

CO₂ and Ion Transport via Plant Aquaporins

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ABSTRACT

CO₂ diffusion across membranes is one of the rate limiting steps during photosynthesis, therefore understanding the process of CO₂ permeation across membranes is important. The question of whether CO₂ transport across membranes can be facilitated by aquaporins is very controversial. Previous research where aquaporins were heterologously expressed in either *Xenopus* oocytes or yeast protoplasts showed that some plasma membrane intrinsic proteins (PIPs) or animal aquaporins could facilitate CO₂ transport. However, others have demonstrated using molecular simulation approaches and biophysical calculations that the unstirred layer poses the major rate limiting step for CO₂ diffusion across membranes, and that it is unlikely that CO₂ permeates via the water pathway in aquaporins, because this pathway exhibits a greater energy barrier compared to that for the lipid bilayer.

If water and CO₂ share the same pathway through aquaporins or if the presence and activity of aquaporins somehow affects CO₂ permeation, there should be a correlation between water permeability and CO₂ permeability. Therefore, by employing the stopped-flow technique and using pea plasma membrane vesicles isolated from pea leaves, this thesis explored the links between CO₂ permeability and water permeability. Plasma membrane vesicles from pea plants that were grown in different conditions showed considerable variability in water permeability. The very high and variable (between preparations) water permeability (0.06 to 0.18 m s⁻¹) plus the low activation energy (10.8 KJ mol⁻¹) of water transport indicated aquaporins dominated water flow, yet there was no significant correlation between water permeability and CO₂ permeability (1.49 x 10⁻² cm s⁻¹). The activation energy for CO₂ permeation was 37 KJ/mol which is about double that for CO₂ diffusion in water. Also the aquaporin inhibitor silver sulfadiazine resulted in a large inhibition of water permeability but this did not affect CO₂ permeability. Similar results were obtained for plasma membrane

vesicles isolated from *Arabidopsis* leaves though the water permeability was lower. In performing these measurements care was taken to exclude artifacts caused by the concentration of carbonic anhydrase (CA) and its temperature dependence, since vesicular entrapped CA was required to measure CO₂ permeability via changes in vesicular pH.

Because there are not many aquaporins that have been identified in pea, some *Arabidopsis* aquaporins that have been suggested to be involved in CO₂ transport were expressed in *Xenopus* oocytes for further investigation. Water transport via these aquaporins was first studied. It was demonstrated that PIP2s were functional water channels when expressed alone, while PIP1s were not. However when PIP1 and PIP2 aquaporins were co-expressed in *Xenopus* oocytes a greater than additive effect on water permeation was observed for some combinations. This suggested that AtPIP1;2 and AtPIP2;1, and AtPIP1;5 and AtPIP2;1 interact. A previously identified natural mutation in the pore region of VvPIP2;5 from grapevine (G100W), which prevented water flow, was used to probe AtPIP2;1 and its interaction with AtPIP1;2. This showed that the interaction still occurred despite the lower water permeation of the combined pair when expressed in *Xenopus* oocytes.

Originally, the CO₂ permeabilities of the *Arabidopsis* aquaporins of interest were intended to be tested using the external pH micro-electrode technique which was first employed to test CO₂ transport across *Xenopus* oocyte plasma membrane. However, one of the criteria for using this technique is that the expression of the aquaporins should not induce any ion conductance, which would potentially alter external pH either directly or indirectly. Therefore, electrophysiology experiments were conducted to test whether the expressed aquaporins induced any ionic currents. It was found that AtPIP2;1 indeed induced ionic currents selective to anions including HCO₃⁻ when expressed in *Xenopus* oocytes. It was

demonstrated that AtPIP2;1 homotetramer was likely to function as an ion channel since when co-expressed with its interacting partner (AtPIP1;2) this abolished the anion conductance. Furthermore the G100W mutation also prevented anion conductance of the AtPIP2;1 indicating that the pathway may be via the water pore. Expression of AtPIP2;1 in *Saccharomyces cerevisiae* was undertaken to test a potential anion sensitivity induced by the expression of AtPIP2;1. The expression of AtPIP2;1 induced increased water permeability of the yeast spheroplast as it does in *Xenopus* oocytes, and gave a low growth phenotype on all media tested, however this could not be linked to increased anion transport.

This thesis has demonstrated that measurements of CO₂ permeability are extremely difficult and likely to be limited by factors not always controlled for in previous experiments. Furthermore it has been demonstrated that some plant PIP aquaporins may function as anion channels and that this could complicate the interpretation of CO₂ permeation particularly when the HCO₃⁻ anion can permeate as was demonstrated for AtPIP2;1.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

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