

Electromagnetic Probes of Biological Molecular Motors

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Abstract—Researchers are conducting measurements of harmonics generated by biological systems in response to applied sinusoidal electric fields. The frequency and amplitude-dependence of the induced harmonics exhibit features that correlate with physiological processes. For example, budding yeast cells (*S. cerevisiae*) produce numerous harmonics, the amplitudes of which depend strongly on frequency. Thus far, we have observed harmonics that appear to be generated by active molecular motors, such as membrane pumps, in the plasma membrane and in internal organelles, including mitochondria and chloroplasts (for plant cells).

AMONG THE FORCES OF nature, electromagnetic interactions play a dominant role in biological processes. An applied low-frequency electric field, for example, polarizes live cells in suspension, resulting in an enormous dielectric response¹ and also modulates each cell's membrane potential by an amount given by, for a spherical cell,² $U_m(\omega, \theta) = 1.5E_c R \cos\theta [1 + i\omega\tau_m]^{-1}$, where E_c is the external field amplitude, R is the cell radius, θ is the polar angle from the field direction, and τ_m is a relaxation time. Thus, a field of only 5 V/cm modulates the normal membrane potential of a 10- μm radius cell by up to 7.5MV/m across a 10-nm thick plasma membrane of a resting cell.



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Membrane proteins³ are candidates for nonlinear behavior⁴ since they cannot rotate within the membrane and dissipate energy through Debye-like relaxation and, also, since any transmembrane domains with dipole moments interact with the greatly amplified ac field. Modulated transmembrane potential drives membrane proteins to change their conformational states and can even induce pumps to transport ions.^{5,6} This combination of protein conformational changes and ion translocation creates a nonlinear response manifested by the generation of harmonics.^{7,8} An externally modulated membrane potential may, therefore, affect enzyme activity, transport, and conformational behavior. Cyclic processes, such as ion transport and ATP consumption, are of special interest because they are likely to be sensitive to synchronization by an oscillatory field.

Cation pumps known as P-type ATPases,^{9,10} H⁺-ATPase in particular, have been reported to generate harmonics based on stud-

ies¹¹ with mutant strains of *S. cerevisiae*. H⁺-ATPase shows nonlinear behavior due to its structural and mechanistic similarity to Na, K-ATPase, and is thus expected to transduce exogenous electric fields in a similar manner to that demonstrated by Tsong and colleagues.¹² The best understood P-type ATPase is Ca-ATPase, which exists in two main conformational states, E₁ and E₂, while H⁺-ATPase and other P-type pumps are believed to be similar. The structure¹³ is like that of a hand in a mitten that opens and closes. An oscillatory potential across the membrane can induce time-dependent conformational changes that alter the energy landscape seen by the cations.¹⁴ Once the ions become trapped in the well, a conformational change facilitated by ATP hydrolysis or an external field takes place. The modulated potential U_m affects the potential barrier seen by the ion, thus increasing (or decreasing) the probability for the ion to hop into the adjacent well. Additional molecular motors of interest include ATP synthase and other complexes in the mitochondrial inner membrane, as discussed in a companion report. (See p. 33.)

Goal of the Project

The goal of this project is to utilize nonlinear harmonic response to study active bio-molecular motors. Biological systems contain numerous mobile charged particles, including cations, anions, electrons, and electrically charged proteins. Thus, when a sinusoidal electromagnetic field is applied, these charges react to yield a linear response that can be measured using impedance (or dielectric) spectroscopy and a nonlinear response, which can be probed by measuring induced harmonics, as done in the study reported here, or using related techniques (e.g., mixing or inter-modulation).

Results

A companion report discusses measurements of nonlinear harmonic response produced by complexes in extracted mitochondria and chloroplasts. Here we discuss harmonics of oscillatory

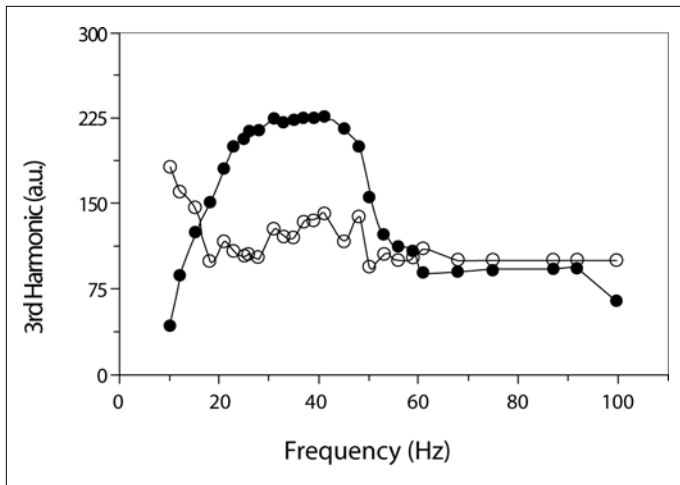


Figure 1. Induced third harmonic response of budding yeast (*S. cerevisiae*, 10^8 cells/ml) as a function of applied fundamental frequency before (closed circles) and after (open circles) adding 0.8 mM of the P-type ATPase inhibitor sodium metavanadate. The field amplitude was fixed at 3 V/cm.

electric fields at low frequencies (< 100 Hz), which appear to be generated by active membrane pumps in the plasma membrane of whole budding yeast cells.

Frequencies well below 1 kHz are required to obtain sufficient transmembrane field amplification should the nonlinear response be dominated by complexes in the plasma membrane. A problem that plagues measurements at low frequencies is the generation of harmonics by the electrical double layer at each electrode.^{15,16} In order to reduce such spurious harmonics, we use only two electrodes to apply the electric field and employ a high- T_c superconducting quantum interference device (SQUID) magnetometer to probe the time-dependent magnetic field generated by the resulting currents, while the output of SQUID electronics is fed into a spectrum analyzer. The SQUID magnetometer eliminates the need for additional measurement electrodes and any resulting interface effects. Harmonics induced by the cell suspension are measured by applying a sinusoidal signal in the frequency range 10-100 Hz and field amplitude range 0-5 V/cm; the response of a conductivity-matched reference medium is subtracted to further reduce any spurious harmonics. Further details, including the measurements and preparation of budding yeast suspensions (*S. cerevisiae*, 10^8 cells/ml) are described elsewhere.¹⁷

For sufficient field amplitudes, a series of harmonics are observed, the largest of which, in the absence of glucose, are the odd harmonics. Figure 1 shows the measured third harmonic response vs. applied frequency for fixed amplitude of 3 V/cm, before and after adding 0.8 mM of sodium metavanadate, an inhibitor of P-type ATPases. Note that the inhibitor suppresses the third harmonic response for the same amplitude, consistent with previous observations. Figure 2 depicts the amplitude-dependent third harmonic response for a fixed applied frequency of 23 Hz, before (closed circles) and after (open circles) adding sodium metavanadate. The data before adding the inhibitor exhibit a prominent peak centered around 2.75 V/cm

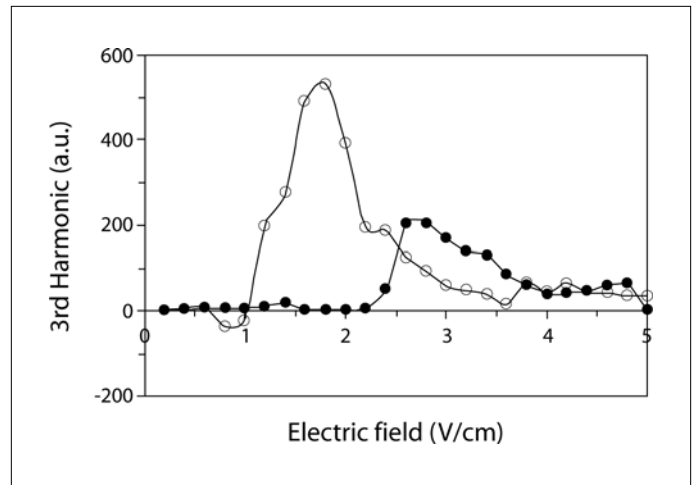


Figure 2. Third harmonic response induced by budding yeast cells (*S. cerevisiae*, 10^8 cells/ml) as a function of applied field amplitude before (closed circles) and after (open circles) adding sodium metavanadate. Here the frequency was fixed at 23 Hz.

amplitude and a major threshold field of around 2.2 V/cm, below which little response is observed except for a minor peak at around 1.4 V/cm. In addition, the sodium metavanadate appears to reduce this threshold field and greatly increases the third harmonic response for field amplitudes of around 1.8 V/cm, while the third harmonic response is suppressed somewhat for amplitudes in the range 2.5-3.5 V/cm.

The observed increase in harmonic response at lower field amplitudes after adding sodium metavanadate is somewhat surprising because previous studies with mutant strains of *S. cerevisiae* strongly indicate that H^+ -ATPase is a dominant contributor to the nonlinear harmonic response of these organisms, and sodium metavanadate is an inhibitor that interacts selectively with the E_2 conformation of the enzyme. Vanadate is known to interact with the enzyme conformation in the absence of the cation (e.g., H^+ or Ca^{2+}) by acting as a phosphate group (P_i) analog. It has been suggested¹⁸ that uncoupled (e.g., E_2-E_2) cycles can occur in addition to the coupled E_1-E_2 cycle. Thus, although ATP hydrolyzing reactions will be inhibited by vanadate, since it stabilizes the E_2 state, it may also enable an oscillatory field driven E_2-E_2 cycle to take place. If the major threshold field for the E_1-E_2 cycle was due to the energy barrier for driving cations from the potential well, then perhaps the threshold field for driving cations back-and-forth over the E_2 barrier in a field-driven E_2-E_2 cycle might be reduced. Moreover, oscillatory fields can drive conformational state changes and cation transport even in the absence of ATP. Since these measurements were done without glucose, it is likely that the E_1-E_2 changes in a conformational state prior to adding vanadate were, indeed, field driven rather than driven by ATP. The potential energy barrier to drive the E_1-E_2 conformational change and cation transport without ATP hydrolysis is likely larger than simply driving cation transport over the barriers in the E_2 conformation, which would lead to a larger threshold field in the absence of either vanadate or glucose.

The addition of glucose to yeast cells has been shown to cause a two- to three-fold increase in plasma membrane ATPase activity.¹⁹ In order to study the effects of glucose in real time, we automated the data acquisition so that specific harmonics could be plotted as a function of time. A companion report shows measured second and third harmonics as functions of time after adding D-glucose for a fixed frequency of 45 Hz and amplitude of 3 V/cm, where $t = 0$ represents the time when glucose was added. We find that, after several minutes, the second harmonic increases dramatically, by more than 400 percent after 10 minutes, but then reduces in amplitude after about 25-30 minutes as the glucose concentration becomes depleted. By contrast, the third harmonic actually reduces in amplitude over the same time period when the second harmonic is larger and then recovers to about its original size after 30 minutes.

Several features of our harmonic generation experiments warrant explanation, including the strong frequency dependence, with threshold-like behavior in the amplitude dependence, and the observed increase of even harmonics when ATP production is taking place. In order to capture the essential behavior, we proposed a model²⁰ that explicitly introduces a threshold field, similar to those observed in density waves, where fields above threshold drive charge transport through an energy landscape with multiple wells, and in Coulomb blockade tunnel junctions, recently exploited to define the current standard. In our model, a P-type enzyme complex is represented as a junction, through which cation (eg. H^+ or Ca^{2+}) transfer can be driven by a sufficiently large voltage. First, the displacement current is taken to be zero when the modulated voltage across the membrane is in the range $-V_2 \leq V \leq V_1$, where V_1 and V_2 are threshold voltages and $V_2 > 0$. When V falls outside this range, the displacement current is assumed to persist for a time $\tau_{1,2}$ reflecting the time for the system to change conformational states and for cation transfer to take place. The displacement current is taken to be

$$I = \frac{dQ}{dt} \sim \pm f(v_{\pm}) g(t_{\pm}), \quad (1)$$

where $v_+ = (V - V_1)/V$ when $V > V_1$, $v_- = (|V| - V_2)/V_2$ when $V < -V_2$, and $t_{\pm} = [t - t(V_{1,2})]/\tau_{1,2}$ is the normalized time period after the voltage crosses the threshold value in either the positive or the negative direction. We take $f(v)$ to peak for a finite v , in this case using the simple form

$$f \sim v \exp[-v] \Theta(v), \quad (2)$$

where $\Theta(v)$ is the unit step function. In order to reflect the time required for conformational changes and cation transfer to take place and to avoid discontinuities, the function $g(t_{\pm})$ is taken to be

$$g \sim \{1 - \cos[2\pi t_{\pm}]\} \Theta(t_{\pm}) \Theta(1 - t_{\pm}). \quad (3)$$

Finally, taking the voltage $V(t) = V_0 \sin \omega t$ to be sinusoidal, the displacement current $I(t)$ is determined using the above expressions, and the various Fourier components of the current response are computed.

We find that the dc current and all even harmonics are zero in

this model, as expected, when the junction is perfectly symmetric, i.e., when $V_1 = V_2$ and $\tau_1 = \tau_2$. However, the theoretical third harmonic response is found to be nonvanishing, exhibiting a threshold voltage in its amplitude dependence. We obtain theoretical plots (see Ref. 20) qualitatively consistent with the experimental data shown in Figs. 2 and 3. We also find that the predicted second harmonic response increases significantly as the asymmetry in time scales increases, suggesting that such an asymmetry is sufficient for an ac field to drive ion transport through a membrane pump. Similarly, asymmetry in threshold voltages, V_1 and V_2 , enables an ac field to induce both a second harmonic response and a dc current in our model. These results suggest that the production of ATP from glucose makes the pump more asymmetric by enabling ATP induced ion transport along a preferred direction.

Conclusion

The ability to noninvasively monitor active physiological processes *in vivo* is of potential importance for biophysics, biomedicine, and pharmaceutical development. For example, proton pump inhibitors are widely employed to treat acid reflux disease and related stomach disorders. In addition, we have recently observed, in the nonlinear harmonic responses at kilohertz frequencies of whole cells, mitochondria, and chloroplasts, evidence that the technique can monitor internal metabolic events that would be extremely difficult to study using invasive (e.g., patch clamp) methods. These results are discussed in a companion report. (See p. 116.) Clearly, this topic constitutes a potentially exciting field of study in biophysics.

References

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SPECTROSCOPY—Dr. John Miller is holding a liquid sample cell for dielectric spectroscopy measurements.

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Presentations

See companion reports “Martian Soil Biosensors Based on Dielectric Spectroscopy” and “Low-Frequency Dielectric Spectroscopy of Martian Soil Samples.”

Funding and Proposals

Proposals are under development for submission in 2006.