Plant Plasma Membrane Protein Isolation: Ultracentrifugation vs. Spin-Column

(Invent Biotechnologies Inc.)

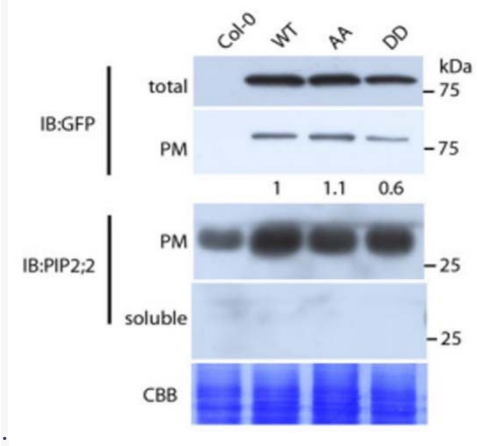
The plasma membrane is a vital organelle in plant cells, responsible for regulating the exchange of molecules between the cytoplasm and the extracellular environment. To understand the molecular mechanisms underlying plant growth and development, it is crucial to isolate and characterize plasma membrane proteins from plant tissue. In this mini-review article, we discuss commonly used methods for the isolation of plasma membrane proteins from plant tissues, along with their advantages and disadvantages.

One of the most widely used methods for plasma membrane protein isolation is differential centrifugation. This method involves sequential centrifugation at various speeds to pellet plasma membranes from homogenized plant tissue, followed by further purification using density gradient ultracentrifugation or affinity chromatography. While this method has the advantage of high yield and low cost, it is complex, time-consuming, and requires specialized equipment. Additionally, cross-contamination is a common concern due to the large sample size required (1).

Another commonly used method is aqueous two-phase partitioning (ATPP), which is based on the differential partitioning of proteins between two immiscible aqueous phases formed by polymer solutions. Plasma membrane proteins can be extracted from the upper phase, but clear separation of upper and lower phases is required, and cross-contamination can be an issue (2).

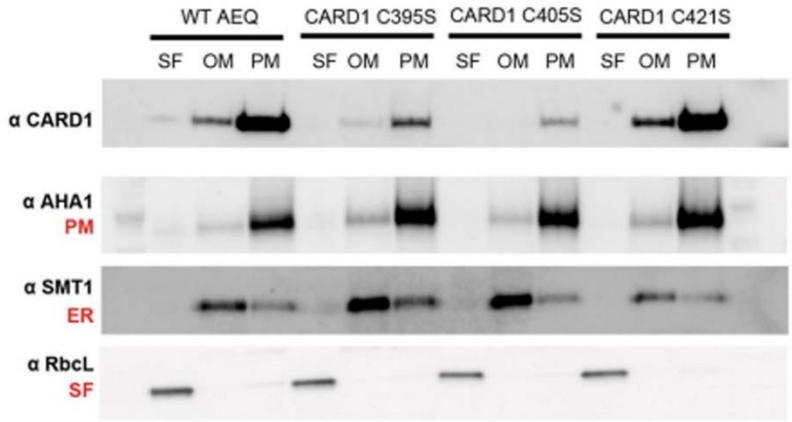
The latest technology for plasma membrane protein isolation is the spin-column based method, which features a specially designed filter cartridge and proprietary detergent-free buffers for rapid isolation of plasma membrane vesicles with associated proteins in native form, without using ultracentrifugation. This method requires only a table-top centrifuge, and starting material as small as 200-300 mg of tissues.

A study by Brillada C. et al. demonstrated the effectiveness of this method by investigating the relationship between exo70B2 and immune signaling in Arabidopsis seedlings using PIP2;2 in immunoblotting. Using the spin-column method (SM-005-P), they were able to significantly enrich plasma membrane protein with a clear separation from soluble fraction, and confirm the association of GFP-Exo70B2 with the PM while the phosphomimic S554/567A display reduced accumulation in PM (3).

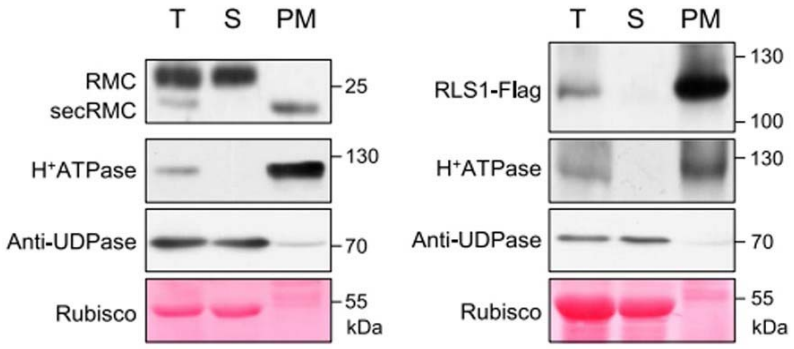


Similarly, Laohavisit A. et al. used the spin-column method to investigate the role of quinone signaling and card1 function in parasitic and nonparasitic plants (4). Seedlings of Arabidopsis were separated into PM, organelle membrane (OM), and soluble fraction (SF) using SM-005-P, which are further probed with

Card1, AHA1 (PM marker), SMT1 (ER marker) and RbcL (soluble fraction marker) in Western blotting. Results showed that PM protein was significantly enriched, and card1 was mainly found in the PM fraction, with no cross-contamination between PM and soluble fraction.



The spin-column method has also been used to investigate the mechanism of NB-ARC protein and cysteine-rich receptor-like protein RMC to trigger cell death in rice (5). The data showed that PM protein marker (H+-ATPase) is significantly enriched as compared to total cell lysate (T) and there is minimum cross-contamination between PM and soluble supernatant fraction(s).



Moreover, the spin-column method has been successfully applied in various plant species, including Tobacco (7), Mulberry (8), Cucumber leaves (9), Rosa hybrida cv. Samantha (10), PUT3OE transgenic line (12), Sedum plumbizincicola (13), and soybean (14). PM proteins isolated by the spin-column method have also been analyzed by mass spectrometry in proteomic applications (6, 11, 13, 14).

In summary, the spin-column based technology has proven to be a simple, rapid, and consistent method for plasma membrane protein isolation in plant species, and its effectiveness has been validated by numerous studies conducted across a broad range of plant species.

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