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Sodium Transport in Salt Tolerant Algae: *Chara longifolia*

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Abstract

Salt tolerant alga *Chara longifolia* shows a difference in Na^+ efflux due to pH dependence and inhibitor sensitivity when grown in fresh water or in high salt. Most of the Na^+ which enters the cell is removed by efflux through Na^+/H^+ antiport. When *C. longifolia* was cultured in freshwater, sodium efflux elevated at pH 5 and did not show a significant change from pH 7 to pH 9. Salt water adapted *C. longifolia* showed higher Na^+ efflux than fresh water adapted species. Amiloride inhibition showed a decrease in the efflux at pH 5 in fresh water adapted species. Salt cultured *C. longifolia* showed an increase in efflux with pH change over time. Therefore, in conclusion, fresh water adapted *C. longifolia* showed a large percentage increase in Na^+ efflux from pH 7 to 5 and small percentage decrease from pH 7 to 9 whereas salt water adapted *Chara* showed large Na^+ efflux percentage increase form pH 7 to 5 as well as large percentage decrease pH 7 to 9 over time. It is expected to see similar inhibition profiles in both fresh water and salt water adapted *C. longifolia*.

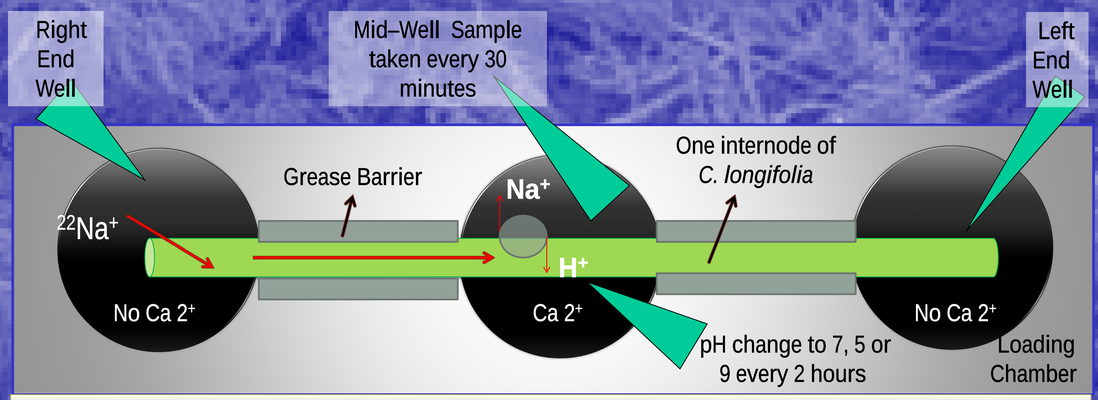

Fig. 1: Schematic diagram of method of efflux measurement

Materials and Methods

- Internodal cells were cut from the growth tank and allowed to recover in distilled water for 24 hours. Prior to the experiment, chambers were loaded with the cells and the connections between the wells were sealed with corning #7 release compound and Dow Corning high vacuum grease.
- Cells were checked for healthy cytoplasmic streaming and the diameter and length were measured. End wells were loaded with $^{22}Na^+$ in a 20 mM NaCl solution lacking Ca^{2+} to facilitate uptake of Na^+ by increasing Na^+ permeability.
- The sampling chamber (middle well) was loaded with different pH solutions containing 0.1 mM $CaSO_4$, 20 mM NaCl, 0.05 mM K_2SO_4, and 5 mM buffer (pH 5, Mes; pH 7, Hepes; pH 9, Caps), titrated to desired pH using BisTrispropane.
- Total mid-well solution is removed for counting every 30 minutes and new solution added. Radioactive $^{22}Na^+$ is measured as counts per min (cpm) of the sample using a gamma counter. The specific activity is calculated by sampling 2µl of solution diluted in double distilled water from both right and left end wells.
- For experiments with salt water adapted C. longifolia, osmolarity of all the solutions were adjusted to 375 milliosmoles kg^{-1} using sorbitol.

Introduction

- Sodium is a large component of plants' ionic environment. Cells must maintain low cytoplasmic sodium ion (Na^+) concentration for proper functioning.
- ***Chara longifolia*** is salt tolerant, growing in either salt or freshwater. In this experiment, mechanisms leading to sodium homeostasis were observed in both freshwater and salt water-grown *C. longifolia*.
- Na^+ efflux through the plasma membrane of single internodal cells was monitored. When grown in freshwater, *C. longifolia* has a higher efflux than a related salt sensitive species, *Chara australis*.
- Sodium efflux is mainly through Na^+/H^+ antiport.

Mechanism:

- $^{22}Na^+$ comes to isotopic equilibrium with cytoplasmic Na^+ ~90 min after loading from a 0 Ca^{2+} solution.
- Once in the cytoplasm, Na^+ moves by cytoplasmic streaming to the part of the cell in the mid-well. Grease barriers prevent the extracellular movement of sodium between the wells.
- The mid-well solution has varying H^+ concentration. Na^+ moves out of the cell in exchange H^+ moving in (antiport). Therefore the mid-well solution is sampled to track the efflux.
- Amiloride and lithium are known inhibitors of Na^+/H^+ antiport at pH 5.
- Na^+ efflux is up-regulated in the cells from salt water adapted tanks.

Results

A. Na^+ efflux increased from pH 7 to pH 5 and decreased at pH 9 in freshwater *C. longifolia.*

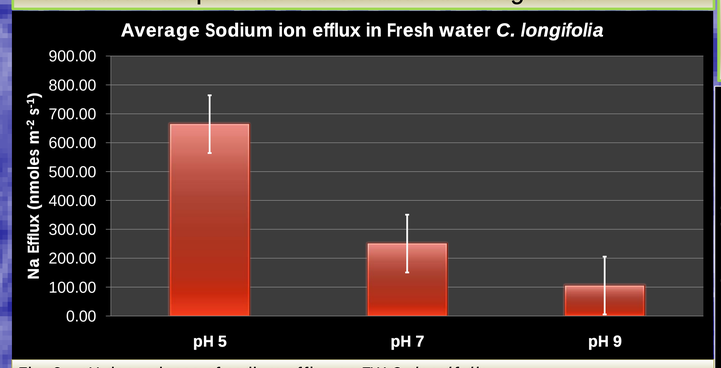

Fig. 2. pH dependence of sodium efflux on FW *C. longifolia*.

B. Freshwater *C. longifolia* was salinized over a period of 5 days and efflux measured over 92 days. Efflux increased from Day 17 to Day 27, but was, unexpectedly, lower than freshwater efflux.

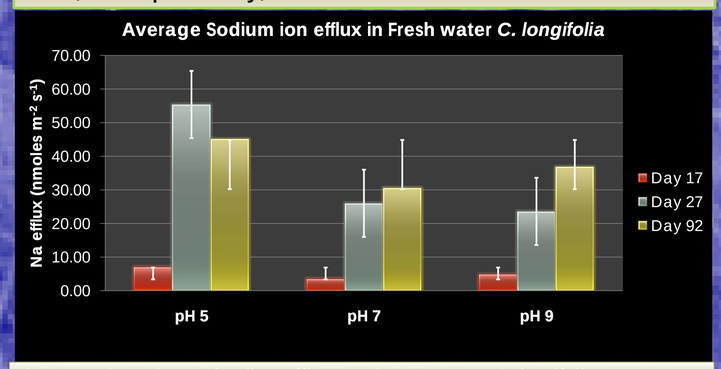

Fig. 3. pH dependence of sodium efflux on salt water grown *C. longifolia*.

C. The percent change in efflux with changes in pH was too variable to allow us to draw any conclusions with confidence. More experiments need to be done to clarify this question.

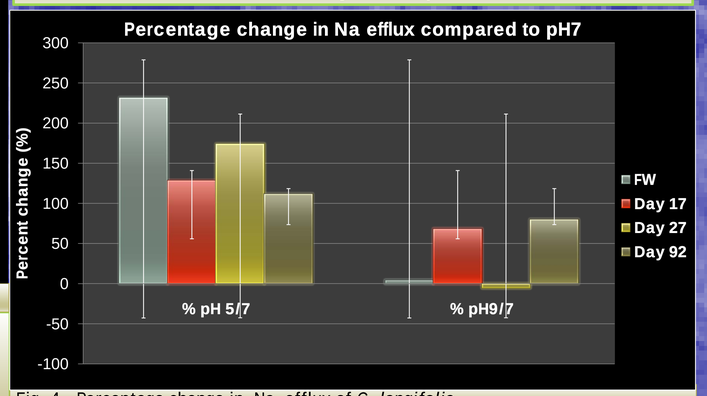

Fig. 4. Percentage change in Na efflux of *C. longifolia*.

Conclusions

- Efflux increased between 17 and 27 days after salinization.
- Efflux is higher at pH 5 than at pH 7 under both growth conditions.
- Efflux is higher at pH 9 than at pH 5 on in salt-adapted *Chara longifolia*

Future work

- Continue experiments on cells from salt water
- Inhibition studies to assess mechanism

Acknowledgements

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Works Cited

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