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Application Note

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Vivapure® S Ion Exchange Membrane Adsorbers For the Rapid Fractionation of Peroxidases from Plant Calluses

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Introduction

Ion exchange chromatography is one of the most used techniques in protein biochemistry. However, as a tool for the analysis of multiple samples, there has been a lack of commercially available devices which offer the necessary speed and performance. Recently, our laboratory has become interested in a 40 kDa basic peroxidase from grapevine callus, which has a role in rapid cell wall defence against pathogen ingress. A detailed study of this peroxidase has been limited by its low yield from callus cultures, from which only minor quantities could be purified. We are looking to identify callus cultures that secrete higher levels of this peroxidase and which could be utilized as a more convenient tissue source for the purification of this molecule on a larger scale. We therefore intend to use cation exchange chromatography to isolate this peroxidase from other cell wall peroxidases, and subsequently quantify its abundance in different callus cultures by SDS-PAGE and measurements of peroxidase activity. Our initial assay of different grapevine material utilized conventional SP-Sepharose column chromatography. However, we found this technique to be time-consuming and very few samples could be processed per day. It is also not amenable for the fractionation of low volume samples, which we have instead attempted to process by batch purification with sulphonic acid based resins. In this case, high recovery of target proteins required extensive washing of the resin, resulting in sample dilution.

A recent innovation from Sartorius - Vivapure® - enables ion exchange purification of proteins by centrifuge-assisted passage of samples through membrane adsorbers. These devices are available in two sizes and with a choice of three functional groups, and the 400 µL "Mini" units are ideal for the processing of several small volumes in parallel, using a bench-top centrifuge.

The high binding capacity and 3-5 µm pore size of the membrane adsorbers used in Vivapure® devices, ensures rapid sample processing with high yield and concentration of target proteins. We report here on the use of Vivapure® S devices for the isolation of a 40 kDa peroxidase from grapevine cell walls, and their relevance to rapid screening and detection of this peroxidase in different callus cultures.

Materials and Methods

To isolate cell wall proteins from grapevine cultures, 0.5 g of callus material was incubated in 0.6 mL of 1 M KCl for a short period and then centrifuged for 2 min at 5,000 *g*. The supernatant (500 µL) was concentrated and equilibrated in 20 mM sodium acetate buffer (pH 4.5) using Vivaspin® 500 (10 kDa MWCO PES).

The cell wall extract (250 µL) was loaded into a pre-wetted Vivapure® S device (500 µL capacity) and centrifuged at 10,000 *g* for 5 minutes. Non-bound proteins were removed by applying 500 µL sodium acetate buffer and centrifuging for further 5 min. The membrane adsorber insert was transferred to a fresh tube and fractionated with 500 µL acetate buffer containing 0.3 M NaCl by further centrifugation. A further fraction was obtained by repeating this procedure with buffer containing 0.5 M NaCl. The fractions obtained (0 - 0.3 M and 0.3 - 0.5 M NaCl) were equilibrated in 20 mM sodium acetate buffer (pH 4.5) and concentrated to a final volume of 50 - 100 µL using Vivaspin® 500 devices (10 kDa MWCO PES).

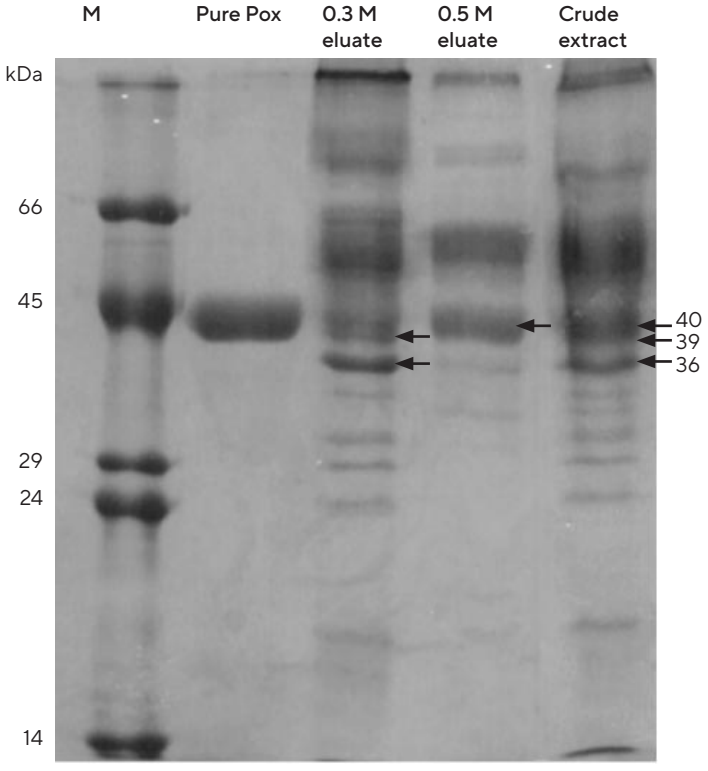
Results

SDS-PAGE analysis of cell wall proteins isolated from grapevine cultures (Figure 1, crude extract), showed that the 40 kDa peroxidase of interest migrated close to two other known peroxidases with molecular weights of 39 and 36 kDa.

Eluent fractions were also analyzed on the same polyacrylamide gel, showing that the contaminating 39 and 36 kDa peroxidases were removed, along with the majority of other cell wall proteins, in the 0 – 0.3 M NaCl fraction (Figure 1, 0.3 M eluate). In contrast, the 0.3 – 0.5 M NaCl fraction (0.5 M eluate) contained comparatively few proteins and was highly enriched with the 40 kDa peroxidase.

Figure 1.

SDS-polyacrylamide gel showing fractionation of cell wall proteins using Vivapure S centrifugal ion exchange membrane devices. Lane M = molecular weight markers.

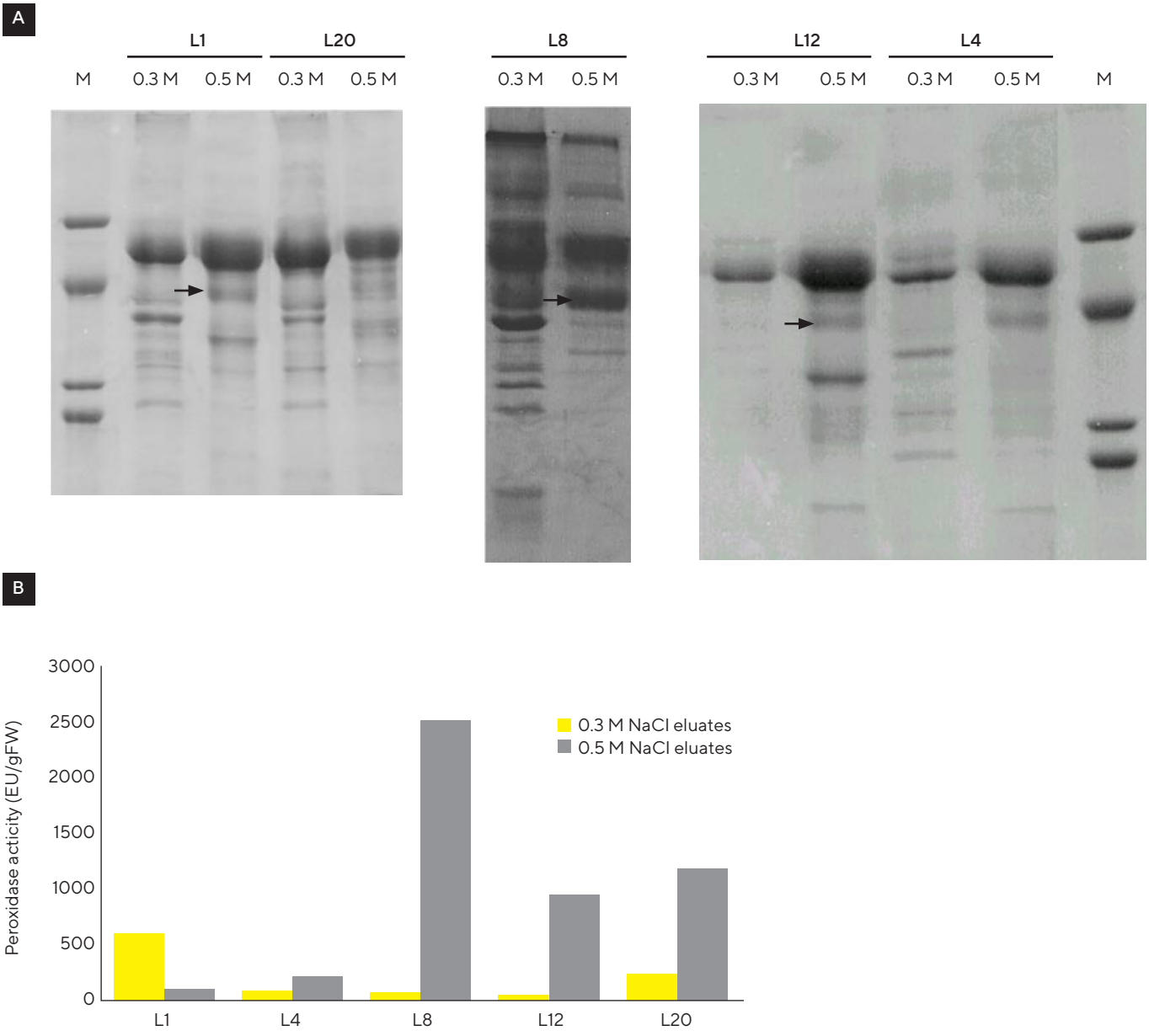


Our initial experiment to determine fractionation conditions demonstrated that we could utilise Vivapure® devices to rapidly obtain a highly enriched fraction of the 40 kDa peroxidase without contamination by other cell wall peroxidases. This enabled us to accurately quantify the abundance of this peroxidase in cell walls through measurements of its peroxidase activity. We therefore applied the same procedure to assay the relative abundance of this peroxidase in several different types of callus derived from grapevine. An analysis of the fractions obtained from some of these cultures is depicted in Figure 2.

SDS-PAGE analysis (Figure 2A) clearly indicates that the abundance of the different peroxidases varies substantially between grapevine cultures. Measurements of peroxidase activity in each fraction (Figure 2B) confirms this. Interestingly, some cultures secreted higher quantities of the 36 and 39 kDa peroxidases, while others secreted very little peroxidase of any type. Several cultures secreting the 40 kDa peroxidase as the major form were identified. Of these, callus line 8 (L8) yielded the highest quantity (enrichment) and activity of this peroxidase.

Figure 2.

A: SDS-polyacrylamide gels showing enrichment of 40 kDa peroxidase (arrows) from 5 different grapevine callus lines (L1, L4, L8, L12 and L20) after stepwise elution with 0.3 and 0.5 M NaCl. M = molecular weight markers. B: Total soluble peroxidase activity is correlated with corresponding callus lines



Conclusions

Our fractionation experiments with Vivapure® S have enabled us to identify an appropriate tissue source (callus line 8, L8) for the large-scale purification of a 40 kDa peroxidase.

We have shown that Vivapure® ion exchange devices can be used to rapidly obtain enriched fractions of target proteins from complex samples, and allow the processing |of multiple samples in parallel over a short period. Sartorius also offers the same devices with different functional groups - S, Q and D - allowing for the adaptation of this technique to proteins with different isoelectric points. In addition, models with sample capacities up to 19 mL facilitate the use of membrane adsorber technology for purification and fractionation of proteins on a larger scale.

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