Electrical Vibrations of Yeast Cell Membrane

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Abstract— Cytoskeleton with microtubules is the main organization structure of the eukaryotic cell. Possible sources of vibrational excitations of electrically polar cytoskeleton components are enumerated. Temperature stabilized, triple screened box (electrically and electromagnetically by mumetal) with point sensor and preamplifiers was used for measurement of electrical oscillations of yeast cell. Preliminary findings of the electrical measurement and local nanomechanical AFM measurements are presented. Findings correspond to the Fröhlich’s postulate of coherent electrically polar longitudinal vibrations in biological systems.

1. INTRODUCTION
Fröhlich postulated electrically polar longitudinal vibrations in biological systems [1–4]. The vibrations generate endogenous electromagnetic field. The electromagnetic field is generated in a broad frequency spectrum. The oscillations in megahertz [5], gigahertz [6, 7], far infra red [8], visible and UV [9, 10] range are detected. Sources of the electromagnetic fields vary over the spectrum.

Pohl observed attraction of small dielectric particles to cells [11, 12]. He explained this effect as a result of dielectrophoresis, what is attraction of polarized particles in a non-uniform electric field. This effect was greatest during the M-phase of the cells, when the rate of polymerization and depolymerization (treadmilling) of microtubules in the mitotic spindle is enhanced compared with the rate in the interphase (dynamic instability).

Local nanomechanical motion of yeast cell (\textit{Saccharomyces cerevisiae}) membrane was measured by Pelling et al. [13, 14] with atomic force microscope (AFM). By means of the Fourier transform of the measured signal they found oscillations with amplitude of a few tenths of nm to a few nm’s in the frequency range below 2 kHz. Frequency of these oscillations was temperature dependent. Background noise of the AFM was of the order of magnitude of 10^{-2} nm. Oscillations at a single frequency have been detected on the normal yeast cell wall and those at multiple frequencies different from the frequency on the normal cell wall have been detected on the bud scar. Oscillations of the yeast cell wall and of the bud scar ceased after addition of metabolic inhibitor, which suggests cellular metabolism is involved in the generation of motion.

This paper describes possible sources of vibrational excitations of polar cellular membrane through the cytoskeleton and refers about the electrical and AFM measurements of vibrations of yeast cell membrane.

2. CYTOSKELETON AND ITS EXCITATION BY CELLULAR METABOLISM
Cytoskeleton is organizing network of the eukaryotic cell, which helps the cell to move, to maintain its shape and to transport molecules and cellular organelles. Cytoskeleton is comprised of three types of protein filaments: actin filaments (microfilaments), intermediate filaments and microtubules [15].

Microtubules are highly polar, deformable and dynamic structures. They resemble hollow tubes with inner and outer diameter of 17 nm and 25 nm, respectively (Fig. 1(a)). Microtubule consists of 13 protofilaments. The building subunits are tubulin heterodimers composed of \(\alpha\)-tubulin and \(\beta\)-tubulin (Fig. 1(b)). These heterodimers have high electrical dipole moment of over 1000 Debye (10^{-26} C.m); they are responsible for the high electrical polarity of microtubules. In the interphase of the cell cycle, microtubules are radially organized with their minus ends embedded in a centrosome, which is located in the center of the cell near the cell nucleus. There are approximately 400
microtubules in a cell, depending on the organism and the cell type. In the interphase microtubules undergo dynamic growth (polymerization) and shrinkage (depolymerization), so-called “dynamic instability” (Fig. 1(c)). In the M phase microtubules are subject to treadmilling, i.e., polymerize at the plus end and depolymerize at the minus end (chemical plus and minus).

![Figure 1: (a) Dimensions of microtubule, (b) Structural subunits of microtubules: protofilaments composed of tubulin heterodimers, (c) Detail of plus end of a microtubule undergoing dynamic instability.](image)

There are three possibilities how the vibrations of microtubules can be excited:

1. release of energy stored in microtubules by hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP) [17–19]
2. microtubule motor proteins (dyneins and kinesins) “crawling” along the microtubule [13, 14, 16]
3. vibrational energy released from mitochondria during the production of ATP by oxidative phosphorylation in the Kreb’s cycle (citric acid cycle)

Microtubules can be considered as a longitudinally vibrating chain of dipoles which are source of oscillating electric field. Effect of electromagnetic field of microtubules on transfer of mass particles and electrons has been analyzed in [20, 21].

Actin filaments are polar structures too, and undergo hydrolysis of ATP (built in the actin molecules) shortly after polymerization. This process is similar to hydrolysis of GTP in microtubules, however in much smaller rate than in microtubules [15]. Actin filaments may be sources of electric vibrations as well.

3. VIBRATIONS OF THE CELL MEMBRANE

Possibilities of the excitation of vibration in the microtubules have been discussed. If the vibrations are excited in the cytoskeleton, they may cause vibration of the cell membrane because of the bonding of cytoskeleton to the cell membrane. If the measured mechanical vibrations of the cell membrane are caused by the vibrations of microtubules, it is likely that electromagnetic oscillations of microtubules at the membrane are detectable. At the frequency measured by Pelling et al. [13, 14] (~ 1 kHz) the electric field would be screened in a close distance by activity of ions in the extracellular medium (mostly Na+ and Cl–). Therefore, to measure this electric field the sensor must be in the vicinity of a cell membrane [5]. Furthermore, the large number of vibrational and electric sources may create high order spherical harmonics structure (of zonal type) of the vibrational and electrical field on the membrane. The measurement of both types of oscillations requires “point” detector with the size smaller than the half of the wavelength of the surface wave, otherwise the effective amplitude would be zero, since the negative and positive peak of the surface wave would cancel out.

4. MATERIALS AND METHODS

4.1. Yeast Cells

Cold sensitive β-tubulin mutant tub2-401 of yeast cells Saccharomyces cerevisiae (strain CUY67 Mata tub2-401 ura3-52 ade2-101) was used. Evolution of the cells in the cell cycle can be synchronized by cultivation at the restrictive temperature (14°C) when the microtubules cannot be formed. The mutant cells at the restrictive temperature continue in their pathway along the cell cycle up to the point before entering the M phase, whose processes depend on the microtubules. Thus after certain time period all the mutant cells are stopped at the same point of the cell cycle. When the temperature increases to the permissive temperature (≥ 25°C) microtubules are reassembled and the mitotic spindle is formed. Therefore, start of the M phase in the cells cultivated under
the restrictive temperature is triggered by the temperature increase above 25°C. Thus the cells are synchronized. Evolution of the M phase after the warm-up above the permissive temperature is described in detail in [5].

We measured synchronized and non-synchronized cells in suspension. The cells were suspended in the aqueous sucrose solution. After warming to the permissive temperature, synchronized cells synchronously enter the M-phase, in which they are more active in generating the electric field in their vicinity than in the interphase.

4.2. Measurement System

A schematic diagram of the measurement system is shown in the Fig. 2. The crucial parts are the sensor (schematically in Fig. 3.) and the preamplifiers, which are located in the temperature stabilized and triple shielded box (electrically and electromagnetically by mumetal box). The effectiveness of the screening was verified in [5]. The batteries for the power supply of the amplifier are located inside the screened box, too. At the bottom of a small cuvette there is an evaporated Pt layer forming one electrode. Detecting wire electrode cut at an angle to obtain a point end (about 50 nm) is at a distance of 8 μm above the bottom of the cuvette. Dimension of 8 μm corresponds approximately to the diameter of a cell.

Figure 2: Schematic depiction of the used measurement system.

Figure 3: Dimensions of the used sensor.

The sensor with the preamplifier is connected to spectrum analyzer through semirigid coaxial cable. Control of the spectrum analyzer is provided by a PC program via GPIB interface. Spectrum analyzer used is R&S FSEA 30 (20 Hz–3.5 GHz). Detected cellular signal is amplified by two preamplifiers; the first one provides transition of “nano” to macroscopic signals. We performed measurements in the frequency range 0.5–2.5 kHz.

4.3. Measurement Protocol

Suspension with synchronous cells was cultivated at the temperature of 14°C. Suspension with non-synchronous cells was cultivated at the temperature of about 30°C. Before measurement the test tubes with the suspension were warmed in water bath of 28°C for 3 minutes. Optical density (OD 600) of the suspension was 4.5 [5], which corresponds to concentration of about 2 × 10^8 cells per milliliter. Afterwards, the cuvette was filled with the 60 μl of suspension. Measurement was started immediately after filling the cuvette. Cells sediment at the bottom of cuvette. Similar measurements of non-synchronous cells were performed, too.

4.4. AFM Measurements

We realized the measurements with AFM device similar to those published by Pelling et al. [13, 14]. Noise was on the level of 10^{-1} nm.

5. RESULTS

Results of preliminary measurement of electrical oscillations of yeast cells are presented. Fig. 4 shows measured spectrum in the frequency bandwidth from 1280 to 1400 Hz.

When a high amplitude spectral line was observed the frequency of the line was evaluated (1315.5 Hz) and the spectrum analyzer was switched to a 2.5 Hz bandwidth. Fig. 4(b) shows shifted 2.5 Hz bandwidth spectra with selected spectral line in time t_1 = 0, t_2 = 33 s, t_3 = 53 s, t_43 = 73 s.
Figure 4: Frequency of electrical oscillations of yeast cells in the range 1280–1400 Hz. RBW and VBW of the spectrum analyzer was 1 Hz. The sweep time was 600 s. (a) Frequency spectrum before detailed examination. (b) The successively measured 2.5 Hz bandwidth spectra shifted according to the time of measurement.

Temperature in the box with yeast cell suspension was 27.6–28°C. Preliminary result from AFM is presented in Fig. 5.

Figure 5: Frequency of local nanomechanical oscillations of synchronized yeast cell in the range 0–3 kHz as measured with AFM.

6. DISCUSSION & CONCLUSIONS

Our findings correspond to the Fröhlich’s postulate of coherent electrically polar longitudinal vibrations in biological systems.

The electrical measurement system used does not enable precise spatial localization of the tip of electrode. Since the cells exhibit a movement, we cannot assure that cell is under the sensor when the spectrum analyzer sweeps through the frequency of cell oscillations.

The electrical measurements were performed in temperature stabilized box, but the character of the measurement setup and protocol (opening of the box in order to change of the measured cell samples) introduced slight temperature changes in the environment of cells. Temperature changes cause shift in spectra of a signal, according to [14, 15], the shift is about 135.5 Hz/1°C.

Implications of existence of endogenous electromagnetic field in biological systems are far-reaching in terms of structural organization in organisms and possible mutual electromagnetic interactions of organisms.

REFERENCES