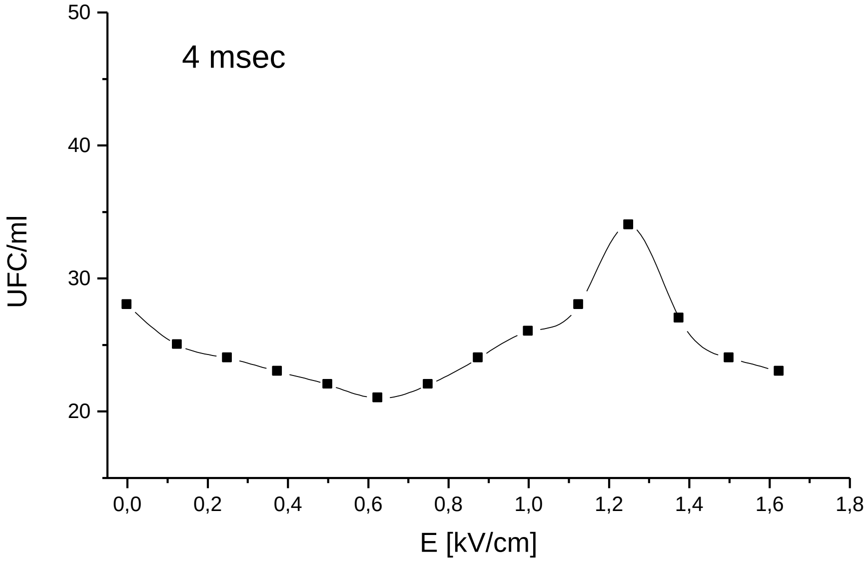

Figure 1. UFC/ml vs electric field intensity for 4 msec bipolar electric pulses train length

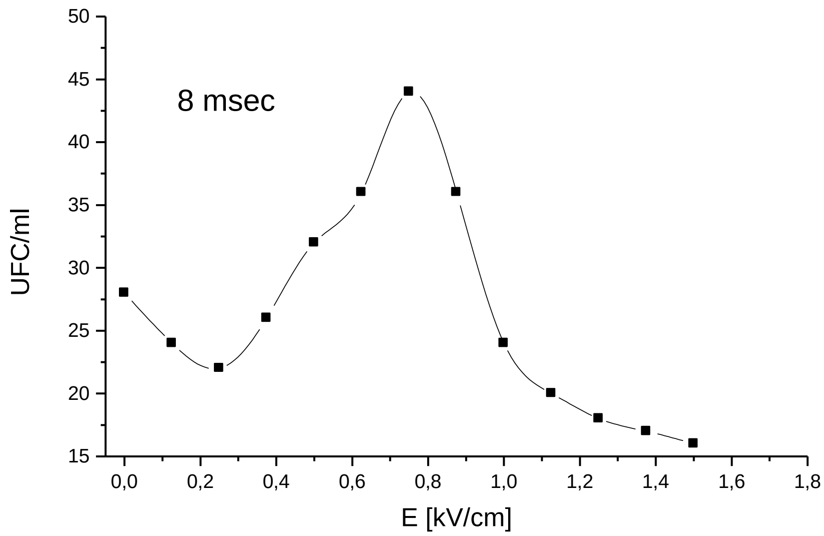

Figure 2. UFC/ml vs electric field intensity for 8 msec bipolar electric pulses train length

According to Brown [8], for both cases, the induced potential is lower than the electroporative one, but the UFC is still decreasing at low electric field. This effect could be explained by taking into account the fact that cell populations are not homogenous in size or even in physiological state [5], so the cellular response could be described using an appropriate Gaussian distribution [9]. As a result, the shapes of the curves from Figure 1 and 2 could be easily explained. For a low electric field, there is no stimulation effect, only a weak lethal one is present (the induced potential will be critical only for cells with large radius or in a certain physiological state). Then, the stimulation effect will be present, the corresponding electric field depending of train pulses length. Increasing the electric field, only the lethal effect of the electric field could be recorded.

The exact mechanisms of electrostimulation are still unknown. A possible mechanism may be related to the increased flow of nutrients and to changes in the activity of some membrane proteins.

Conclusions

Following an electric treatment with bipolar electric pulses, yeast division rate is obviously increased. There is an electrical window for such a response, limited by lethal effect of electric field. However, the exact mechanism of electrostimulation is still unknown, some practical applications of this effect could be imagined, for example biomass increasing.

Acknowledgement

The current work was partially supported by 02-2-PED-414 BIOTECH contract.

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