REVIEW ARTICLES

Non-invasive scanning ion-selective electrode technique and its applications to the research of higher plants*

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Accepted on November 7, 2006

Abstract The process of various ions and molecules getting into and out of cells is critical for plant survival. The non-invasive scanning ion-selective electrode technique (SIET) is a non-invasive method to obtain the information of ions/molecules across membranes in plant. This technique can measure the absolute concentration of ions and molecules, and also their fluxes and directions of movement. The samples to be analyzed can be a single cell, a piece of tissue, a whole organ and even an intact seedling. This article reviews the recent progress made in plant physiology by using this technique and discusses its potentials in future studies on plant physiology.

Keywords: non-invasive electrophysiology technique, ion-selective electrode, ion transport across membrane, SIET.

Crossing of ions and molecules through biological membranes is vital to plant growth and development. In the post-genomic era, it is a challenge to understand and identify unknown proteins involved in the activities of the plants, especially ion transporters involved with the plasma membranes, in order to understand their biological functions. The non-invasive scanning ion selective electrode technique (SIET), a newly-developed electrophysiological technique, is an ideal tool to tackle the task.

Development of SIET was based on the original work done by Kühtreiber and Jaffes[1]. The essential part of the SIET is a microelectrode with some ionic and molecular properties, including a set of automatic positioning and measuring system with a computer control unit (Fig. 1). Many improvements have been made on it, such as computerization, signal amplification and 3D measurement capability[2]. SIET can non-invasively detect the fluxes of ions and/or molecules going in and out of the samples by its high sensitivity to the concentrations of varieties of ions/molecules with the improvement of electronics and the enhancement of computer hardware/software. SIET has been widely used in many research fields including fundamental biology, physiology, neurology, space biology, clinical medical science, agriculture and forestry study[3,4].

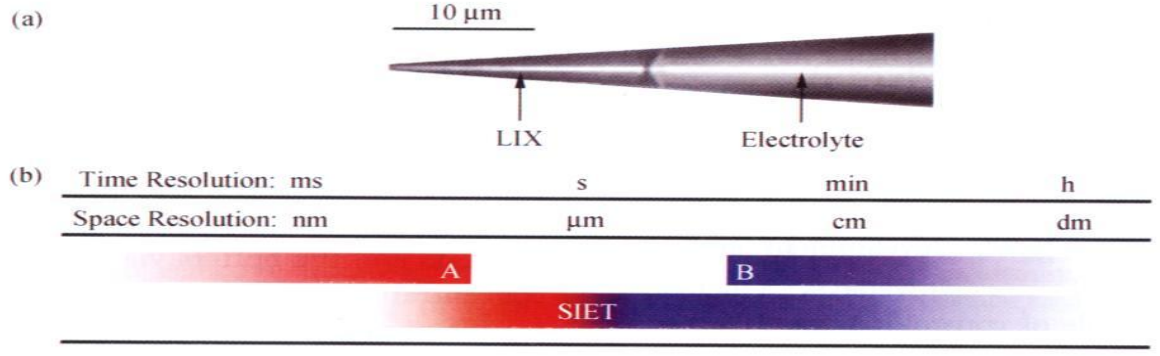

Fig. 1. Ion-selective microelectrode and comparison of temporal and spatial resolutions among SIET, patch clamp and chemical analyses. (a) The photograph of a Ca^{2+} microelectrode with liquid ion exchanger (LIX) and electrolyte; (b) letter A represents the temporal and spatial resolutions covered by fluorescence microscopy and patch-clamp; letter B stands for chemical analyses. SIET as an open experiment platform bridges the gap between A and B.

* Supported by National Natural Science Foundation of China (Grant No. 30170552), Beijing Municipal Natural Science Foundation (Grant No. 5042004), Academic and Technical Development Key Project of Beijing Education Community (Grant No. KZ200710028013)

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Some information of ionic/molecular distributions and movements has been obtained by measuring membrane potentials with techniques such as patch-clamp[5,6] and fluorescence microscopy. As a complementary tool to the above techniques with its unique spatial and temporal resolutions, SIET has become an indispensable tool to identify or verify some functions of transplasma membrane system. In this review, we will introduce the theory of the SIET method and its applications into the research of higher plant cells.

1 SIET fundamentals

1.1 Physics and mathematics

Ions and/or molecules diffuse from higher concentrations to lower concentrations in aqueous media. Although charged particles have also tendencies to move from higher electrochemical gradients to lower ones, it has been proved that if an ion-selective electrode moves less than ten micrometer (Fig. 2 dx), the electrochemical gradient due to the ionic motion can be neglected. Therefore, the flux of ion mobility can be calculated by Fick's Law—the first law of diffusion[7] (Fig. 2).

The ion-selective electrode is composed of a glass microelectrode, Ag/AgCl wire, electrolyte (e.g. 100 mmol/L $CaCl_2$) and the liquid ion exchanger (LIX). The voltages, V_1 and V_2, can be obtained by moving the electrode to two points over a predefined distance dx. The concentration difference dc of the two points can be calculated based on the calibration, correlated voltages and concentrations of particular ions/molecules. D is a constant of diffusion for a particular ion or molecule (unit: $cm^{-2}\cdot sec^{-1}$). Plugging them into the Fick's law of diffusion: $J_0 = D * dc/dx$, the ionic/molecular flux (unit: picomoles $\cdot cm^{-2} \cdot sec^{-1}$) can be calculated.

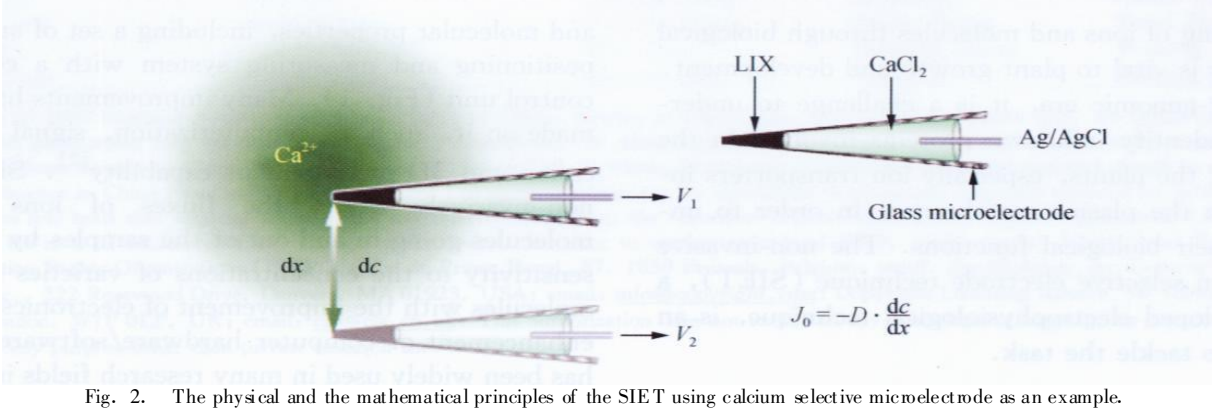

Fig. 2. The physical and the mathematical principles of the SIET using calcium selective microelectrode as an example.

The liquid ion exchanger is an organic compound composed of a neutral molecular carrier, which is commercially available. Some researchers design their own LIX to meet their specific needs as more neutral carriers are being developed.

1.2 Computer technology and system integration

The development of computer technology plays a critical role in the invention, development and improvement of the SIET[2,8]. Nowadays, a personal computer is well capable of controlling the 3 sub-systems of the SIET, which are a 3D-manipulation system, imaging system and signal amplification system. The fact that the ion selective electrodes need a few hundred milliseconds to settle down before they can collect data leaves the computer plenty of time to cope with the electrode movements and image manipulations. The use of a personal computer makes SIET a more affordable technique to common laboratories.

1.3 SIET buffers

In the use of SIET, buffers are normally added into the solutions, such as MES, Tris or EDTA etc., with intent to stabilizing the ionic measuring environment[9—12]. However, an incorrect choice or design of buffer(s) could lead to interference between ions of interests and the buffers via a breakdown of the ionic concentration gradient which would inevitably result in inaccurate or even false data interpretations. Kunkel et al. have recommended the so-called "Good" buffers specifically for SIET[13]. It has also been demonstrated that well designed buffers can make ionic flux measurements more efficient. Therefore, attention should be paid to the choice of buffers for SIET experiments. A buffer should be suitable for

your biological sample, sufficient to maintain the ionic/molelcuar gradient while having the least interference with the liquid ion exchanger (LIX).

1.4 Geometry in SIET

There are three models of spatial ionic/molecular distributions based on (1) 1—2 μm diameter ion electrodes; (2) the distance between the electrodes and the samples is 2—20 μm and (3) 5—30 μm in excursion dx. They are point source/sink, planner surface and sphere. However, it is always thought to be a planner distribution pattern when the distance between the sample and the electrode is less than 5 μm.

It is worth mentioning that the SIET is the only experimental system so far in the world that enables a researcher to approach their samples from various angles manually or using predefined programs. An excellent example would be the Ca^{2+} influx measurements at the tip of growing pollen tubes done by Kunkel et al.[13]. They found out the close correlation between growing pollen tube and Ca^{2+} influxes. Namely, the Ca^{2+} influxes were only detected around the tip region of rapidly growing pollen tubes while almost no Ca^{2+} influxes were measured from mature elongated ones. They further concluded that there was a disc-like structure on the pollen tube tip that was responsible for the intake of Ca^{2+} based on a mathematical modeling method.[14].

2 Applications of SIET

Due to the presence of cell wall, which makes the plant cells more difficult to be studied by other means such as patch-clam, the momentum of invention and development of SIET has come from plant physiology research. Realizing that SIET can obtain ionic/molecular activities in a non-invasive way, Kochian et al. developed more ion selective electrodes, such as H^+, K^+, Al^{3+} and Cd^{2+} based on the original design of Ca^{2+} microelectrode by Kutreber and Jaffe[1]. These ion selective microelectrodes have been successfully applied in the studies of corn roots and plant toxicology followed by applications in animal research areas[15—17]. Thus, the application of SIET into the research of whole roots, root hairs and the pollen tubes has clarified the relationship between calcium ion transport activities and plant growth[14, 18—24]. Messerli et al. successfully correlated the pulsatory growth of pollen tubes and the frequency of ion fluxs by using SIET[25].

2.1 Simultaneous measurement of both proton and molecule fluxes proved the existence of constitutive alkaline band of the pollen tubes

A constitutive alkaline band in the clear zones of growing pollen tubes has been found by Feijo et al.[23] who further proposed that the alkaline band formation is due to the existence of condensed mitochondria in the region. With specially designed simultaneous 2-electrode measurement system, Xu et al. have demonstrated the close correlation between H^+ efflux and O_2 influx, which could result from the energy demands from the growth of pollen tubes via oxidative phosphorylation in mitochondria. Thereby, the Hepler group's hypothesis has been supported by the measurement of a H^+ efflux that results in the alkalization of the pollen tube in cooperation with mitochondrial activities[23] (Fig. 3).

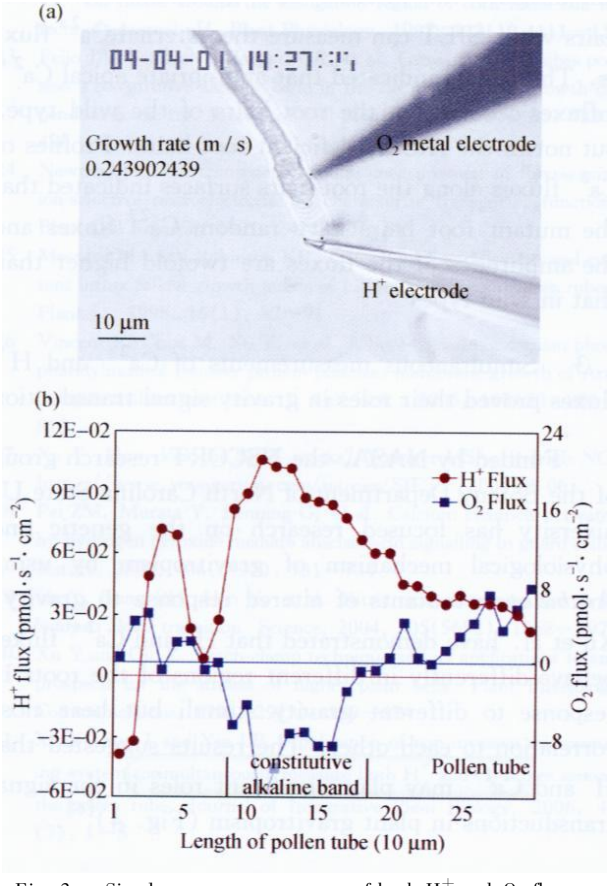

Fig. 3. Simultaneous measurements of both H^+ and O_2 fluxes. (a) Screenshot of both H^+ and O_2 electrode configurations along with the growth rate calculated from a sequence of images captured during the same experiments. (b) Correlation between H^+ efflux and O_2 influx around the constitutive alkaline band of the pollen tubes.

2.2 SIET supported fluorescence microscopy data that phosphatidylinositol transfer protein correlates with root hair polarity

Combining with fluorescence microscopy, Vincent et al. used SIET to demonstrate that phosphatidylinositol transfer protein (PITPs) has effects on the Ca^{2+} distributions internally and transportations externally. PITPs is a member of AtSfh1p family which regulates both intracellular and plasma membrane phosphoinositide polarity transportation, Ca^{2+} signaling, and cytoskeleton structures in the growing root hairs. The combined use of SIET and microscopy has provided new insights in the research of plant cell polarity.

Arabidopsis thaliana mutant *AtSfh1p* not only has root hair morphology defects, i.e. short and fat, but also has lost its gravitropism along with abnormal Ca^{2+} transport[26]. The Ca^{2+} fluorescence microscopy can measure the internal Ca^{2+} gradient in the root hairs while SIET can measure the external Ca^{2+} fluxes. The results indicated that appropriate apical Ca^{2+} influxes occurred in the root hairs of the wild type, but not in the AtSfh1-deficient root hairs. Profiles of Ca^{2+} fluxes along the root hairs surfaces indicated that the mutant root hairs have random Ca^{2+} fluxes and the amplitudes of the fluxes are twofold higher than that in wild types[26].

2.3 Simultaneous measurements of Ca^{2+} and H^+ fluxes proved their roles in gravity signal transduction

Funded by NASA, the NSCORT research group of the Botany Department of North Carolina State University has focused research on the genetic and physiological mechanism of gravitropism by using *Arabidopsis* mutants of altered response to gravity. Xu et al. have demonstrated that H^+ and Ca^{2+} fluxes behave differently in different regions of the roots in response to different gravity stimuli but bear close correlation to each other. The results suggested that H^+ and Ca^{2+} may play significant roles in the signal transductions in plant gravitropism (Fig. 4)[6].

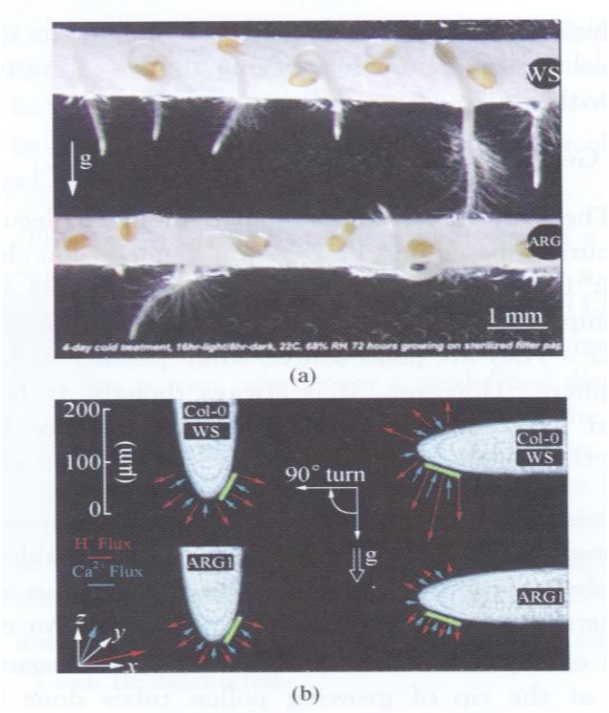

Fig. 4. Simultaneous measurements of both Ca^{2+} and H^+ fluxes using the SIET. (a) *Arabidopsis thaliana* mutant (ARG: altered response to gravity) and wild type (WS); (b) the differences of both Ca^{2+} and H^+ fluxes between the ARG and the wild type responding to the stimulation of gravity.

3 Summary and perspective

SIET has undergone a continuous development in the areas of data acquisition, data collection and calibration. By taking advantage of its 3D measurement capability, scientists can detect ionic/molecular activities near specific points of interests non-invasively, which is almost impossible to do with other electrophysiological methods[8, 27–29]. One concludes that SIET and patch clamp[30, 31] are very complementary to each other in terms of both spatial and temporal resolutions. Research on specific ionic/molecular transport systems has been facilitated by using SIET because of its improvements in sensitivity, spatial and temporal resolutions along with the use of cellular and molecular biology techniques, other electrophysiology techniques and fluorescence microscopy. It is predictable that SIET will play a significant role in the study of ionic/molecular active transport and co-transport pumps and carriers.

The progress of molecular biology enables us to identify, clone and control membrane transport genes. These transport process genes can be transfected into yeast or other egg cells or plants followed by functional characterization. After clarifying the gene structure and localizing its gene product in the cell, its role can be explored using non-invasive, multi-functional probes in 3D mode of SIET. Thus, SIET currently aids in the study of physiological functions at the cellular and tissue levels and, may be even considered indispensable in the future.

Acknowledgment Many thanks to Prof. Tingyun Kuang's support for SIET's promotion in China despite the fact that she was just diagnosed with breast cancer when she read this manuscript. Professional English assistance and polishing from Xu Yue (Beijing) Sci. &Tech. Co. Ltd. is also highly appreciated.

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