

Electric activity of yeast cells in low kHz region

Michal Cifra¹, Jiří Pokorný², František Jelínek³, Jiří Hašek⁴, Jan Šimša⁵

¹ Dept. of Electromagnetic Field, Czech Technical University, Technická 2, 166 27 Prague, Czech Republic,
email: cifra@ufe.cz, 2nd affiliation as 2

² Institute of Photonics and Electronics, Academy of Sciences of the Czech Republic, Chaberská 57, 182 51, Prague
8, Czech Republic, pokorny@ufe.cz

³ Same affiliation as 2, email: jelinek@ufe.cz

³ Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4-Krč,
Czech Republic, email: hasek@biomed.cas.cz

⁵ Same affiliation as 2, email: simsa@ufe.cz

Abstract

Nonlinear electrical polar vibrations and generation of endogenous electromagnetic field in living cells excited by metabolic energy supply were postulated by H. Fröhlich. Pelling et al. detected mechanical vibrations of yeast cell membrane by Atomic Force Microscope (frequency range below 2 kHz, amplitude about 1 nm). Electric activity of temperature dependent tubulin mutant *tub2-401* of *Saccharomyces cerevisiae* was measured in the frequency range 0.4 - 1.6 kHz. The cells synchronized in the M phase have higher electric activity than the nonsynchronized cells which is consistent with Pohl findings of increased electromagnetic activity of yeast cells in the M phase.

1. Introduction

Electromagnetic field generated by biological systems was detected in the optical [1], infrared [2] and MHz [3, 4] bands of the spectrum. Estimated generation mechanism varies in dependence on the frequency of the field. Nanomechanical vibrations of the yeast cell membrane of the order of magnitude of 1 nm were detected in the region of 0.5 – 2 kHz by Atomic Force Microscope (AFM) [5]. The authors estimate the origin of these nanomechanical vibrations in the coordinated action of many proteins working in a concerted and cooperative manner and relate them to the metabolic activity of the cell. Levin et al. [6] observed nano-scale oscillations (at low frequencies of 0.2 – 30 Hz) of human erythrocytes membrane with point dark field microscopy and linked them to MgATP-dependent dynamic assembly of the sub-membrane skeleton. Spatial and time coherent properties of the erythrocyte membrane motions were described from the results of ms time and nm spatial resolution measurements with diffraction phase microscopy [7].

Fröhlich postulated electrical polar vibrations in biological systems giving rise to endogenous biological electromagnetic field [8-11]. The coherent vibrations are excited in systems with nonlinear coupling between elastic and polarization fields excited by metabolic energy supply.

The majority of proteins are electrically polar, for instance tubulin heterodimers. The static electric field is effectively screened by mobile ions under physiological conditions in a distance of a few nm. However, recently it was found that the strong electric field around the mitochondrial membrane may extend up to few μm into cytosol [12] and the authors conclude that cytosol cannot be considered to be a simple ionic solution but more likely a heterogeneous hydrogel.

Microtubules, a part of the cytoskeleton [13], are highly polar, deformable and dynamic structures. They resemble hollow tubes with inner and outer diameter of 17 nm and of 25 nm, respectively (Fig. 1a). Microtubule consists of 13 (or 14) protofilaments. The building subunits are tubulin heterodimers composed of α-tubulin and β-tubulin (Fig. 1b). Heterodimers have high electrical dipole moment of over 1000 Debye (10^{-26} C.m) and are responsible for the high electrical polarity of microtubules. In the interphase, 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 can exist as dynamically stable with a turnover rate of about 18 hrs or dynamically unstable with a turnover rate of 5-10 mins. The unstable microtubules undergo dynamic growth (polymerization) and shrinkage

(depolymerization), so-called “dynamic instability” (Fig. 1c.). 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).

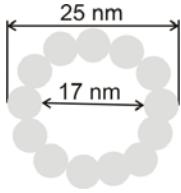


Fig 1a. Dimensions of microtubule.

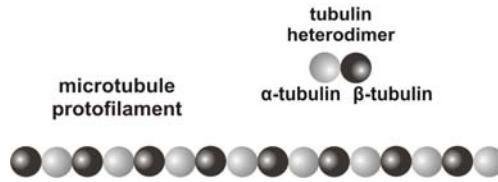


Fig 1b. Structural subunits of microtubules: protofilaments composed of tubulin heterodimers.



Fig. 1c. Detail of the plus end of a microtubule undergoing dynamic instability.

Excitation of mechanical vibrations in microtubules (which are electrically polar) can give rise to electrical oscillations. Mechanical vibrations may be excited by:

- release of energy stored in a microtubule by hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP) [14]
- microtubule motor proteins (dyneins and kinesins) “crawling” along the microtubule [5, 15]
- wasted energy in the form of heat released from mitochondria during the production of ATP by oxidative phosphorylation in the citric acid cycle

Energy supply is a necessary condition for existence of the vibration states in the cytoskeleton. Extraordinary properties of microtubules (high shear deformability), layers of structured interstitial water in cytoplasm and high static electric fields created by surrounding mitochondria may participate in forming nonlinear conditions which comply with the Fröhlich’s requirements for coherent vibrations.

In this paper, we present a direct experimental proof of existence of yeast cell electrical activity in kHz region.

2. Materials and methods

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, which 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 is increased to the permissive temperature ($\geq 25^{\circ}\text{C}$) 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 [4].

We measured synchronized and non-synchronized cells in suspension. The cells were suspended in the aqueous sucrose solution. After warming to the permissive temperature, the cells synchronously enter the M-phase.

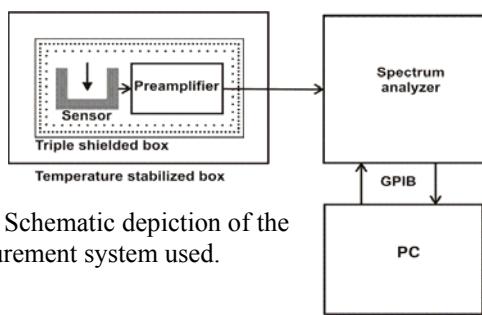


Fig 2. Schematic depiction of the measurement system used.

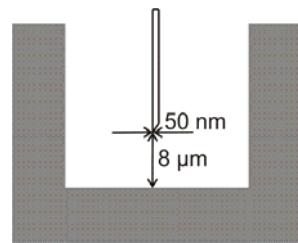


Fig 3. Dimensions of the sensor used.

Measurement system: A schematic diagram of the measurement system is shown in the Fig. 2. The crucial parts are the sensor and the preamplifiers (Fig. 3), which are located in the temperature stabilized and triple shielded box (electrically and magnetically by mumetal box). The effectiveness of the screening was verified in [4]. 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 yeast cell. After sedimentation cells form a layer at the bottom of the cuvette. Signal from the sensor is amplified inside the screened box. The preamplifier is connected to the spectrum analyzer through semirigid coaxial cable. Control of the spectrum analyzer is provided by a PC program via GPIB interface. Spectral analyzer Agilent E4448A has been used.

Measurement protocol: Suspension with synchronized cells was cultivated at the temperature of 14°C. Suspension with non-synchronized cells was cultivated at the temperature of about 30°C. Before measurement the test tubes with the suspension were warmed in a water bath of 28°C for 3 minutes. Optical density (OD 600) of the suspension was 4.5 [4], which corresponds to concentration of about 2×10^8 cells per milliliter. Afterwards, the cuvette was filled with the 60 μl of suspension. Measurement started immediately after filling the cuvette. Cells sedimented at the bottom of cuvette. Similar measurements of non-synchronized cells were performed, too.

Data processing: Measured data have been processed with specified scripts of Python programming language. Modules used: matplotlib, pylab, Numeric, SciPy

3. Results

Measurements of the electrical activity of 25 synchronized cell suspensions and 25 non-synchronized cell suspensions have been carried out. Each measurement contained at least 400 double sweeps in the region 0.4 – 1.6 kHz (measured in two subbands 0.6 kHz each) with resolution bandwidth 1 Hz. Each double sweep and transfer of data to PC took 6s. Measured power is of the order of magnitude of 10^{-18} W.

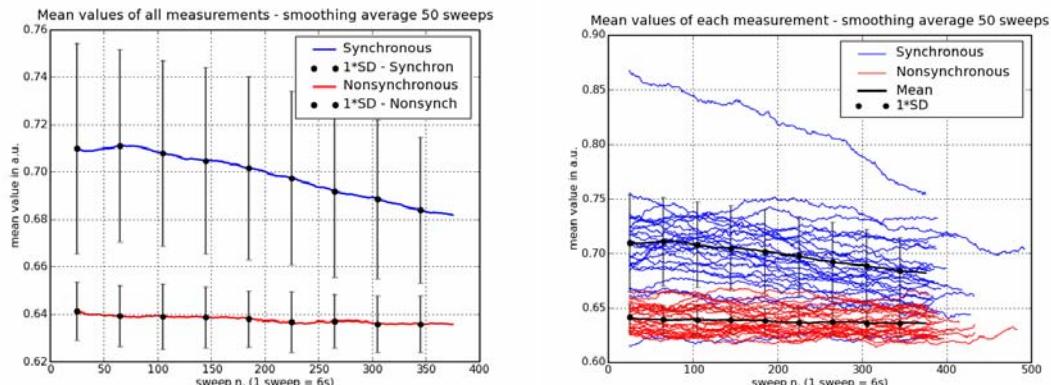


Fig. 4 – Left: Mean power of cellular electrical activity in a frequency band 0.4–1.6kHz, error bars – symmetric SD
Right: 25 measurements of synchronized and nonsynchronized cells each.

Fig. 4 on the left hand side shows average power of synchronized and nonsynchronized cells. Two extreme cases in the measurements of synchronized cells, one of unexpectedly low power and one of unusually high power increased standard deviation (SD) by cca. 0.02 a.u. Each measurement was smoothed by moving average of 50 double sweeps wide windows to suppress the noise before taken for calculation of mean and SD.

Figure 4 on the right hand side depicts 25 measurements of synchronized and nonsynchronized cells each. In some cases, the measured powers of synchronized cells are of similar level or lower than that of nonsynchronized cells. The cells are cultured and treated under the same experimental conditions and the measurements are carried out always under the same protocol. Biological variability probably plays a role here. When we evaluate the cells that come from the same inoculation, electrical activity of the synchronized cells is higher than that of nonsynchronized cells (data not shown).

4. Discussion & conclusion

Measurements described in this contribution show that the electrical activity of synchronized cells in the M-phase is greater than that of the nonsynchronized cells. It corresponds to the experimental findings of Pohl et al. [3] based on dielectrophoretic attraction of dielectric particles to yeast cells indicating that their electromagnetic activity is greatest in the M phase. However, in our measurements, lower electrical activity of nonsynchronized yeast cells may be also related to the energy depletion, since synchronized cells saved their energy when they are kept under restrictive temperature before measurement while nonsynchronized cells were normally active at the room temperature.

Electrical processes seem to be important for cell activity. Cell cytoskeleton is a probable source of electrical polar vibrations. Endogenous biological electromagnetic field may play important role on temporal and spatial organization of structures and processes in living systems at least on cellular level. Effects on transfer of reaction components, of charge and mass particles were theoretically analyzed [16-18]. Disturbed electromagnetic field can be related to pathological processes. One of the deepest disturbances in organization and regulation in multicellular systems is cancer. In cancer cell, regardless of their viral or other mutagenic origin, dysfunction of mitochondria and disintegration of the cytoskeleton are observed [19, 20]. Vibration states and generated electromagnetic field may be disturbed. The static electric field around mitochondria (and around microtubules too) is diminished, and the wasted energy efflux is cut off (the wasted energy may be used in microtubules in nonlinear processes). Restoration of mitochondrial function in cancer cells by treatment with dichloreacetate results in restoration of normal cell function or in apoptosis of aberrant cells [19].

5. Acknowledgments

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6. References

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