FFE Weber GmbH

ProMetHEUS™ FFE

Fractionation of Saccharomyces cervisiae in 8M urea by isoelectric focusing free-flow electrophoresis

Introduction

The preparative separation of proteins is a tedious problem. Existing chromatographic or electrophoretic methods like size-exclusion chromatography or preparative continuous elution PAGE electrophoresis, respectively, are very time-consuming and/or labour intensive. Furthermore, reproducibility is low and quantitative recoveries are rare due to the matrix that is used. Free-flow electrophoresis (FFE) is perfectly suited to solve these drawbacks. The isoelectric focusing separation of proteins under native as well as denaturing conditions guarantees fast, highly reproducible results in combination with nearly complete sample recoveries. To demonstrate the separation qualities of FFE under denaturing conditions, this application note describes the fast separation of *Saccharomyces cervisiae* by isoelectric focusing using media containing 8M urea.

Methods

Sample preparation Saccharomyces cervisiae type II (Sigma) was grounded mechanically under liquid nitrogen. Afterwards, it was suspended in lysis buffer containing urea (7 M), thiourea (2M), CHAPS (4% w/v), DTT (1%), and Pharmalyte® 3–10 (2% v/v). Cells were sheared by passing through a syringe needle and sonication for 15 min. After centrifugation at 25000 g for 1h, the supernatant was diluted 1:4 with separation media (see E6 below). Traces of the red, acidic dye 2-(4-Sulfophenylazo)-1,8-dihydroxy- 3,6-naphthalenedisulfonic acid (SPADNS, Aldrich) were added to ease the optical control of the migration of the sample within the separation chamber. Final protein concentration was approximately 1–2 mg/ml.

Free-flow electrophoresis FFE separations were conducted at 15°C using the following media:

Anodic stabilization medium (I1) 14.5% (w/w) glycerol; 42% (w/w) 8M urea; 0.12% (w/w) HPMC; 100 mM H_2SO_4

Separation medium (I2) 14.5% (w/w) glycerol; 42% (w/w) 8M urea; 0.12% (w/w) HPMC; 14.5% (w/w) Prolyte™ 1

Separation medium (I3-5) 14.5% (w/w) glycerol; 42% (w/w) 8M urea; 0.12% (w/w) HPMC; 14.5% (w/w) Prolyte™ 2

Separation medium (I6) 14.5% (w/w) glycerol; 42% (w/w) 8M urea; 0.12% (w/w) HPMC; 14.5% (w/w) Prolyte™ 3

Cathodic stabilization medium (I7) 14.5% (w/w) glycerol; 42% (w/w) 8M urea; 0.12% (w/w) HPMC; 100 mM NaOH

Counterflow medium 14.5% (w/w) glycerol; 42% (w/w) 8M urea

Anodic circuit electrolyte 100 mM H_2SO_4

Cathodic circuit electrolyte 100 mM NaOH

The experiments were run in a horizontal separation using a 0.5 mm spacer. A flow rate of ~ 75 g/h (Inlet I1-7) was used in combination with a voltage of 1350 V which resulted in a current of 24 mA. Samples were perfused into the separation chamber using the cathodal inlet at ~ 2.0 g/h. Residence time in the separation chamber was ~ 20 min. Fractions were collected in polypropylene minititer plates, numbered 1 (anode) through 96 (cathode).

Data analysis

The pH-values of the individual microtiter plate fractions were measured manually. Horizontal IEF-PAGE analysis of protein fractions was done using a Desaga electrophoresis device HF 210 (Sarstedt) in combination with Servalyt®-based ultrathin polyacrylamide gels (5% T, 3% C). The gels were cast according to the instructions from Serva. The silver-staining procedure was based on the method of Blum (see references).

Results

The ProlytesTM-based separation media containing 8M urea establish a perfectly linear pH-gradient within the FFE separation chamber (Figure 1). This allows the isoelectric focusing FFE separation of the Saccharomyces cervisiae proteins. The silver-stained IEF-gel in Figure 2 exemplifies the fast (residence time only 20 min!) fractionation of the proteins with a nice distribution over the whole pH-range that reflects perfectly the pHgradient in the FFE-chamber.

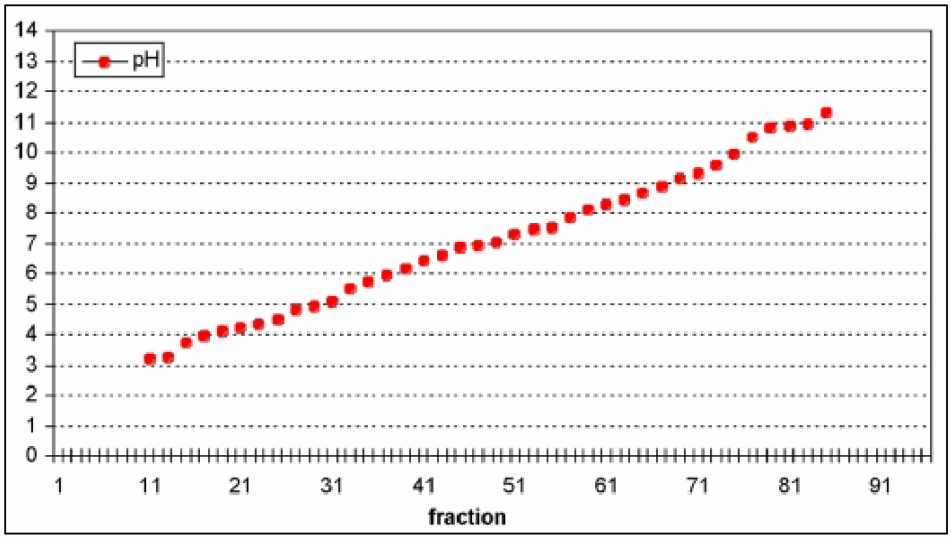

Figure 1: pH-Gradient within the FFE separation chamber.

Figure 2: Silver-stained IEF-PAGE analysis of IEF-FFE-fractionated Saccharomyces cervisiae proteins using 8M urea. Lanes from left to right: Crude sample; fraction 11 (pH = 3.17); 13; 15; ...; 81; 83; 85 (pH = 11.31).

Conclusion

FFE in the isoelectric focusing mode is an indispensable tool for the fractionation of proteins. The absence of any matrix (like a polyacrylamidegel) guarantees highly reproducible results combined with quantitative sample recoveries in a fast and straight forward manner.

References

1) Blum, H., Beier, H. and Gross, H. J. (1987) Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels, Electrophoresis 8 93-99.
2) Weber, G. and Bo_ek, P. (1998) Recent developments in preparative free flow isoelectric focusing, Electrophoresis 19(10) 1649–1653.