Harmonic generation by yeast cells in response to low-frequency electric fields

D. Nawarathna,[1] J. R. Claycomb,[1,3] G. Cardenas,[1] J. Gardner,[2] D. Warmflash,[1] J. H. Miller, Jr.,[1] and W. R. Widger[2]
[1]*Department of Physics and Texas Center for Superconductivity, University of Houston, Houston, Texas 77204-5005, USA*
[2]*Department of Biology and Biochemistry, University of Houston, Houston, Texas 77204-5001, USA*
[3]*Department of Mathematics and Physics, Houston Baptist University, Houston, Texas 77074-3298, USA*
(Received 30 January 2006; revised manuscript received 27 March 2006; published 23 May 2006)

We report on harmonic generation by budding yeast cells (*Saccharomyces cerevisiae*, 10^8 cells/ml) in response to sinusoidal electric fields with amplitudes ranging from zero to 5 V/cm in the frequency range 10–300 Hz. The cell-generated harmonics are found to exhibit strong amplitude and frequency dependence. Sodium metavanadate, an inhibitor of the proton pump known as H^+-ATPase, and glucose, a substrate of H^+-ATPase, are found to increase harmonic production at low amplitudes while reducing it at large amplitudes. This P-type proton pump can be driven by an oscillatory transmembrane potential, and its nonlinear response is believed to be largely responsible for harmonic production at low frequencies in yeast cells. We find that the observed harmonics show dramatic changes with time and in their field and frequency dependence after perturbing the system by adding an inhibitor, substrate, or membrane depolarizer to the cell suspension.

DOI: 10.1103/PhysRevE.73.051914 PACS number(s): 87.50.Rr, 87.16.Uv, 82.39.Jn

I. BACKGROUND AND MOTIVATION

The interiors of intact biological cells are negatively charged, and the motions of charged ions and macromolecules in the viscous medium cause them to respond to an applied oscillatory field in a strongly frequency-dependent fashion. Cation and anion gradients across the cellular plasma membrane are controlled by membranous ion pumps, which establish a membrane potential difference that attracts ions dissolved in the extracellular medium. An externally applied oscillatory electric field, with a frequency of several kilohertz or lower, causes these counter-ions to migrate along the cell wall to create a net dipole moment, the magnitude of which decreases with frequency, in the direction of the applied field [1–3]. In addition, at low frequencies, the induced oscillatory field across the plasma membrane is greatly amplified as compared to that in the bulk medium due to the membrane's insulating properties [4]. Membrane-bound proteins and enzymes can effectively harness the electric field energy to perform biologically useful work, such as the pumping of cations [4,5]. Such phenomena can be probed by monitoring harmonics generated in response to a sinusoidal excitation field. The induced harmonics can be attributed to changes in conformational states, which have distinct electric dipole and higher multipole moments. Unlike patch- and voltage-clamp measurements, harmonic response measurements do not require pipettes or electrodes to be inserted through the cell membrane, thus providing a potentially powerful noninvasive technique for monitoring active physiological processes.

Theoretical predictions by Tsong and Astumian [6] show that membrane proteins generate harmonics in response to ac fields, in part because they cannot rotate freely and dissipate electrical energy by simple Debye-type rotation. It has been shown [7] that the interaction of protein dipole moments with the amplified transmembrane field may generate harmonics. Westerhoff *et al.* [8] have simulated harmonic generation using a model based on conformational changes in membrane proteins containing dipolar molecules spatially distributed in a lipid bilayer with low relative dielectric permittivity. Woodward and Kell [9,10] have experimentally demonstrated harmonic production, resulting from plasma membrane pumps, in yeast cells. Induced membrane potentials may change enzyme activity, transport, and conformational behavior. Among membrane proteins, ion transporters exhibit cyclic and time-dependent processes (e.g., dipole orientation and charge displacement) likely to be sensitive to synchronization by an externally applied ac electric field.

At frequencies above ~1 kHz, the external field capacitively couples through the plasma membrane and polarizes structures within the cellular cytoplasm. Thus, in the kilohertz frequency range, electrically excitable internal organelles (mitochondria, etc.) may generate harmonics due to processes within complexes of the mitochondrial electron transport chain and other enzyme complexes [11]. In this study, we focus on the membranous proteins and enzymes located in the plasma membrane of yeast cells by working in the frequency range 10–300 Hz. No harmonics are expected from internal organelles and cellular processes at these low frequencies, because most of the induced voltage drop appears across the insulating plasma membrane while the field in the interior is small.

In budding yeast (*S. cerevisiae*) cells, the observation [12] that harmonic generation by a mutant strain containing vandate-resistant H^+-ATPase is also highly resistant to sodium metavanadate, as compared to normal cells for which vanadate is an H^+-ATPase inhibitor, suggests that these membranous proton pumps are major sources of nonlinear harmonic production at low frequencies. H^+-ATPase (its main isoform being PMA1 ATPase) is the major plasma membrane proton pump essential to growth of fungal cells, such as yeast [13,14]. Addition of glucose to yeast cells has been found to cause a twofold to threefold increase in PMA1 ATPase activity [15]. However, the net efflux of H^+ per ATP split by H^+-ATPase is a flexible rather than fixed parameter. As a result, glucose has been found to stimulate increased proton extrusion from yeast cells by, in some cases, more than 50-fold [16]. This pump exhibits nonlinear behavior in

 ©2006 The American Physical Society

response to an applied field and, due to its structural and mechanistic similarity to Na, K-ATPase and related P-type ATPases [17,18], is expected to transduce exogenous electric fields in a manner similar to that discussed by Tsong and Astumian [19].

P-type ATPases are especially important because they carry out fundamental ion transport processes in cells, which ultimately control the membrane potential, generation of action potentials for muscle contraction, and removal of toxic ions. The structures of several members of this enzyme family have been found using x-ray crystallography [20], which shows that P-type ATPases consist of four principle domains, labeled P, N, A, and M. The P domain is the catalytic core of the enzyme. N is a large insert in the P domain, linked by a strongly conserved hinge consisting of two antiparallel peptide strands. The A (actuator) domain is the smallest cytoplasmic section of the enzyme. The M (membrane) domain consists of ten membrane-spanning helices that surround the ion binding sites, as well as short connecting loops on the outer membrane surface. During biological processes, P-type ATPases undergo transformations between two major conformational states, called El and E2, each having different affinities for nucleotides and transported ions. In a previous paper [21], we discussed a possible mechanism of ac field-induced transport and harmonic production by P-type ATPases.

II. EXPERIMENTAL METHODS, RESULTS, AND INTERPRETATION

A low-frequency (10–300 Hz) electric field is needed to obtain sufficient transmembrane field amplification for generation of induced harmonics resulting from complexes in the plasma membrane. At such low frequencies, an electrical double layer forms in an interfacial region near the boundary between the conductive biological cell suspension and the metal electrodes. In our setup, the double layer arises from an excess of charge, which may be electrons, ions, or oriented dipoles, in this interfacial region. The resulting nonlinear current-voltage relation at the electrode-medium interface may generate spurious harmonics.

To minimize this effect, we use two gold-plated tungsten electrodes to apply the sinusoidal excitation, since gold has been found to reduce the electrical double layer as compared to other electrode materials. In addition, a contactless procedure, employing a high-T_c superconducting quantum interference device (SQUID), is used to measure the response of the suspension to the sinusoidal excitation, which further reduces spurious harmonics as compared to the use of additional contacting measurement electrodes. The SQUID magnetometer, acting as a flux-to-voltage transducer when operated in a flux-locked loop, directly senses the time-dependent magnetic field generated by the oscillatory currents, whose spectral content is perturbed by the cells in the suspension. The voltage output of the SQUID (flux-locked loop) electronics is fed into a spectrum analyzer to measure the harmonics produced by the cell suspension. Although the SQUID magnetometer eliminates the need for measurement electrodes, it does not completely eliminate harmonics produced at the source electrodes. This is achieved by recording the spectrum from a conductivity-matched reference medium and subtracting the measured harmonics from that of the biological cell suspension, thereby eliminating the nonbiological harmonic contribution from the electrode interface. Further details of the experimental setup, measurement procedure, and preparation of budding yeast suspensions are described elsewhere [22].

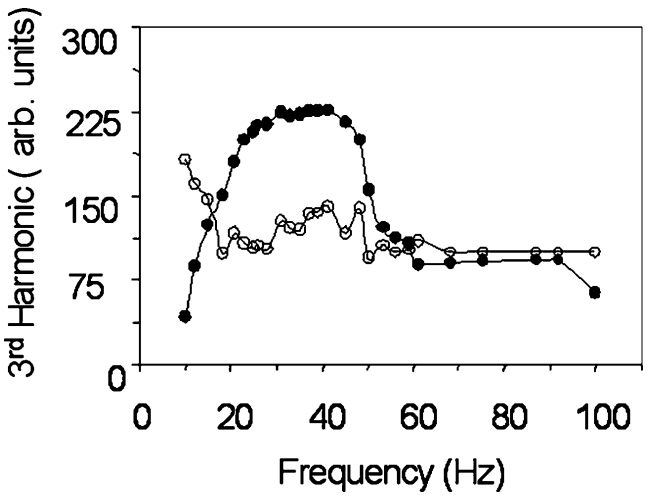

FIG. 1. Induced third-harmonic response of budding yeast as a function of applied frequency before (solid circles) and after (open circles) adding 0.8 mM of the H^+-ATPase inhibitor sodium metavanadate. The field amplitude was fixed at 3 V/cm.

In the experiments discussed here, we initially measured a resting cell suspension of budding yeast cells (*Saccharomyces cerevisiae*, 10^8 cells/ml) without any inhibitors or substrates. A series of odd harmonics, the largest being the third harmonic, was observed for sufficiently high field amplitudes. Figure 1 shows the measured third-harmonic response versus applied frequency for a fixed applied field amplitude of 3 V/cm. The same measurement was then repeated after adding 0.8 mM of sodium metavanadate, an inhibitor of H^+-ATPases. Note that the inhibitor suppresses the third-harmonic response for the same amplitude, consistent with previous observations [9,10].

We then set the applied fundamental frequency to 23 Hz and measured the harmonic response as a function of field amplitude to further explore the cells' behavior and possible H^+-ATPase activity, in response to external perturbations. This frequency, of 23 Hz, was selected based on theoretical work carried out by Westerhoff *et al.* [8] and previous experimental studies carried out by Woodward and Kell [9,10]. In addition, we numerically solved the equations discussed in Ref. [8] to calculate the induced third harmonic, obtaining a peak around 20–25 Hz (data not shown). Figure 2 shows the amplitude-dependent third-harmonic response for a fixed applied frequency of 23 Hz before (solid circles) and after (open circles) adding sodium metavanadate. The data before adding the inhibitor exhibit a prominent peak centered around 2.75 V/cm 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. Addition of sodium metavanadate appears to suppress the response at

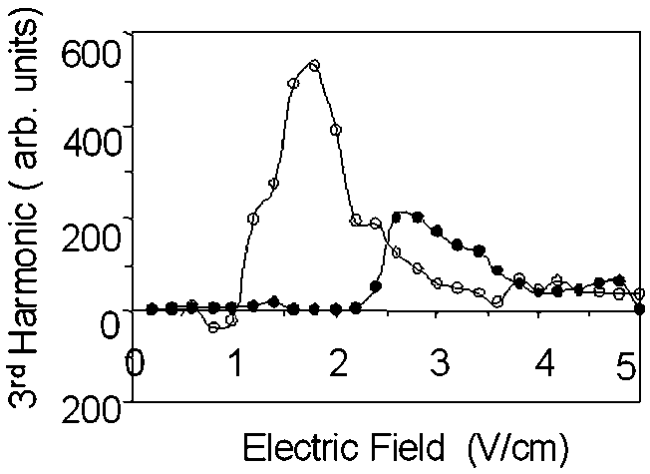

FIG. 2. Third-harmonic response induced of budding yeast cells as a function of applied field amplitude before (solid circles) and after (open circles) adding sodium metavanadate. The frequency was fixed at 23 Hz.

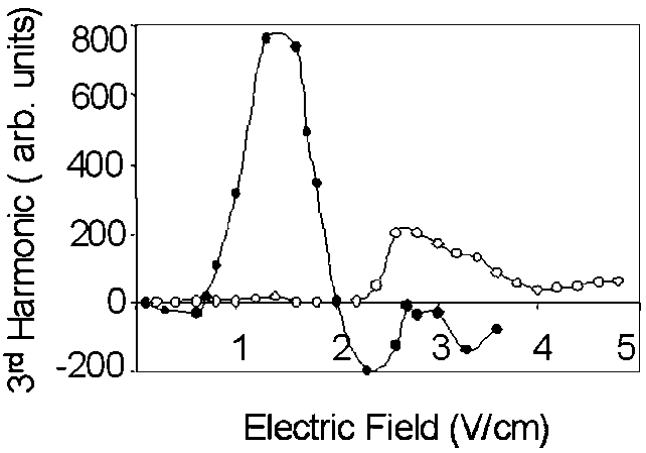

FIG. 3. Amplitude variation of the third harmonic with (solid circles) and without (open circles) glucose, a substrate that activates H^+-ATPase. The frequency of the applied signal was set to 23 Hz during the experiments. The plot with solid circles shows the response after adding 100 mM glucose.

2.75 V/cm and greatly increases the third-harmonic response for field amplitudes of around 1.4 V/cm. One might, at first glance, expect sodium metavanadate to suppress the harmonics for all applied field amplitudes, so the observed increase in harmonic response at low field amplitudes, after adding metavanadate, may appear surprising. However, it is likely that metavanadate shifts the kinetics of each change in conformational states such that the most electrogenic ones now occur at different amplitudes and frequencies than without metavanadate. Moreover, as Woodward and Kell point out [11], no other vanadate-sensitive, membrane-bound enzymes, besides H^+-ATPase, are known in *S. cerevisiae*.

The effects of glucose are of interest, since it has been shown experimentally that addition of glucose can increase ATP hydrolysis twofold to threefold [14] and total H^+-ATPase activity (proton pumping) more than 50-fold [15]. Therefore, we measured the harmonic response of the yeast cells after the addition of glucose. Figure 3 depicts the amplitude-dependent third-harmonic response for a fixed applied frequency of 23 Hz before (closed cycles) and after (open cycles) adding 100 mM glucose. As seen in Fig. 3, enzyme activity due to glucose generates a peak around 1.4 V/cm, while the third-harmonic response is suppressed for amplitudes of about 2.75 V/cm or greater. Furthermore, an additional negative peak appears around 2.25 V/cm, where we should note that the harmonics generated by a conductivity-matched reference medium have been subtracted. Figures 2 and 3 are similar because metavanadate is a competitive inhibitor that binds to the substrate (glucose) binding site. The third harmonic peak at 1.4 V/cm may represent the nonlinear response of the substrate-binding site of the enzyme. The response at 2.75 V/cm is inhibited by metavanadate, so it may probe the ion-binding site of the enzyme.

We have also measured the second harmonic versus field amplitude after the addition of both vanadate and glucose. Figure 4 shows that the addition of glucose causes a pronounced second harmonic response between 2.5 and 3.5 V/cm. The second harmonic appears only after the addition of glucose. We have previously proposed a P-type ATPase junction model [21] to explain the generation of odd and even harmonics above a threshold field. Similar thresholds are observed in density waves, where fields above a certain threshold drive charge transport through an energy landscape with multiple wells [23,24] and in Coulomb blockade tunnel junctions [25]. When the junction is perfectly symmetric, the predicted third-harmonic response is nonvanishing and exhibits a threshold voltage in its amplitude dependence. When the junction becomes asymmetric, both the dc current and the second harmonic begin to appear. The model suggests that the production of ATP from glucose makes the pump more asymmetric by enabling ATP induced ion transport along a preferred direction.

In order to investigate how the membrane pump enzyme reacts with inhibitors and substrates and how those reactions affect the induced harmonics (especially the third) with time, we have measured the temporal harmonic behavior after the addition of inhibitors and substrates. Figure 5 shows the effects of adding 0.8 mM sodium metavanadate (vanadate) to

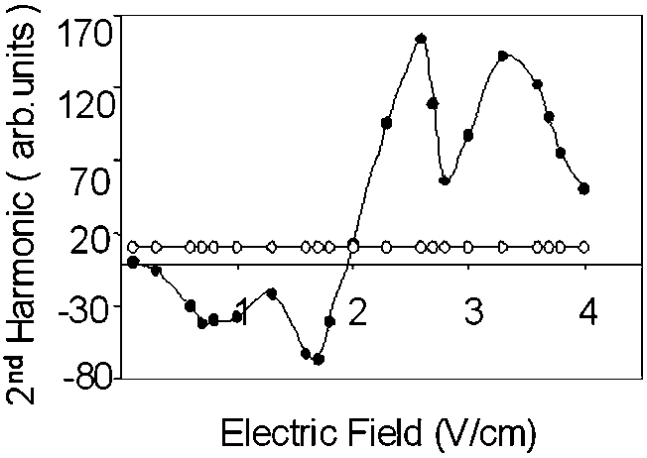

FIG. 4. Second harmonic after glucose is added. The plot with solid (open) circles shows second harmonic vs field amplitude with (without) glucose. The frequency of the applied signal was 23 Hz.

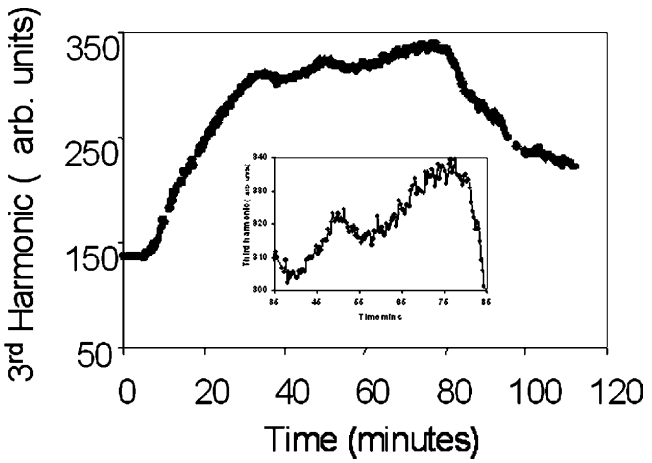

FIG. 5. Temporal behavior of the third harmonic after addition of 0.2 mM metavanadate to the resting yeast cell suspension at 3 min. The frequency of the excitation signal was set to 23 Hz with amplitude of 1.4 V/cm. Inset shows the zoom-in view of the 35–85-min section. An oscillatory like behavior was observed during a 10-min interval.

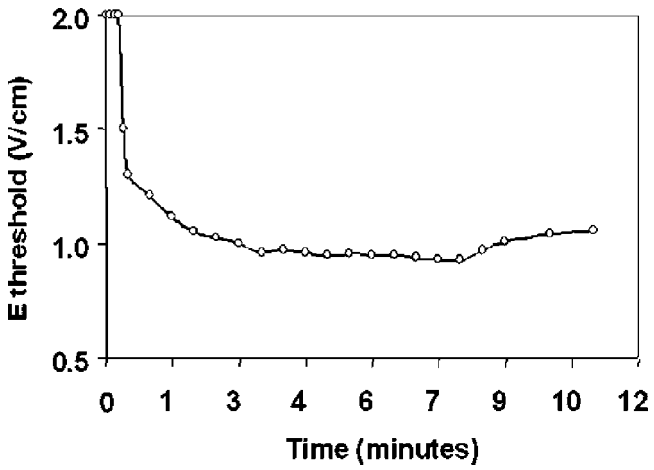

FIG. 6. Estimated time-dependent threshold field amplitude for selected times, based on the data in Fig. 5 and using the model in Ref. [21] assuming a relaxation time of 20 ms.

cell suspension after an initial time interval of 3 min. Note that a third harmonic appears when vanadate is added to the resting yeast cells and the applied field amplitude and frequency are fixed at 1.4 V/cm and 23 Hz, respectively. Distinct behaviors are observed during three major time intervals, which can be labeled as interval 1 (5–30 min), interval 2 (30–80 min), and interval 3 (80–115 min). Interval 1 corresponds to an inhibition time, where vanadate effectively binds to the binding site of the protein. During interval 1, the harmonic increases with time. Interval 2 corresponds to a recovery time, where the inhibition of vanadate weakens with time and the harmonic continues to grow with time, but at a slower rate. In this interval, equilibrium between inhibition and noninhibition may occur. Also note that the third harmonic exhibits oscillatory behavior, which may shed light on how this enzyme reacts with vanadate. The inset shows a zoom-in view of the oscillatory behavior in interval 2, with a period of oscillation of about 10 min. Interval 3 corresponds to a second recovery time, where the enzyme recovers completely from the inhibition from metavanadate, during which time the third harmonic decreases.

Much of the increase in harmonic response observed in Fig. 5 likely results from a reduction in threshold field amplitude, as discussed in our previous work [21] and suggested by the plots in Fig. 2. In order to estimate the time dependence of the threshold based on the data in Fig. 5, we ran the model discussed in Ref. [21], where we assumed that the initial threshold field amplitude was 2 V/cm (based on the plot in Fig. 2 and assuming a symmetric junction—i.e., $V_1=V_2=V_T \rightarrow E_1=E_2=E_T$) and the frequency was fixed to 23 Hz. The relaxation time ($\tau_1=\tau_2=\tau$) was taken to be 20 ms, comparable to the inverse angular frequency following Ref. [21]. The initial value of the measured third harmonic was taken to represent a background value to be subtracted off, based on our measurements with a matching reference medium and also suggested by Fig. 2, in which the reference-generated harmonic is already subtracted off. Figure 6 shows the estimated threshold field versus time, at selected time periods, based on the above assumptions, and using the model in Ref. [21]. Note that slight changes in threshold voltage can sometimes have a dramatic effect on the induced harmonic response. Figure 6 thus shows an initial rapid drop in threshold field amplitude, followed by much smaller changes with time.

Figure 7 shows how the third harmonic (for an applied field amplitude of 1.4 V/cm) changes upon the addition of glucose at $t=5$ min, after which two major intervals can be identified. Interval 1(10–30 min) represents a glucose-affected time interval, during which the third harmonic increases. In this interval, at least two subintervals with slightly different rates of change can be seen. Interval 2 (30–80 min) represents a recovery time, during which the harmonic decreases with time. Several subintervals with slightly different rates of change can be seen, as before, possibly due to the different reactions taking place during glucose metabolism.

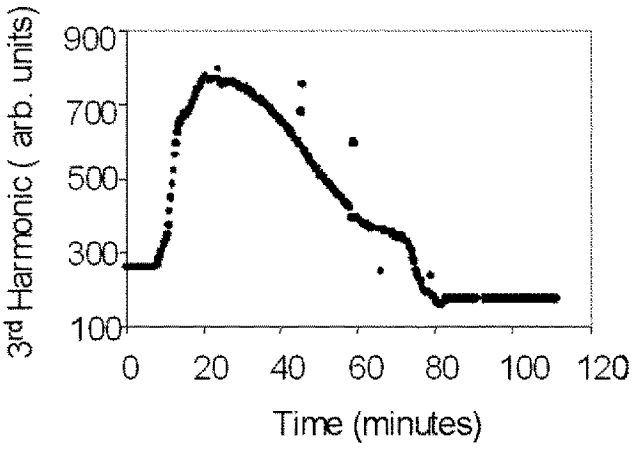

FIG. 7. Temporal behavior of the third harmonic after addition of glucose. Glucose was added to a resting cell suspension of yeast at 5 min. The frequency of the signal was 23 Hz and the applied electric field amplitude was 1.4 V/cm.

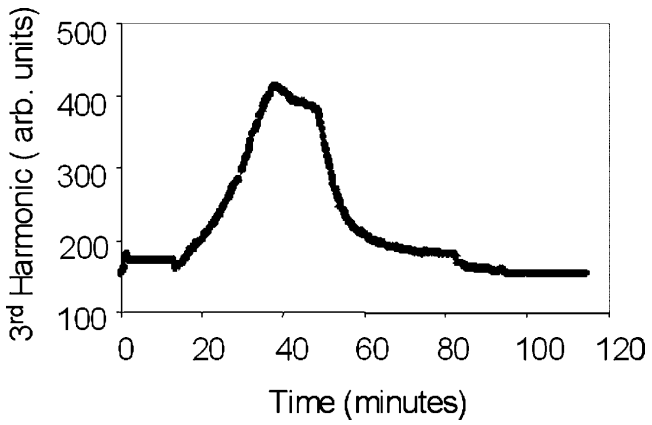

FIG. 8. Variation of the third harmonic with the membrane potential of the cells. The electric field frequency was 23 Hz and field amplitude was 1.5 V/cm. The addition of glucose at 15 min causes the third harmonic to increase dramatically. At 40 min 100 μm gramicidin, a membrane depolarizer, was added and at $t=50$ min another dose of 100 μm gramicidin was added to the cell suspension. Membrane depolarization caused the third harmonic to disappear. At 80 min, 100 mM of glucose was added but a third harmonic was not generated.

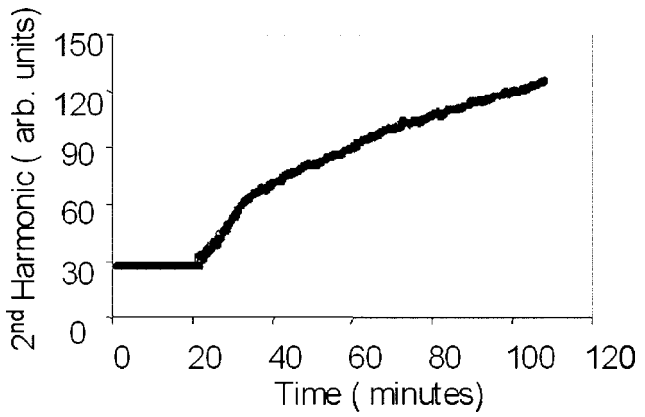

FIG. 9. Second-harmonic variation with the change in membrane potential of the cells. The electric field frequency was 23 Hz and the field amplitude was 3 V/cm. The addition of glucose at 20 min caused a second harmonic to appear, while the addition of membrane depolarizer (100 μM gramicidin) at 40 min caused the second harmonic to increase in magnitude.

We have also measured how the induced transmembrane potential affects different harmonics by adding very low concentrations of gramicidin to the cell suspension to depolarize the membrane. Gramicidin is a channel-forming lipid-soluble, hydrophobic molecule that enhances the permeability to specific ions. Two gramicidin dimers are thought to come together end to end across the lipid bilayer to form transmembrane channels that allow monovalent cations to flow down their electrochemical gradients by depolarizing the membrane potential [26]. Figure 8 shows the development of the third harmonic at 1.5 V/cm and 23 Hz. At $t=20$ min, glucose was added to the resting cell suspension and at $t=40$ min a 100 μM gramicidin membrane depolarizer was added. Note that gramicidin reduces the third harmonic. Another dose of 100 μM gramicidin added to the suspension at $t=50$ min completely suppressed the third harmonic as gramicidin further depolarized the membrane. An additional 100 mM dose of glucose added to the suspension at $t\sim 80$ min failed to generate harmonics. These results confirm that the membrane potential drives the H^+ ATPase transport of ions across the membrane. Figure 9 shows the development of the second harmonic at 3.0 V/cm field amplitude. Glucose was added at $t=20$ min, and 100 μM gramicidin was added at $t=35-40$ min. Note that gramicidin causes the second harmonic to appear more strongly. This shows that both the second and third harmonics depend strongly on membrane potential. Gramicidin is seen to promote the second harmonic whereas it suppresses the third harmonic.

III. CONCLUSIONS

We have measured the harmonics generated by H^+-ATPase membrane pumps in budding yeast cells under various conditions. It has been shown previously that almost all P-type ATPases share the same basic mechanism of ATP-driven ion translocation [27,28]. A variety of P-type ATPases (Ca^{2+}, Na^+/K^+, H^+, etc.) perform ion transportation in mammalian cells, suggesting future potential for clinical and pharmaceutical applications. Developing minimally invasive or noninvasive techniques for studying these pumps and understanding their basic mechanisms of cation transport are thus important to biology, medicine, and biophysics. For an example, H^+-ATPase (H^+/K^+-ATPase), located in parietal cells, is responsible for acid secretion in the human stomach. Malfunction of this pump causes acid reflux disease in humans. Various theoretical works have been carried out on ion transporters such as H^+-ATPase, including our earlier work [20,29–31] modeling the membrane enzyme by analogy to a junction with a threshold voltage, which showed that the type of harmonics (even versus odd) generated depends on the symmetry of the junction.

In this study, we have probed the reactions of H^+-ATPase with substrate (glucose) and competitive inhibitor (metavanadate). Fundamentally, it is known that both substrate and inhibitor bind to the same site of the enzyme but the temporal dependences of the harmonics after the addition of substrate or competitive inhibitor are quite different from each other. It can be concluded that, even though glucose and metavanadate bind to same site, they participate in entirely different processes during the reactions. Moreover, since we are performing whole cell measurements, there will be additional effects due to the different levels of glucose and vanadate acting on the physiology of each cell, which may affect our results. In Figs. 5 and 7 different rates of increase with time and oscillations (Fig. 5) of the harmonics with time may correspond to different reactions taking place in the enzyme. Finally, the harmonic response measurements reported here show how the enzyme is inhibited by vanadate and glucose, followed by subsequent recovery. This method may shed light on understanding the basic mechanisms by which enzymes transduce electric field or ATP energy to perform biological work. The observation of a strong second harmonic after the addition of glucose is in accordance with Astumian and Robertson's four-state enzyme model [32], which pre-

dicts that even harmonics appear only when the enzyme performs biological work. The membrane potential is also likely to play a major role in the enzyme activity of ion transporters such as H^+-ATPase. Our data show that the third harmonic indeed depends on the membrane potential, whereas the second harmonic does not.

In conclusion, the ability to noninvasively monitor active physiological processes *in vivo* is of potential importance for biophysics, biomedicine, and pharmacology. Furthermore, this work should shed light on further understanding of the mechanism of P-type motor proteins involved during ion transport. The experimental technique used here is relatively simple and, in principle, can be applied over a wider frequency range including the kilohertz region, in which we have observed, for instance, the nonlinear harmonic response of eukaryotic cellular organelles.

ACKNOWLEDGMENTS

The authors gratefully acknowledge helpful comments by D. B. Kell. This work was supported by the Texas Center for Superconductivity at the University of Houston (TcSUH), the Robert A. Welch Foundation (E-1221), and the Institute for Space Systems Operations (ISSO).

[1] H. P. Schwan, Adv. Biochem. Psychopharmacol **5**, 147 (1957).
[2] C. L. Davey, H. M. Davey, and D. B. Kell, Bioelectrochem. Bioenerg. **28**, 319 (1992).
[3] M. E. Van Der Rest, A. H. Kamminga, A. Nakano, Y. Andraku, B. Poolman, and W. N. Konings, Microbiol. Rev. **59**, 304 (1995).
[4] C. Grosse and H. P. Schwan, Biophys. J. **63**, 1632 (1992).
[5] R. D. Astumian, P. B. Chock, T. Y. Tsong, and H. V. Westerhoff, Phys. Rev. A **39**, 6416 (1989).
[6] T. Y. Tsong and R. D. Astumian, Prog. Biophys. Mol. Biol. **50**, 1 (1987).
[7] T. Y. Tsong, Methods Enzymol. **157**, 240 (1988).
[8] H. V. Westerhoff, R. D. Astumian, and D. B. Kell, Ferroelectrics **86**, 79 (1988).
[9] A. M. Woodward and D. B. Kell, Bioelectrochem. Bioenerg. **24**, 83 (1990).
[10] A. M. Woodward and D. B. Kell, Bioelectrochem. Bioenerg. **25**, 395 (1991).
[11] J. H. Miller, Jr., D. Nawarathna, V. Vajrala, J. Gardner, and W. R. Widger, J. Phys. IV **131**, 363 (2005).
[12] A. M. Woodward and D. B. Kell, FEMS Microbiol. Lett. **84**, 91 (1991).
[13] A. Goffeau and C. W. Slayman, Biochim. Biophys. Acta **639**, 197 (1981).
[14] R. Serrano, Biochim. Biophys. Acta **947**, 1 (1988).
[15] P. Supply, A. Wach, and A. Goffeau, J. Biol. Chem. **268**, 19753 (1993).
[16] K. Venema and M. G. Palmgren, J. Biol. Chem. **270**, 19659 (1995).
[17] G. Scarborough, J. Bioenerg. Biomembr. **34**, 235 (2002); G. A. Scarborough, Trends Biochem. Sci. **28**, 581 (2003).
[18] W. Kühlbrandt, Nat. Rev. Mol. Cell Biol. **5**, 282 (2004).
[19] T. Y. Tsong and R. D. Astumian, Annu. Rev. Physiol. **50**, 273 (1988).
[20] C. Toyoshima, M. Nakasako, H. Nomura, and H. Ogawa, Nature (London) **405**, 647 (2000). Also see Refs. [16,17] for reviews.
[21] D. Nawarathna, J. H. Miller, Jr., J. R. Claycomb, G. Cardenas, and D. Warmflash, Phys. Rev. Lett. **95**, 158103 (2005).
[22] D. Nawarathna, J. R. Claycomb, J. H. Miller, Jr., and M. J. Benedik, Appl. Phys. Lett. **86**, 023902 (2005).
[23] G. Grüner, Rev. Mod. Phys. **66**, 1 (1994).
[24] J. H. Miller, Jr., C. Ordóñez, and E. Prodan, Phys. Rev. Lett. **84**, 1555 (2000).
[25] J. Bylander, T. Duty, and P. Delsing, Nature (London) **434**, 361 (2005).
[26] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter, *Molecular Biology of the Cell*, 4th ed. (Garland Science, New York, 2002).
[27] A. W. Woodward, E. A. Davis, S. Denyer, C. Oliliff, and D. B. Kell, Bioelectrochemistry **51**, 13 (2000).
[28] K. Munson, R. Garcia, and G. Sachs, Biochemistry **44**, 5267 (2005).
[29] R. D. Astumian, I. Derenyi, Eur. Biophys. J. **27**, 474 (1998).
[30] R. D. Astumian, Biophys. J. **64**, 7 (1993).
[31] R. D. Astumian, Ann. N.Y. Acad. Sci. **720**, 136 (1994).
[32] R. D. Astumian and B. Robertson, J. Chem. Phys. **91**, 4891 (1989).