Electrolyte-concentration Cell using *Bruguiera Gymnorrhiza*

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An electrolyte-concentration cell was fabricated and its power generating characteristics were evaluated for the first time using salt-tolerant plant *Bruguiera gymnorrhiza*. This plant is known to grow in sea water and the chloride concentration in sap is lower than that in the saline surrounding the root.\(^1\)\(^,\)\(^2\) Plant tissue that separated the saline and the sap usually contains several ions like potassium and calcium, and was thought to show ionic conductivity. An electrolyte-concentration cell was, therefore, thought to be able to fabricate using the chloride ion concentration difference and the ionic conductivity. One of the two Ag/AgCl electrodes was inserted into the petiole of the plant, and the other among the vermiculites that supported the plant root. The vermiculites were soaked in a 400 mM NaCl solution. Two electrodes were connected to a galvanostat and the voltage was measured under a fixed current (20 nA) mode. The voltage gradually decreased with time from 260 mV to ca. 175 mV in two weeks. A periodical change in the voltage was observed. The internal resistance of the system was estimated to be 1.78 Ω.

*Key Words*: Electrolyte-concentration Cell, *Bruguiera Gymnorrhiza*

1 Introduction

Plants have microstructure such as xylem or phloem that contains different ion concentration. The use of such structure will be a key to future biocatalytic micro power generator.\(^3\) Ionic conductivity of the sap is expected to be sufficient to fabricate an electrochemical battery because plants maintain a certain amount of ions *i.e.* calcium, potassium, chloride, nitrate etc. in their sap,\(^4\)\(^,\)\(^5\) and among mangrove plants, *Bruguiera gymnorrhiza* is reported to have salt filtration mechanism in the root system.\(^6\) Transpiration is regarded as the main driving force to absorb water from saline.\(^7\) NaCl concentration of the xylem sap of Bruguiera gymnorrhiza is reported to be one thousand times lower than that in sea water.\(^1\)\(^,\)\(^2\) These two characteristics, *i.e.* the chloride concentration difference and the expected ionic conductivity, might allow us to fabricate an electrolyte-concentration cell. An electrolyte-concentration cell with liquid junction can be fabricated when two NaCl solutions with different chloride concentration are separated by a liquid junction with a sufficient ionic conductivity.\(^8\) Electromotive force between the two Ag/AgCl electrodes is measured using a voltmeter with large internal resistance (ca. 10\(^{12}\) Ω or more) when the electrodes are placed separately to the solutions. In our previous works we have already succeeded in measuring the voltage *in vivo* between two Ag/AgCl electrodes, one inserted into the pith cavity of azuki bean (*Vigna angularis*) and the other into the saline surrounding the root.\(^9\)\(^,\)\(^10\) In the previous work we have found that the voltage profile reflects the salt permeation process into the plant. Encouraged by the result, we then focused on *Bruguiera gymnorrhiza*. In this report, an Ag/AgCl electrode is inserted inside the petiole and the other into the saline surrounding the root. Tissue that separated the sap and saline acted as the junction. The electrode inserted among the vermiculites is surrounded by 400 mM of chloride, and at the surface the oxidation of silver into silver chloride initiated as following formula.

\[
\text{Ag} + \text{Cl}^- \rightarrow \text{AgCl} + e^-
\]

As the chloride concentration in the sap surrounding the electrode in the petiole was relatively smaller than that in saline, the possible initiating reaction occurred at the electrode in the petiole was reduction of silver chloride into silver.

\[
\text{AgCl} + e^- \rightarrow \text{Ag} + \text{Cl}^-
\]

The electric potential difference between the two electrodes can be calculated using Nernst equation, and one can measure this experimentally as an electromotive force (emf) using a voltmeter. When the electrodes are connected to a circuit with lower resistance, current flows and the voltage decreases compared to the emf. This can be characterized by the output current-voltage plot.

In this work two miniature Ag/AgCl electrodes were used to form an electrolyte-concentration cell. One was inserted into the petiole of the mangrove plant. The other was placed among the vermiculite where the plant was potted, holding 400 mM saline. Power generation of this "Mangrove battery" was characterized for the first time.
2 Experimental

All the experiments, including the germination and growth of the plant, were performed in a room with controlled temperature (25°C) and humidity (70%). Lighting was controlled to make 12 h of light and 12 h of dark condition.

*Bruguiera gymnorrhiza*:

The propagules (seeds) of *Bruguiera gymnorrhiza* was potted among vermiculites soaked in tap water. After 6 months, when the seedling became ca. 30 cm in height above the pot, tap water was exchanged into saline containing 400 mM of sodium chloride. After adaptation to the saline for 2 months, the electrode was inserted and measurements were started.

*Electrodes*:

Silver wires partly covered with silver chloride (200 µm in diameter, 50 mm in length, and 35 mm of it was covered with AgCl) were purchased from TOA-DKK company (Tokyo, Japan). A hole was drilled manually into a petiole using a 0.5 mm diameter drill, and the electrode was inserted into this hole. The “wound” was covered using a sealing film. (Fig. 1 (a)) The other electrode was inserted among the vermiculite ca. 10 mm away from the stem as illustrated in Fig. 1 (b).

*Power generation evaluation*:

Two electrodes were connected to a galvanostat (HA 151, Hokuto Electric Company, Tokyo, Japan) as illustrated in Fig. 1 (b). Current was set at 20 nA and the voltage was measured for two weeks. Voltage data was recorded every one hour using a voltage data logger (VL 3635-05, As One, Osaka, Japan).

*Inner resistance evaluation*:

Being conscious of the application to power generation, internal resistance of the system was evaluated. On the 14th day of measurement, two electrodes were connected to galvanostat and the current was set at 0, 20, 40, 60, 80, 100, 120 and 140 nA. Voltage measured at each current was plotted and the slope was regarded as the inner resistance as in

\[ E_{\text{measured}} = E_{\text{mf}} - ir \]

where \( E_{\text{measured}} \), \( E_{\text{mf}} \), \( i \), and \( r \) stand for the measured voltage, electromotive force, set current and internal resistance, respectively.

*Microscopic observation*:

Cross section of the petiole after the removal of the electrode was observed using microscope (DML, Leica Microsystems Wetzlar GmbH, Wetzlar, Germany). After all the measurements, the petiole was stained using safranin, an effective material to stain the vascular bundle red. The structural change in the vascular system after insertion and removal of the electrode was observed.

3 Results and Discussion

Plants are reported to recover generally from wounds by secreting products for the xylem to be incrusted. Surface of the petiole where the electrode was inserted became brown and dry. Microscopic image of the petiole cross section is shown in Fig. 2. From this figure, safranin-stained part (dark part (a), presumably the vascular bundle) seemed to face the hole (b) where the electrode had been inserted. Xylem is rich in lignin and is stained red by safranin. This indicated that the sap transferred from the root contacted the electrode. In the figure the vessel structure (c) can be seen at the edge of the hole. It is not reasonable to conclude that the electrode had been in contact with xylem sap. Making a drilled-hole in the petiole certainly will cause cavitation in the xylem or phloem sap.11 Such cavitation might cause snapping of the circuit and no voltage would be measured. It is therefore rational to consider that the inserted electrode was in contact with all the sap, xylem or phloem or other. Admitting the fact that absorbed water flows from the root along the xylem and is distributed to the cells via phloem,11 the use of xylem sap is, therefore, favorable to fabricate a battery, because the ionic conductivity is better than the phloem sap. Voltage profile was shown in Fig. 3 (a). In the beginning the voltage reached 0.26 V and this value agrees with the calculated result using Nernst equation. Voltage decreasing tendency is
recognized in the voltage curve. Several reasons can be considered for this. Firstly the set current might be larger than the reaction-induced flow of electron. The 20 nA current was employed because this was the minimum value with the galvanostat used. Setting of less current with the galvanostat or allowing larger electrode surface area facing the sap might result in the stable voltage without decreasing tendency. Secondly, recovery of the vessel system from the wound was thought to be the reason for the voltage decrease.

When the sealing of the vessel or sieve tube proceeded in the recovery process, the amount of sap that contacted the electrode should be decreased. Adsorption of some polymer on the electrode surface is also imagined. Third possible reason is that the condensation of the saline due to the evaporation gave ionic stress to the plant. We let the saline evaporate because the root respiration is necessary for the plant. Generally in the artificial condition many seedlings gradually lost the turgor. Salt accumulation by leaves is found in mangroves and the leaf drop occurs when the leaf ceases to accumulate salt. Before the drop the vascular bundle in petiole must be closed to prevent sap leak. Temperature and humidity data (Fig. 3 (b)) showed that during the day time the former increased and the latter decreased. The effect of such differences in temperature, humidity and light on the Ag/AgCl electrode was proved to be negligible after the control measurement (data not shown). The periodic increase during the daytime in the voltage curve is therefore thought to be mainly the result of more active transpiration compared to the one at nighttime. This is possibly related to the water transport characteristic of the plant. Generally stomata open in the daytime to absorb carbon dioxide as a result of photosynthesis. Through these open stomata vapor is released into the air and transpiration is activated in the daytime. The movement of water inside the plant is therefore activated in the daytime and this may be one of the reason for the periodical perturbation in the voltage profile (Fig. 3(a)). Measurement of electromotive force between the two Ag/AgCl electrodes by setting the current to zero indicated that the sap contacted the electrode contained ca. 0.02 mM of chloride. This concentration is one order smaller than the one in xylem sap reported. The electrode, therefore, possibly contacted not only with xylem sap but also with others. The relationship between the set current and the measured voltage was shown in Fig. 4. The internal resistance calculated from the slope of the dotted line in the figure was 1.78 GΩ. After the measurement we found no accumulation of cation/anion at the interface between the root and the saline. When we cut the root of Bruguiera gymnorrhiza and placed it in a saline solution, no significant ionic concentration difference was observed between inside/outside the root. (data not shown). In our system, the internal resistance resulted in far large value for a power generator probably due to the long distance (20-30 cm) between the two electrodes. This work, however, indicated that the use of electrodes with smaller diameter will enable the power generation using tubular structure in plants.

4 Conclusion

We have fabricated the electrolyte-concentration cell using a mangrove plant Bruguiera gymnorrhiza and evaluated its power generating characteristics for the first time. The Ag/AgCl electrode that was inserted into the petiole contacted probably both with the xylem and the phloem sap that contained far less concentration of chloride than the saline. Voltage profile obtained under a fixed current (20 nA) showed gradual decreasing tendency from 260 to 175 mV in two weeks. Further efforts should be made to realize an effective battery by miniaturizing the electrode until it can be inserted into the vessel.
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