Separation of Native Basic Proteins by Cathodic, Discontinuous Polyacrylamide Gel Electrophoresis

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Abstract

An efficient and easy method for separation of native basic proteins (pI >8.0) by polyacrylamide gel electrophoresis is described. In this system, potassium is the leading ion and histidine is the trailing ion, with TAPS (N-tris[hydroxymethyl] methyl-3-aminopropane-sulfonic acid) as the common, buffering ion. Native cationic proteins migrate from the anode to the cathode during electrophoresis. The method was tested with various basic proteins with good resolution.

Introduction

The methods for separating native basic proteins described in the literature have several disadvantages. The discontinuous buffer system for the electrophoresis of native cationic proteins at near-neutral pH described by Thomas and Hodes[4] yields poor resolution (Figure 1). The Blue Native Electrophoresis described by Schägger *et al.*[3] can only be used for basic proteins that bind the blue dye Coomassie® G-250. IEF-PAGE yields satisfactory resolution for proteins with a pI <9.5.

The new system described here offers improved resolution of proteins after separation, does not require binding of dyes, and is applicable also for proteins with a pI >9.5.

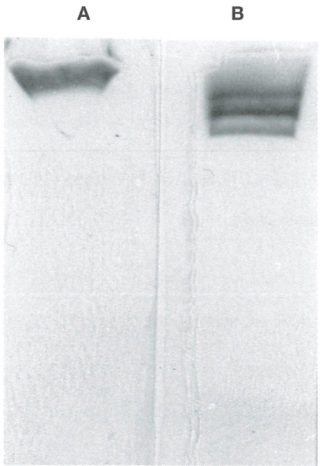

Fig. 1. Comparison of the resolution obtained with system A as described by Thomas and Hodes and with system B, a newly designed system described here. The acrylamide concentrations were 7% each. Each lane was loaded with a peroxisomal preparation containing three isoforms of the enzyme hydroxypyruvate reductase. After activity staining, the isoforms of the enzyme were only detected in lane B.

Methods

ELECTROPHORESIS

The leading ion is potassium, histidine the trailing ion and TAPS the buffer. The composition is presented in Table 1. The electrophoresis runs were performed in a constant voltage mode, starting at 200 V for 20 minutes, followed by 250 V for 40 minutes (the cathode must be at the bottom of the gel). The electrophoresis chamber, Mini-PROTEAN® II cell and the Power Pac 3000 power supply are products of Bio-Rad (München, Germany). L-lactic dehydrogenase from rabbit muscle (pI 8.6, 146 kDa), three isoforms of lentil lectin from lens culinaris (pI 8.7, 8.5, 8.1, 49 kDa), and trypsinogen from bovine pancreas (pI 9.3, 24 kDa) were used as standard proteins. Pyronine Y (0.3 kDa) was used as tracking dye. Standard proteins were stained with Coomassie blue R-250. The *in situ* assay for peroxisomal hydroxypyruvate reductase was adapted from Titus *et al.*[5]

Results

The specifications of Williams and Reisfeld[6] for discontinuous eletrophoresis buffers are met in this new system. The pH of the separating gel decreases by about 0.5 pH units (pH 7.5 to 6.9) during electrophoresis. The leading ion is K^+. The trailing ion, histidine (pK_{a2} 6.0), is a weak base with a pK_a about 1 pH unit lower than that of the separating gel (pH 7.5) and the pK_a of the buffer (TAPS, pK_a 8.4) is about 1 pH unit higher than that of the separating gel (pH 7.5).

Basic standard proteins were used to determine a molecular mass calibration curve with the system described here using several gels from 5% to 10% acrylamide. The results of the separation using 9% acrylamide are given in Figure 2. The results of the relative mobility versus gel concentration and the negative slope versus molecular mass are shown in Figure 3A and Figure 3B, respectively, as described by Hedrik and Smith.[1]

Discussion

Positively charged enzymes dominate in plant peroxisomes.[2] These proteins often have two or more isoforms with minor differences in charge. For isolation and characterization it is necessary to separate the enzymes by electrophoresis followed by electro-elution. We separated a peroxisomal preparation on the electrophoresis system described here. This preparation contains the enzyme hydroxypyruvate reductase with a pI >10.

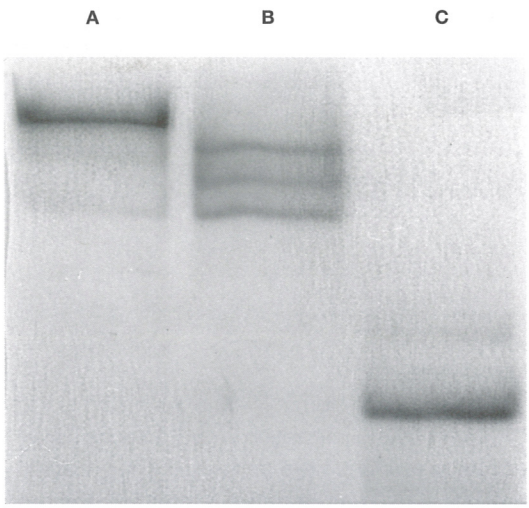

Fig. 2. Native polyacrylamide gel electrophoresis (9% T) of three basic standard proteins. A: L-lactic dehydrogenase (pI 8.6, 146 kDa) B: Lentil lectin (pI 8.1, 8.5, 8.7, 49 kDa) C: Trypsinogen (pI 9.3, 24 kDa) Each lane contained 10 µg of protein and was stained with Coomassie dye.

Table 1. Composition of Gels and Electrode Buffer

	5%	10%	15%
H_2O	3,095 µl	2,470 µl	1,850 µl
TAPS 1.5 M pH 7.5 (adjusted with KOH)	1,250 µl	1,250 µl	1,250 µl
Acrylamide-bisacrylamide (40% solution, ratio 29:1) Solution was degassed for 15 minutes <120 mbar	625 µl	1,250 µl	1,825 µl
TEMED[1]	2.8 µl	2.8 µl	2.3 µl
APS[2] (10% w/v solution)	28 µl	28 µl	23 µl

Electrode buffer: 100 mM histidine–20 mM TAPS (cathode and anode)
pH: about 7.6 (do not adjust)

1 *N, N, N', N' -tetramethylethylenediamine*
2 *Ammonium persulfate*

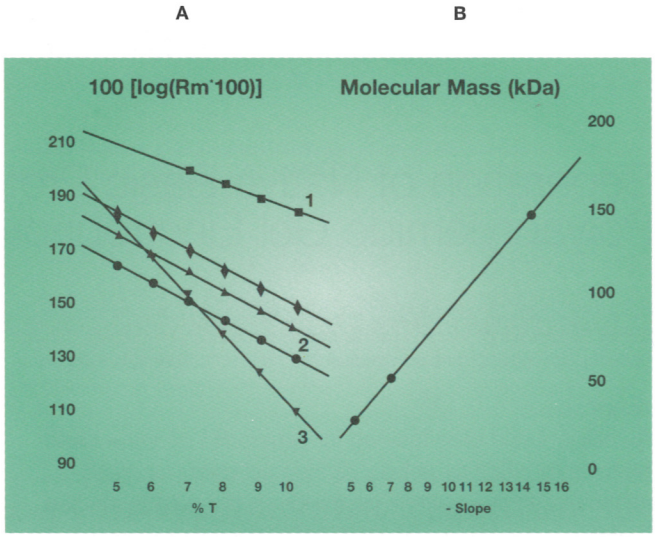

Fig. 3. A. Relative mobility of standard proteins versus polyacrylamide concentration. 1. Trypsinogen, 2. Lentil lectin (three isoforms), 3. L-lactic dehydrogenase. B. Molecular mass versus negative slope of the results shown in A.

Three isoforms of the hydroxypyruvate reductase were detected on the gels after activity staining *in situ* (Figure 1B). The system described by Thomas and Hodes[4] was found to be inadequate for effecting the separations required for peroxisomal proteins (Figure 1A).

The system described here offers improved resolution of cationic proteins and their isoforms. Applying the TAPS buffer system gives access to a broad range of isolation procedures for proteins which cannot be separated by IEF-PAGE or do not bind the blue dye Coomassie G-250.

References

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