# METHODS FOR THE PARTIAL PURIFICATION OF PEROXIREDOXIN-2 IN PORCINE SKELETAL MUSCLE

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## I. OBJECTIVES

Peroxiredoxins are a family of antioxidant proteins ubiquitously expressed in cells and reduce reactive oxygen species, primarily that are $H_2O_2$. Peroxiredoxins exist as a homodimer with 2 identical subunits involved in the active site of the protein. The catalytic cycle of peroxiredoxins involves a peroxidatic cysteine residue which is oxidized to a sulfenic acid. The peroxidatic cysteine can be further oxidized to a sulfinic acid or inactivated to a sulfonic acid. In postmortem skeletal muscle, peroxiredoxin, specifically peroxiredoxin-2 (PRDX-2), is hypothesized to play a critical role in protecting against oxidative damage by reactive oxygen species. In order to test this hypothesis, it is necessary to use purified PRDX-2 in controlled benchtop experiments. Thus, the objective was to define a method to partially purify PRDX-2 from skeletal muscle.

## II. MATERIALS AND METHODS

Porcine *psoas major*, *diaphragm*, and *longissimus dorsi* were collected at about 45 min postmortem. Muscles were immediately trimmed of visible fat and connective tissue, minced, and stored in −80°C. Each muscle (20 g) was homogenized in 3 volumes of ice-cold, low–ionic-strength buffer (50 mM Tris-HCl [pH 8.5] and 1 mM EDTA) and centrifuged at 10,000 × *g* for 30 min at 4°C. The supernatant was filtered through cheesecloth and dialyzed against 40 volumes of 40 mM Tris-HCl (pH 7.4), 1 mM EDTA, and 0.1% 2-mercaptoethanol (TEM pH 7.4). Dialyzed extracts were clarified by centrifugation and filtration. Clarified dialysates were loaded onto separate 20 mL Q-Sepharose Fast Flow anion exchange columns, equilibrated with TEM pH 7.4. Columns were washed, and proteins were eluted with a linear gradient of 75 to 500 mM NaCl in TEM pH 7.4. PRDX-2 eluted in all muscles from approximately 75 to 160 mM NaCl as confirmed by immunoblot analysis. Monoclonal rabbit anti-PRDX-2 antibody (ab109367; ABCam, Cambridge, UK) was used to detect PRDX-2 abundance. Immunoreactive fractions were pooled, dialyzed against 40 vol of TEM pH 8.0, and clarified by centrifugation and filtration. Clarified dialysates were loaded separately on a DEAE-TSK equilibrated with TEM pH 8.0. Columns were washed and eluted with a linear gradient of 0 to 500 mM NaCl in TEM pH 8.0. PRDX-2 eluted from approximately 35 to 120 mM NaCl as confirmed by immunoblot analysis. Immunoreactive fractions were pooled, dialyzed against 40 vol of TEM pH 8.0, and clarified by centrifugation and filtration. Protein concentration of the resulting dialysates was determined by Bradford analysis. The purity of the dialysates was analyzed by resolving with a 15% SDS-PAGE gel and visualized by Silver staining. Images were captured using a ChemiImager 5500, and whole lane analysis was conducted with Alpha Ease FC software. The percent purity of the extract was measured by comparing the densitometry of the 22 kDa PRDX-2 band to the sum of the total area of individual bands within the lane.

## III. RESULTS

The purity of the muscle extracts was determined to be approximately 45%–50% purity in the *longissimus dorsi* and *psoas major* samples and approximately 30% purity in the *diaphragm* sample.

## IV. CONCLUSION

To our knowledge, this is the first report of a method to partially purify PRDX-2 from skeletal muscle. The methods here describe provide a viable method for the partial purification of PRDX-2 in postmortem skeletal muscle to utilize in controlled in vitro experiments.

Keywords: column chromatography, peroxiredoxin-2, protein purification