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ATPase proton translocation across isolated tonoplast vesicles of wheat
by Glenn Michael Magyar

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences
Montana State University
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Abstract:
Proton-translocating adenosine triphosphatases (ATPases) have been shown to exist in the vacuole membrane (tonoplast, (TP)) and the plasma membrane (PM) of many-higher plants including barley, beets, carrots, corn and oats. The PM ATPase of wheat has been characterized, but to date there have been no reports concerning the TP ATPase in this important crop plant.

A crude membrane preparation isolated from wheat (Triticum aestivum L. cv. Winalta) roots was separated by differential and sucrose density gradient centrifugation into three fractions which have different SDS-PAGE protein profiles. One of these fractions is enriched in both nitrate sensitive ATPase activity and nitrate sensitive, orthovanadate insensitive, (tonoplast type) ATP dependent proton translocating activity. Another fraction of this crude membrane preparation is enriched in orthovanadate sensitive (plasma membrane type) ATPase activity.

The presumptive TP enriched fraction is also enriched in a 70 kD polypeptide which strongly cross-reacts with antiserum developed against an amino terminus peptide of the 70 kD subunit of the carrot (Daucus carota) TP ATPase.

AlF_4^- (fluoroaluminate), which strongly inhibits the orthovanadate sensitive PM ATPase activity, has relatively little effect on the nitrate sensitive TP ATPase activity.

This work shows for the first time, the identification and characterization of a nitrate sensitive, proton translocating ATPase activity in the vacuolar membrane of wheat.

ATPase PROTON TRANSLOCATION ACROSS ISOLATED

TONOPLAST VESICLES OF WHEAT

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency and is ready for submission to the College of Graduate Studies.

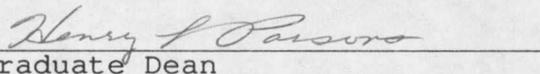3/7/87
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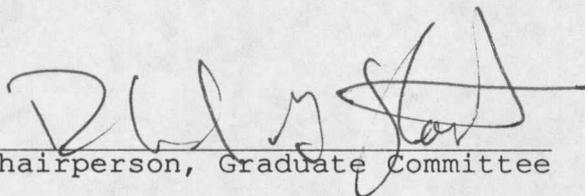
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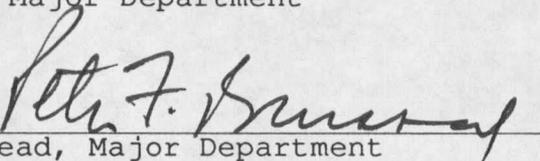
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INTRODUCTION

Plant Cell Vacuoles

One of the major differences between plant and animal cells is the existence of a prominent vacuole found in most plant cells. This organelle is bounded by a lipid bilayer membrane known as the tonoplast. In some cells the vacuole is so large that nearly 90% of the total cell volume lies within it whereas the cytoplasm constitutes only about 4% (10). This organelle is so large, that it accounts for the majority of eukaryotic biomass on the earth (20). Because of the sugars and other nutrients that accumulate within the vacuole it has been argued that this is one of the most important parts of the entire plant. If this concentration of cell sap did not occur, most fruits and vegetables would have no appealing taste at all (30).

Vacuolar Functions

Perhaps the most important function of the plant cell vacuole is the maintanance of cell turgidity. A high concentration of solutes is commonly found within the vacuole, which results in a subsequent influx of water. This causes the vacuole membrane to be pressed against the

cytoplasm which in turn is pressed against the cell wall. This pressure enables non-woody plants to stand erect and is also responsible for cell enlargement in growing plants (12, 15, 22, 28).

There is strong evidence to support a lytic function of the vacuole thus making it similar to the lysosomes that are found in animal cells (14, 15, 19, 22). The various types of hydrolytic enzymes that have been found within the vacuole include proteinases, acid phosphatase, phosphodiesterase, RNase, DNase, alpha and beta-amylases, beta-glucosidase and beta-galactosidase. These enzymes are all specific to different cells and physiological functions, e.g. leaf abscission, plant senescence and seed germination (15, 19).

Another function of the vacuole is storage. Many different types of biomolecules can be found within the vacuole, including: sucrose; organic ions such as malate, citrate and oxaloacetate; inorganic ions such as K^+, Na^+ and Cl^- and also various amino acids and proteins. The high concentration of these organic acids contributes to the low pH of the vacuole, which is typically around 5.5 (31). Many of these metabolites are stored within the vacuole until they are needed by the plant (15,19).

The vacuole is involved in at least one other function of importance to the plant cell. It has been proposed (19) that the vacuole serves the same extracytoplasmic purpose in

the plant cell as the intercellular spaces do in animal cells. In other words, they are able to act as a compartment that mediates the exchange of components to and from the cytoplasm. The concentrations of these various components are maintained by transport systems at both the plasmalemma and the vacuole membrane.

Vacuolar Biochemistry

The vacuole membrane is also called the tonoplast, a name that implies a very elastic nature. This appears to be true as tonoplasts have been shown to be able to increase in surface area by 1.5 times upon uptake of water into the cell and also to withstand the intense shearing forces of cytoplasmic streaming (30).

The tonoplast is an asymmetrical lipid bilayer that surrounds the plant vacuole. It has been shown to be composed of a high percentage of saturated fatty acids, a property which is thought to be important in maintaining the fluidity of this membrane (20,30).

The proteins that are associated with the vacuole membrane consist of both integral and peripheral proteins on both faces. It has been shown with freeze-fracture faces from electron microscopy that the cytoplasmic side of the membrane appears to contain more globular proteins than the exoplasmic or interior side. However these proteins do not

appear to be anchored into place by microfilaments as they tend to line up into a row when the membrane is ruffled (30). Polyacrylamide gel electrophoresis, has shown that none of the major polypeptides associated with the tonoplast have a molecular weight of greater than 100,000 (20).

Carbohydrates have also been identified on the interior or exoplasmic side of the tonoplast (20), but not on the cytoplasmic side. Transmission electron micrographs show that the inner portion of the membrane appears thicker than the outer portion.. The carbohydrates associated with the inside face could account for this observation.

Electrogenic Pumps and Proton Gradients

By the 1960's, it was known that differences in electrical potential existed between the vacuole, the cytoplasm and the surrounding medium. Since the differences were greater than what could be attained by simple diffusion, an electrogenic "pump" was postulated to be the cause for the gradients that were observed. Higinbotham (10) stated in 1970 that in the plasma membrane,

> "....an efflux pump for H^+ is a likely pos-
> sibility; this is consistent with the hypothesis
> of Mitchell (21)....the source of energy could
> be the potential energy represented by sharp H^+
> gradients or to reducing energy, e.g., NADH, as
> well as to ATP."

A cellular electrogenic pump is defined as a transport system that directly contributes to the electrical potential across a membrane (25). In 1974, several papers were published from a symposium that dealt with the subject of ion transport in plants (11, 24, 27, 32). Two conclusions drawn from these investigations were that the pH of the cytoplasm remains relatively constant in a cell and that the proper physiological pH must be maintained by the active transport of H^+'s across the membrane by a suitable proton pump (31).

Proton gradients formed by pumps are found in several subcellular locations in plants. Both the mitochondria and the chloroplast produce adenosine triphosphate (ATP) from the flow of H^+'s down a concentration gradient. These gradients are established from the action of an electron transport system. Whereas this type of proton electrochemical gradient is used to synthesize ATP via the action of complex ATP synthetases, there are at least two types of enzymes in plants that hydrolyze ATP to form an electrochemical proton gradient (18, 34, 35). These H^+ translocating adenosine triphosphatases (ATPases) are found in both the plasma membrane (PM) and the tonoplast (TP) of higher plants and are responsible for the establishment of an electrochemical proton gradient across these membranes.

Recently, another type of proton translocating enzyme has been shown to exist in the tonoplast. This pump

utilizes pyrophosphate (PP$_i$) as an energy source and also contributes to the pH gradient that is maintained across the tonoplast (29). It has also been reported that this pump is capable of establishing and maintaining a pH gradient in isolated vacuoles when PP$_i$ is used as the sole energy source for proton pumping (8).

Once this proton gradient is established it subsequently drives a number of important processes such as the transport of nutrients (1) the maintanence of cytoplamic pH (34) and the transport of ions (7).

Tonoplast ATPase

While it has been pointed out that there are discrepancies in the literature regarding the TP ATPase (17), there is enough complimentary information available to review the general characterstics of this enzyme and to compare them to the general characteristics of the PM ATPase.

The primary function of both the TP and the PM ATPase is to establish and maintain a proton gradient within the plant cell by active transport of hydrogen ions. The potential energy of this proton gradient is then used to transport other solutes across the membrane. (6, 7, 12, 14, 18, 34, 35, 37).

These enzymes are oriented so that the ATP binding site faces the cytoplasm (23, 34), this allows for the hydrolysis of ATP which in turn provides the energy needed to power the electrogenic proton transport (18). As the gradient becomes steeper, the rate of ATP hydrolysis slows down and finally stops when the transmembrane difference in pH is about 1.5 - 2.0 units and the membrane potential is about 30 mV. Proton ionophores, (molecules that can dissipate a proton gradient), form holes in the membrane and cause the hydrolysis of ATP to begin again. Therefore it may be inferred that the ATPase is affected by the electrochemical proton gradient (34). Figure 1 shows a schematic diagram of the transport proteins located in both the tonoplast and the plasmalemma.

The ATPase of the tonoplast is distinguished from other known ATPases because of several unique properties. These properties were not very clearly defined before 1980 because of the difficulty encountered in the isolation of viable, intact, non-leaky vacuole membranes from plant cells.

Currently, the tonoplast and other endomembranes are separated from large organelles in homogenized plant tissue by differential centrifugation into a so-called microsomal fraction. The membrane vesicles found in this fraction of plant cells include tonoplast, endoplasmic reticulum, golgi, plasma membrane and small fragments of both the mitochondrial and chloroplast membranes (18, 34, 35). Since the