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Quantitative Preparative Native Continuous Polyacrylamide Gel Electrophoresis (QPNC-PAGE)

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Introduction

The understanding of processes in living systems depends to a great extent on our ability to isolate bioactive compounds (e.g., proteins) in biological samples for more detailed examination of chemical structure and function. As about 30-40 % of all known proteins contain metal cofactors (e.g., Fe, Cu, Zn, Mo, Ni), especially native metalloproteins have to be isolated, identified and quantified in biomatrices. Many of these cofactors play a key role in enzymatic catalytic processes or stabilize globular protein molecules. Therefore, it is urgently demanded to develop native separation methods (e.g., preparative native PAGE or gel permeation chromatography [GPC]) for separating, e.g., metalloenzymes, metallochaperones or prions in complex matrices (e.g., plant samples or clinical biofluids) [1-3].

Summary

In supernatant of the model plant *Arabidopsis thaliana* bioactive high molecular mass cadmium proteins with molecular mass of about 200 kDa were isolated by using a combination of GPC and quantitative preparative native continuous polyacrylamide gel electrophoresis (QPNC-PAGE). Plant supernatant was first subjected to GPC and the high molecular mass Cd proteins in a GPC fraction of this plant were isolated by QPNC-PAGE. As a result, the Cd proteins were eluted as a single peak in the resulting electropherograms (comp. Fig. 2 and 3). It was proved that the chemical structure (native conformation) of the cadmium proteins of this plant did not change during electrophoresis. Furthermore, the separated metalloproteins were not dissociated into metal cofactors and apoproteins [1- 3].

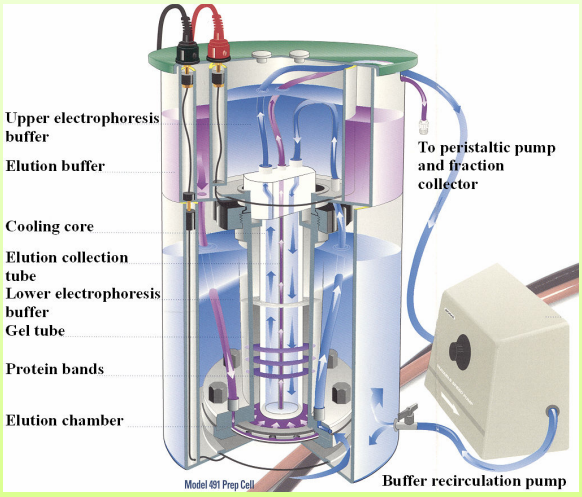

Fig. 1. Electrophoresis chamber *Model 491 Prep Cell* for isolating native metalloproteins in complex biological matrices [1-3].

Methods and Results

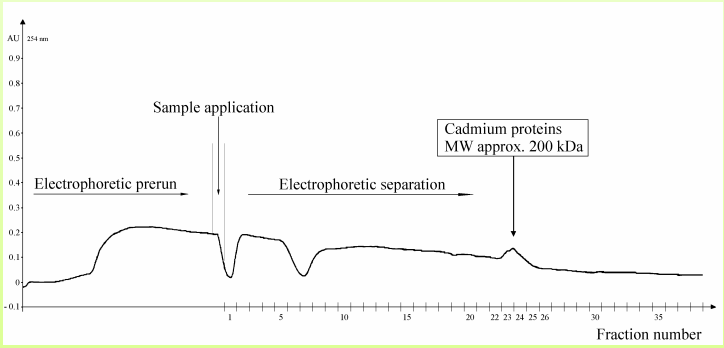

Fig. 2. Electropherogram showing the UV absorption profile of a GPC fraction containing native high molecular mass cadmium proteins of the model plant *Arabidopsis thaliana*. The small UV absorption around PAGE fraction 24 is corresponding with the Cd peak as presented in Fig. 3 [3].

Table 1. The PAGE conditions [1-3]
- Model 491 Prep Cell (all tools listed made by Bio-Rad)
- Electrophoresis buffer: 20 mM Tris-HCl; 1 mM NaN_3; pH 10.00
- Eluent: 20 mM Tris-HCl; 1 mM NaN_3; pH 8.00
- Polyacrylamide gel structure: 4 % T; 2.7 % C
- Gel length: 40 mm
- Inner diameter of the gel column: 28 mm
- Time of polymerization of the gel: 69 h (room temperature)
- Temperature of the separation system: 4° C (constant)
- Power PAC 1000: 5 W (constant)
- Model EP-1 Econo Pump: 1 mL/min; 5 mL/fraction
- Model 2110 Fraction Collector
- Model EM-1 Econo UV Monitor: AUFS 1.0; λ = 254 nm
- Model 1327 Econo Recorder: 100 mV; 6 cm/hr

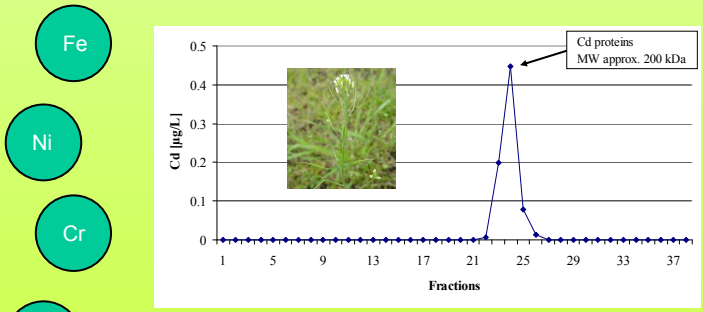

Fig. 3. Electropherogram showing the Cd elution profile (cp. Fig. 2) of a GPC fraction containing native high molecular mass cadmium proteins of the model plant *Arabidopsis thaliana*. Cd concentrations in each fraction were measured by GF-AAS [3].

References:
[1] Kastenholz, B. (2006) Protein Pept. Lett., 13, 503-508.
[2] Kastenholz, B. (2006) Electroanalysis, 18, 103-106.
[3] Kastenholz, B. (2004) Anal. Letters, 37, 657-665.

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