Purification of Marine Algal Vanadate Bromoperoxidase Dodecamer by Preparative Native PAGE on a BioLogic-Driven Mini Prep Cell

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Introduction

Vanadate-requiring haloperoxidases have been described from red, green, and brown algae (Walker and Butler 1993). We are investigating the role of marine algal bromoperoxidases in marine algal propagule adhesion (Vreeland *et al.* 1995). Purified bromoperoxidase was needed for antibody production and amino acid sequencing. Bromoperoxidase from *Corallina officinalis* is known to have multimeric organization (a 740 kD dodecamer; Sheffield *et al.* 1993). We purified native bromoperoxidase multimer, which was much larger than most proteins in extracts and can be readily detected by activity staining in quantities much smaller than by direct protein staining. Activity staining of analytical gels showed that most of the bromoperoxidase in *Fucus gardneri* and *Corallina vancouverensis* occurred as the multimer, with less than 10% in the monomeric form. Rapid purification was needed to minimize crosslinking of bromoperoxidase to extract component materials (proteins, sulfated carbohydrates, and polyphenols). In addition, minimizing the number of purification steps was required to maximize the yield of this sticky protein. A final preparative electrophoresis purification step removed protein contaminants remaining after ion exchange chromatography with little enzyme loss, and also separated bromoperoxidase multimer from enzyme activity in minor bands and in a high molecular weight smear. Preparative electrophoresis was carried out on the Mini Prep Cell operated by the BioLogic Chromatography System (Figure 1).

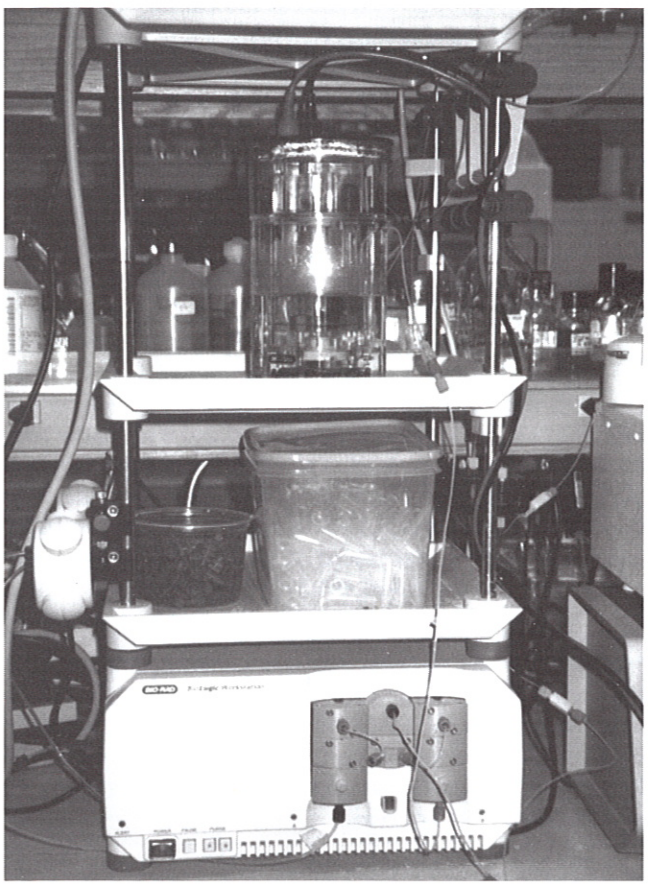

Fig. 1. Mini Prep Cell with elution buffer pumping through Pump B of the BioLogic System.

Method

After extraction of active bromoperoxidase from algal tissue, the enzyme was concentrated in an ammonium sulfate cut (25–90% saturated, or 40–90% saturated, depending on algal source), and desalted and partially purified on an ion exchange column (to be published). Between 95–99% of the 280 nm UV-absorbing material was removed by ion exchange chromatography, and the enzyme was 10–15% of the remaining protein seen on Coomassie® blue-stained mini analytical gels.

Post ion-exchange sample preparation for the Mini Prep Cell included desalting the enzyme by dialysis against 600 mM urea and concentration of the pooled sample (6 ml after the ion exchange run) to 0.5 ml while still in the dialysis tubing. The dialysis tubing was coated with dry polyethylene glycol flakes (Sigma P-2139, 8,000 MW). The dry polyethylene glycol absorbed the liquid and was changed every 10 min until a final

volume of 0.5 ml was obtained. Polyethylene glycol was changed by wiping with a Kimwipe and covering the tubing with fresh polyethylene glycol. It was easier to recover a 0.5 ml sample from one corner of a 2.5 cm flat diameter dialysis tube than from narrower tubing.

Running Conditions

A 2 cm thick stacking gel of 3% acrylamide and a 2 cm thick resolving gel of 4% acrylamide were employed to separate the large enzyme multimer from contaminating proteins, which run at the buffer front on this system. Low ionic strength buffers were used to electrophorese bromoperoxidase (Jordan and Vilter 1990) because bromoperoxidase migrates poorly in standard ionic strength buffers. Buffers used were Tris-HCl, pH 8.0, 10 mM in the stacking and resolving gels and 100 mM in the lower buffer; 10 mM Tris base and 38 mM glycine as the upper buffer; and 600 mM urea as the elution buffer. Urea (0.6–4 M) was added to the gels to prevent enzyme binding to acrylamide. A maximum sample size of 0.5 ml (30–50 µg) in 10 mM Tris, 600 mM urea sample buffer were used. The Mini Prep Cell was placed on a shelf of the BioLogic apparatus, directly above the BioLogic workstation (Figure 1). The elution buffer line from the Mini Prep Cell was connected directly to the inlet of pump B on the workstation, and the B pump outlet was connected directly to the UV detector (bypassing the injector valve). The flow rate of elution buffer was 0.1 ml/min and 0.25 ml fractions were collected. The power source was set at the maximum of 500 V, 5 W, and 10 mA for the 1.5–3 hour Mini Prep Cell run. The Model 491 Prep Cell was run with the BioLogic workstation when larger volumes of sample (4 ml for the smaller-diameter gel and 8 ml for the larger-diameter gel) were used. The Model 491 Prep Cell was placed on a cart immediately in front of the workstation, and the elution line was attached to the inlet of pump B. The buffer recirculating pump was placed on the BioLogic shelf to prevent vibration of the Model 491 Prep Cell on the cart.

The power source was set at 500 v, 10 W, and 40 mA for the Model 491 Prep Cell. Flow rate was 1 ml/min and 1–1.4 ml fractions were collected. The eluted band was recovered in a larger volume for the Model 491 Prep Cell than for the Mini Prep Cell. The gel heights and concentrations, as well as the length of the run, were the same for both the Mini Prep Cell and the Model 491 Prep Cell.

Conclusion

Running the Mini Prep Cell through the BioLogic System was very efficient and provided a printout of the run (Figure 2). The conductivity line showed a two-step decrease when lower molecular weight proteins eluted at the buffer front. Thus, both conductivity and UV detectors provided markers for fractions to test for enzyme activity. No significant UV peak was seen for bromoperoxidase, which eluted after the large protein peak at the buffer front, unless at least several micrograms of bromoperoxidase were loaded on the Mini Prep Cell. Bromoperoxidase was detected by assay of peroxidase activity on dot blots or by antibody binding when inactive. The activity assay utilized o-dianisidine as the substrate (Vilter *et al.*

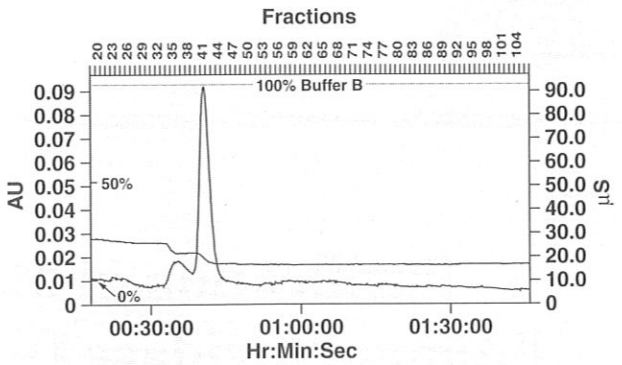

Fig. 2. BioLogic printout of *Corallina vancouverensis* vanadate bromoperoxidase purification on the Mini Prep Cell.

1983) in 100 mM Tris-HCl, pH 8.0, with 1 mM H_2O_2 and 1–10 mM KBr, for a few minutes to overnight.

The purified enzyme was recovered in about six fractions (Figures 2 and 3). Weak activity was sometimes detected in minor bands or in a long tail following the active multimer band, due to enzyme crosslinked to other extract components. When fractions were analyzed on minigels, the first few preparative fractions that contained enzyme activity had sharp bands of enzyme activity, while the activity bands in later fractions were increasingly blurred on the higher molecular weight side due to crosslinking artifacts.

Using the Mini Prep Cell with the BioLogic System was an excellent final purification step for preparing bromoperoxidase multimer in microgram quantities. By immunizing with the first eluted multimer fractions, we prepared polyclonal mouse antibodies specific for the bromoperoxidase multimer and monomer on western blots of crude extracts (to be published).

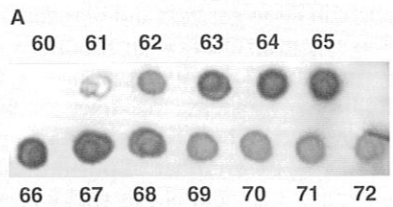

Fig. 3A. Peroxidase activity was detected by o-dianisidine staining of 1 ml aliquots of fractions from the purification shown in Figure 2, dot blotted onto nylon membrane (Biodyne +, Pall).

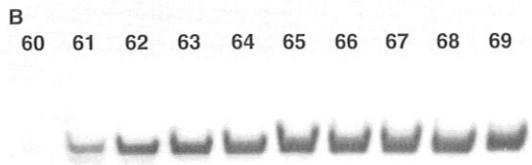

Fig. 3B. Peroxidase activity stained by o-dianisidine on an analytical native urea PAGE gel of Mini Prep Cell fractions. Peroxidase activity bands stained by o-dianisidine.

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