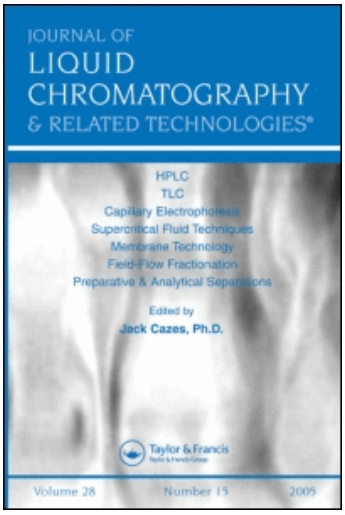

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Rapid Analysis of Protein Fractions From the Hpec™ Using Capillary Electrophoresis

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RAPID ANALYSIS OF PROTEIN FRACTIONS FROM THE HPEC™ USING CAPILLARY ELECTROPHORESIS

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ABSTRACT

Analytical tube and slab gel electrophoresis has frequently been used to monitor protein purification procedures. The ABI Model 230A HPEC™ micropreparative electrophoresis system was used to separate carbonic anhydrase and fractions were analyzed for purity using the ABI Model 270A CE system and compared to silver stained slab gels. Capillary electrophoresis provides a simple, rapid method for the analysis of protein samples.

INTRODUCTION

Protein purification has utilized many different technologies including low pressure chromatography, HPLC, affinity chromatography, preparative electrophoresis, differential precipitation and crystallization, however, following each of these procedures electrophoresis is often used to check the purity of the isolated material. Generally these methods have involved analytical tube or slab gel

electrophoretic systems. Capillary electrophoresis has been shown to be a rapid method for the separation of proteins and peptides (1,2). We evaluated the use of capillary electrophoresis as an analytical tool to evaluate the purity of fractions isolated by automated preparative electrophoresis. The results obtained from capillary electrophoresis were compared with the results from silver stained native slab gels for the same isolated fractions.

MATERIALS AND METHODS

Native gel electrophoresis was performed on the Model 230A HPEC™ (3,4), a continuous elution micropreparative electrophoresis instrument. 50 µg of bovine carbonic anhydrase (Sigma C-7500) was separated using a 5% T, 2.6% C gel (2.5 X 50 mm gel tube). Elution of peaks was monitored at 280 nm and five minute fractions (125 µL) were collected.

Fractions were evaluated, directly from the Model 230A without any additional treatment of the sample, using the Model 270A Capillary Electrophoresis System. In cases where the protein concentration was such that a 1 second injection resulted in offscale peaks, the fraction was diluted in distilled water. A 0.025 M tris - 0.192 M glycine buffer (upper buffer for the Model 230A) was used for the CE analysis. The capillary column was conditioned with 1M NaOH for 10 min and was maintained at 30°C ± 0.1 C. The Model 270A was programmed to perform the following automated analysis cycle: 4 min wash with 0.1 M NaOH, 5 min wash with tris-glycine buffer described above. The samples were introduced to the capillary via vacuum injection at 5" Hg for between 1 - 15 seconds (4 nL/sec).

Polarity was programmed with the anode at the injection point. The electrophoresis was run at 10 kV for 12 minutes. The detector monitored the eluant at 200 nm at a risetime of 0.5 seconds with a range of 0.01 AUFS. Resulting electropherograms were processed with an integrator.

Native protein slab gel (7.5%) electrophoresis was performed in a Hoefer SE 275 Mighty Small™ gel apparatus using the same formula as used for the HPEC™ gels. Samples were prepared in the HPEC™ sample buffer with bromophenol blue and separated in a discontinuous mode. Slab gels were silver stained by the method of Merril et al (5).

RESULTS

Figure 1 compares the electropherograms of carbonic anhydrase separated on the Model 230A HPEC™ (figure 1A) and the Model 270A CE (figure 1B). Although the order of peak elution on the two instruments is reversed, the patterns are essentially identical with three clearly resolved peaks. Quantitation of the peaks separated by CE showed that the third peak represented approximately 3-4% of the total material.

Fractions isolated from the Model 230A, as shown in the electropherogram (figure 1A) were further analyzed on the Model 270A CE for purity. Figure 2 shows the peak fraction for each of the three peaks separated by the Model 230A, and as can be seen, each of the peak fractions contain a single purified species. Even though the first peak separated by the HPEC™ represented only 3 - 4% of the starting material and has been diluted into approximately 250 µl of

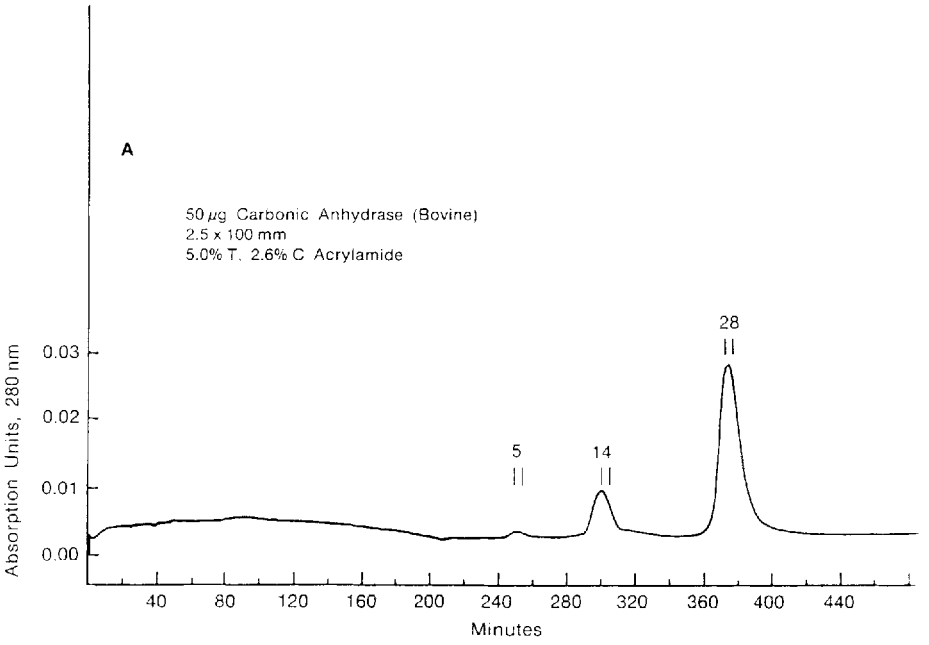

FIGURE 1. Electropherograms of carbonic anhydrase (bovine). FIGURE 1A. Model 230A HPEC™ electropherogram showing the separation of 50 µg carbonic anhydrase. Fractions indicated were analyzed for purity by CE (Figure 2) and native protein slab gel electrophoresis (Figure 3).

elution buffer (2 fractions), it was still readily detectable by the CE (figure 2A) using a 15 second injection (60 nl).

Model 230A fractions analyzed by CE were also characterized using native slab gel electrophoresis stained with silver. The native slab gel (figure 3) also separates the carbonic anhydrase sample into three bands (lanes 1 and 5). Native slab gel electrophoresis also showed that the three fractions each consisted of only a single protein species (figure 3). For the first peak isolated on the Model 230A, 20 µl of sample

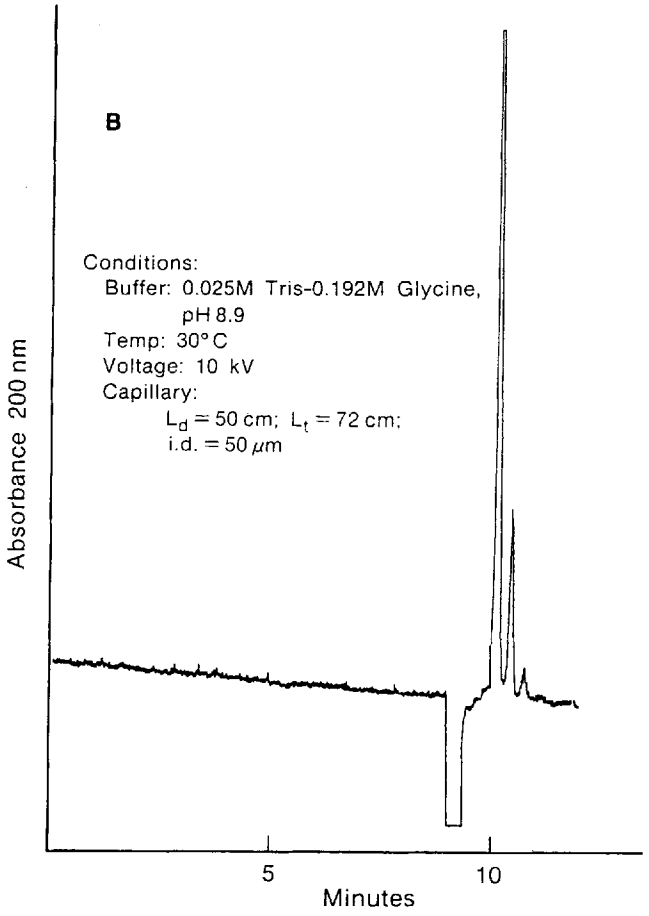

FIGURE 1B. Model 270A CE electropherogram showing the separation of carbonic anhydrase.

was required for the slab gel analysis compared to the 60 nl used for the CE analysis. The other peaks required less material for both types of analysis but capillary electrophoresis consistently required 300 - 500 fold less sample than slab gel electrophoresis.

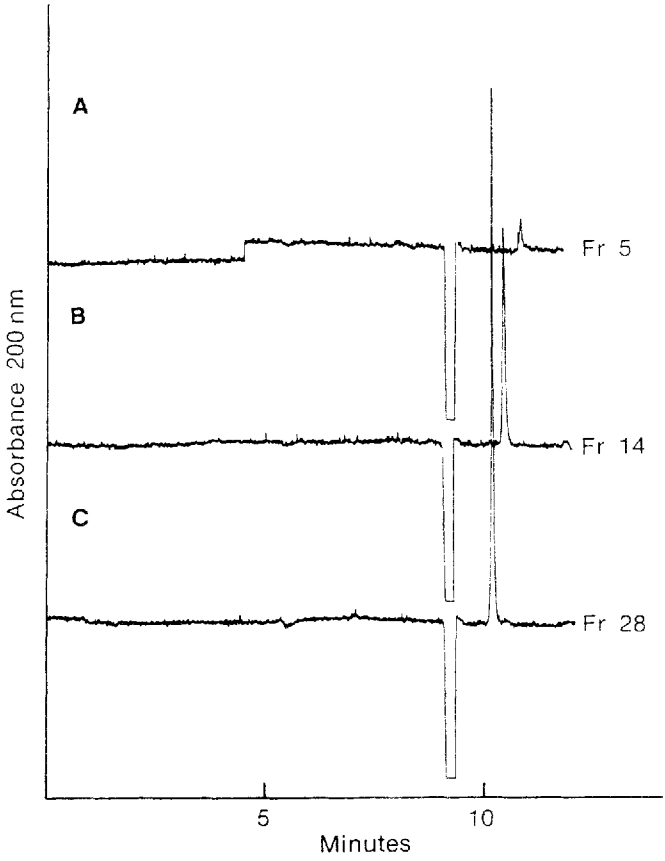

FIGURE 2. Model 270A CE electropherograms of HPEC™ isolated fractions from the isolation of carbonic anhydrase as shown in Figure 1A. Electrophoretic conditions were the same as shown in Figure 1B. A. HPEC™ fraction 5. B. HPEC™ fraction 14. C. HPEC™ fraction 28.

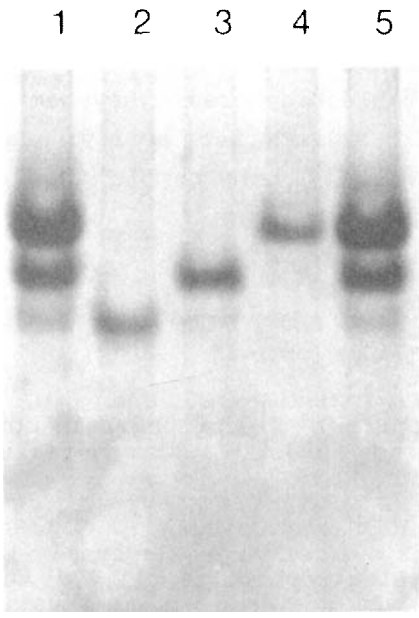

FIGURE 3. Photograph of a native protein slab gel (7.5% T, 2.6% C) of carbonic anhydrase and HPEC™-isolated fractions stained with silver (5). Lanes 1 and 5. Carbonic anhydrase. Lane 2 HPEC™ fraction 5. Lane 3. HPEC™ fraction 14. Lane 4. HPEC™ fraction 28.

DISCUSSION

Analysis of purity is a critical step in the purification of proteins and peptides. Frequently tube or slab gel electrophoresis is used to monitor the purification of proteins and peptides. The short separation times typically found when using CE (1) provides a rapid means for the characterization of isolated fractions during the purification of proteins. The reduced sample requirements also maximizes the sample for any further purification steps that may be necessary.

Direct CE analysis of fractions isolated electrophoretically using the Model 230A HPEC™ micropreparative electrophoresis system provides a simple and rapid method for purity analysis while requiring substantially less sample than conventional slab gel electrophoresis.

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