Isolation of Acidic, Basic and Neutral Metalloproteins by QPNC-PAGE

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Abstract: Quantitative **P**reparative **N**ative **C**ontinuous **P**oly**a**crylamide **G**el **E**lectrophoresis (QPNC-PAGE) is a high-resolution method for the preparative isolation of native or active metalloproteins in biological samples. The method is also useful for resolving properly- and improperly-folded metal cofactor-containing proteins or protein isoforms in complex protein mixtures. QPNC-PAGE is based on the use of a commercial preparative-electrophoresis device. Here we present the standard protocol for QPNC-PAGE applicable to the molecular mass range from 6 to over 200 kD.

Keywords: QPNC-PAGE, Model 491 Prep Cell, Metalloproteins, Methodological Strategy

METHOD

-Recommended Equipment and Run Conditions

Model 491 Prep Cell (*Fig. 1*)
Power Pac 1000: Constant Power: 5 W; Time: 8 hr
Model EP-1 Econo Pump: 1 mL/min; 5 mL/Fraction; 80 mL Prerun V; 480 mL Total V (Elution Buffer)
Model 2110 Fraction Collector: 80 Fractions
Model EM-1 Econo UV Monitor: AUFS: 1.0; Detection Wavelength: 254 nm
Model 1327 Econo Recorder: Range: 100 mV; Chart Speed: 6 cm/hr
Model SV-3 Diverter Valve
Buffer Recirculation Pump: Flow Rate: 95 mL/min (Electrophoresis Buffer)

All instruments are from Bio-Rad Laboratories, Inc.

-Stock Solutions

200 mM Tris-HCl 10 mM NaN_3 pH 10.00 - Store at room temperature
200 mM Tris-HCl 10 mM NaN_3 pH 8.00 - Store at room temperature
40 % Acrylamide/Bis 37.5:1 - Store at 4°C
10 % Ammonium Persulfate (APS) - Store at 4°C (freshly prepared)

-Electrophoresis Buffer

20 mM Tris-HCl 1 mM NaN_3 pH 10.00 - Store at 4°C and degas before use
Upper Electrophoresis Chamber (Prep Cell): 500 mL Electrophoresis Buffer
Lower Electrophoresis Chamber (Prep Cell): 2000 mL Electrophoresis Buffer

-Eluent

20 mM Tris-HCl 1 mM NaN_3 pH 8.00 - Store at 4°C and degas before use
Elution Chamber (Prep Cell): 700 mL Elution Buffer

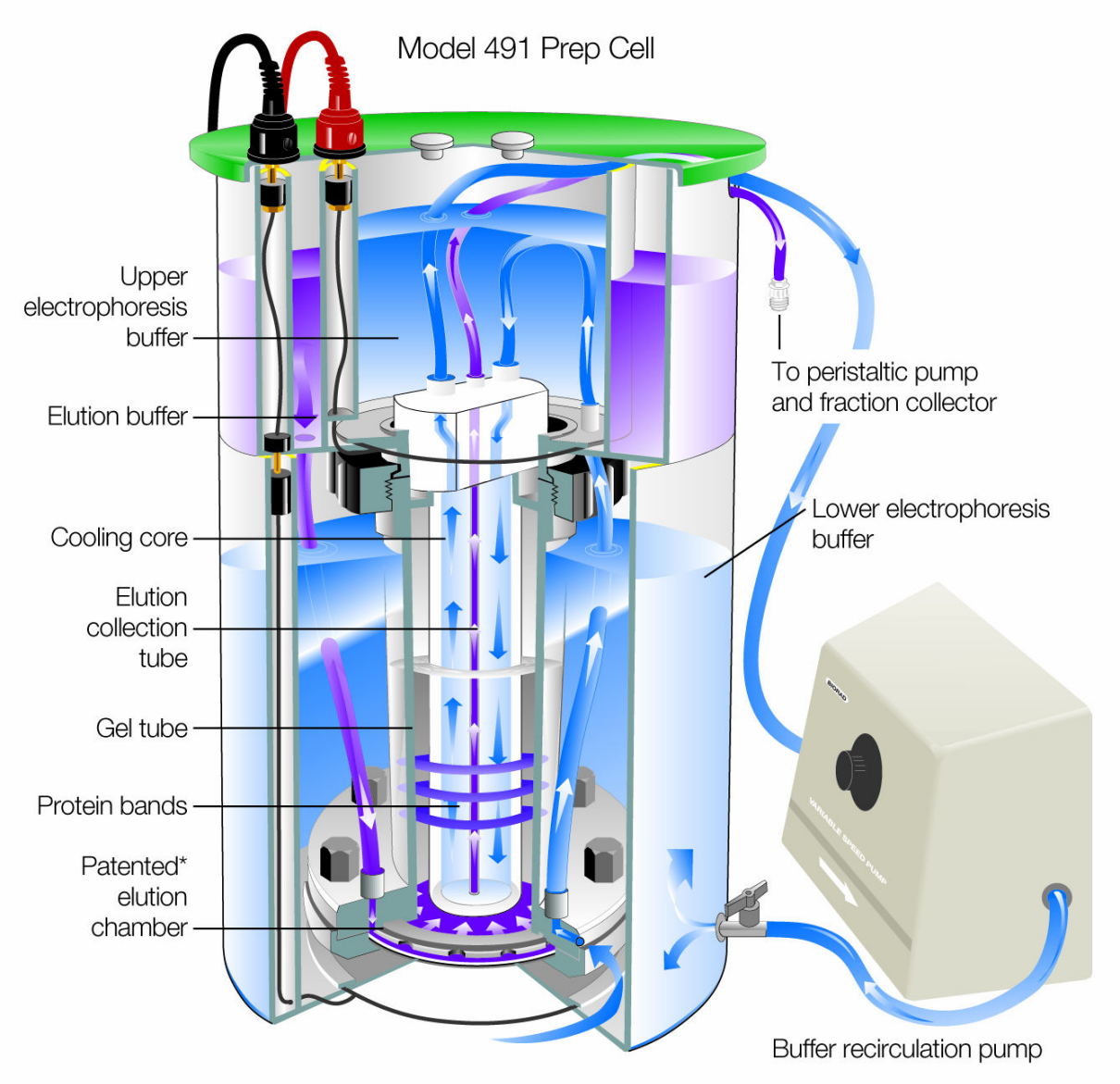

Figure 1: Electrophoresis chamber "*Model 491 Prep Cell*" for isolating quantitative amounts of metalloproteins in complex protein mixtures. This figure is used with the permission of *Bio-Rad Laboratories, Inc.*.

GEL

-Separating Gel

Acrylamide: 4% T - 2.67% C; V = 40 mL:

4 mL 40 % Acrylamide/Bis 37.5:1
4 mL 200 mM Tris-HCl 10 mM NaN_3 pH 10.00
32 mL H_2O
200 µL 10% APS
20 µL TEMED

Use only fresh gels. Add TEMED and APS last. Gently swirl the flask to mix, being careful not to generate bubbles. Pipette the solution to a level of 40 mm in the graduated glass column with an inner diameter of 28 mm (*Model 491 Prep Cell* equipment). Carefully layer 3 mL of 2-propanol on top of the acrylamide solution. After 60 minutes of polymerization time rinse the surface of the gel with electrophoresis buffer and then cover the gel surface with 4 mL electrophoresis buffer. Allow the polymerization reaction to proceed for 69 hr at RT. The heat generated during the polymerization processes is dissipated according to the *Model 491 Prep Cell* instruction manual. There is no stacking gel. At pH 10.0 most proteins are anions.

PROTEIN SEPARATION

Keep liquid samples at 4°C. Gently mix 0.3 mL of glycerol and 2.7 mL of sample containing < 0.5 mg protein. Fill the chambers of the *Prep Cell* with buffer and begin a prerun at 5 W constant power. After 75 minutes interrupt the power for 5 minutes and underlay the sample mixture below the upper electrophoresis buffer on to the gel surface (*Fig. 1*). This gives ample time in which to gently load the sample and for the sample solution to settle on to the top of the PAGE gel. After that begin the electrophoretic separation at 5 W constant power. The protein mixture is separated in ring-shaped protein bands (*Fig. 1*). The separated protein molecules are continuously eluted by the *Prep Cell* into the physiological eluent and transported to a fraction collector (*Fig. 1*). The equipment (except Recorder and Power Pac) is cooled in a refrigerator at 4°C. Protein precipitation or other sample concentration steps should be avoided at all times of the analytical process to avoid the possibility of protein denaturation or aggregation.

QUANTITATION OF METAL COFACTORS

Fe, Cu, Zn, Ni, Mo, Pd, Co, Mn, Pt, Cr, Cd and other metal cofactors can be identified and quantified by ICP-MS (Abbr.: **i**nductively **c**oupled **p**lasma **m**ass **s**pectrometry), for example. Because of high purity and optimized concentration of the native *and* denatured metalloproteins (e.g. Cu chaperone for superoxide dismutase, prions and metalloenzymes) in specific PAGE fractions, the related structures of these analytes can be elucidated by using solution NMR (Abbr.: **n**uclear **m**agnetic **r**esonance) spectroscopy under non-denaturing conditions. This methodological strategy might contribute to the diagnosis and therapy of diseases concerning biometal metabolisms (e.g. Cu, Zn, Fe) and help to develop metal-based medications for the treatment of organisms with protein-misfolding diseases.

EXAMPLE

Figure 2 illustrates the reproducibility that can be expected with QPNC-PAGE. The figure shows the elution profiles of four different runs using gels polymerized for different times. The biological sample was a 200 kD Cd cofactor-containing protein from plant cytosol. Polymerization times were 66, 69, 69, and 93 hours, respectively. Peak-elution times varied over a five fraction range, but the peak shapes are virtually superimposable. The similarities in peak heights and peak widths indicate that the same molecule is being eluted in each case. The reasons for the differences in elution times are unclear, but seem to fall into an acceptable range for this kind of preparative gel electrophoresis. It is possible that the observed elution times reflect variations in polymerization or running conditions, differences in column

heights, or compression of the gels and their pores with the longer incubation times. Modifications to the protein or degradation of the gels are unlikely to have occurred.

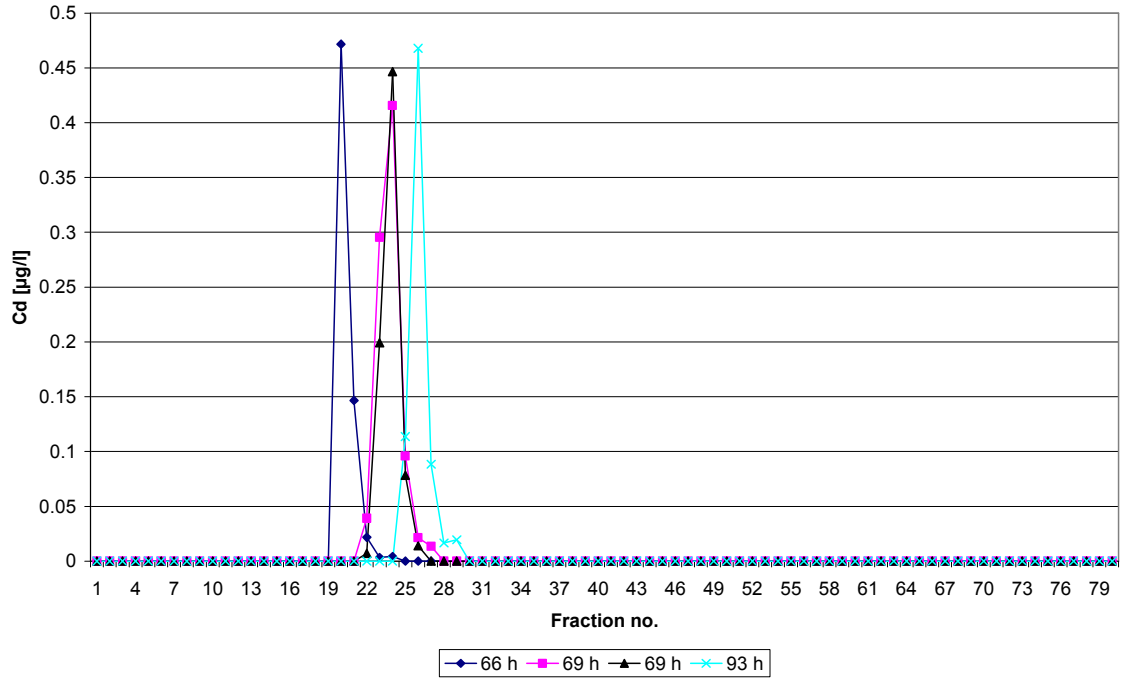

Figure 2: Elution profiles (corrected baselines) of a 200 kD Cd cofactor-containing protein from plant cytosol using PAGE gels polymerized for 66, 69, 69, or 93 hours, respectively. Detection method: Graphite Furnace Atomic Absorption Spectrometry.

REFERENCE

Bernd Kastenholz "METHOD FOR ISOLATING METAL COFACTORS OUT FROM BIOLOGICAL-ORGANIC SYSTEMS INVOLVING THE USE OF PREPARATIVE NATIVE CONTINUOUS POLYACRYLAMIDE GEL ELECTROPHORESIS (PNC-PAGE)" International Patent Application Number: PCT/DE2004/001514; Publication Number: WO/2005/014147; Publication Date: 17 February 2005.

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