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Short communication

Identification and quantitation of human urine proteins by capillary electrophoresis

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Abstract

Capillary electrophoresis, as a fully automatable, powerful analytical technique, has the potential for use in clinical laboratories for separation and identification of protein types in normal and abnormal human urine specimens. Abnormal urine specimens containing previously identified proteins, anion-exchange resin treatment and addition of known components were used to identify and determine migration times of the peaks found in the electropherogram of human urine. The correlation coefficients between capillary electrophoresis and the currently used method of high-resolution agarose gel electrophoresis for Bence Jones protein and albumin were found to be 0.95 and 0.93, respectively.

1. Introduction

The types and amounts of the various proteins present in urine have significant medical significance. In particular, the identification of Bence Jones protein (BJP) is important in the assessment of patients with multiple myeloma, a disorder characterised by the presence of monoclonal protein in their serum. This has been achieved routinely by concentrating urine from these patients and then electrophoresing the concentrated urine on high-resolution agarose gel.

Capillary electrophoresis (CE) is a new technique which allows separations in a buffer-filled, thin-diameter capillary [1]. The automation of the instrumentation has allowed the potential introduction of the technique into clinical laboratories where high throughput is a necessity.

Previous attempts to identify urine proteins by CE have involved the preliminary use of dialysis membrane with a molecular mass cut-off of 14 kDa. The dialysate was then electrophoresed, using UV absorbance detection at 214 nm or 254 nm [2]. The large number of peaks were assumed to be due to small molecules and protein breakdown products, probably peptides. Another group of workers showed urea and creatinine to co-elute, but attempts to identify other urinary constituents by CE were unsuccessful at that time [3]. Jorgensen and Lukacs labelled primary amine-containing compounds in urine with fluorescamine, but were unable to identify the peaks involved [4].

Our aim was to directly quantify the concentrations of albumin and BJP, and identify

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other peaks following CE of human adult urine specimens.

2. Experimental

An Applied Biosystems capillary electrophoresis system Model 270A-HT instrument (AB Foster City, CA, USA) with a standard 72 cm × 50 μm I.D. fused-silica capillary was used for separation. Detection was performed at the cathodic end by on-column measurement of absorbance at 200 nm. For quantitation of Bence Jones protein the area under the curve of the CE electropherogram calculated by on-board software provided with the instrument was compared to the densitometric tracing due to BJP obtained by HRAGE (high resolution agarose gel electrophoresis).

Before each run the capillary was sequentially rinsed for 2 min with 100 m*M* sodium hydroxide, 2 min with distilled water and 3 min with assay buffer (150 m*M* boric acid pH 9.7, containing 1 m*M* calcium lactate).

Electrophoretic comparison by HRAGE was performed using prepared gels (Helena Titan High-Res, Cat. No. 3040; Beaumont, TX, USA). The gels were used according to the manufacturer's instructions with the exception that staining was carried out using 0.2% amido black. The urine electropherogram was scanned using a Helena Cliniscan 2 densitometer at 610 nm.

Urine was concentrated 100-fold using Minicon Macrosolute B15 urine concentrators (Amicon Division, W.R. Grace and Co., Beverly, MA, USA) and then diluted 1:50 in assay buffer. The sample was introduced into the capillary by a five-second $1.27 \cdot 10^2$ mm vacuum injection. Electrophoresis was performed for 18 min by applying 18 kV constant voltage. For urine samples with a protein content greater than 5.0 g, we found that decreasing the sample injection time to 20% of that used for other urines aided separation of intact immunoglobulin and free light chains.

To remove anionic species from specimens a 1-g amount of Dowex 2 strongly basic anion-exchange resin (Sigma, St. Louis, MO, USA) was measured by weight and suspended in 1 ml of deionized water. Equal volumes of urine and resin were added to a conical tube and mixed by inversion. After 30 min the mixture was centrifuged at 9500 *g* for 2 min. Forty microlitres of the supernatant were added to 60 μl of assay buffer, and sampled by the instrument in a manner identical to that used for the concentrated urine. Control urine specimens, not submitted to anion-exchange treatment, but diluted accordingly with assay buffer were also sampled for comparison. Overall, 71 urine samples from 30 different myeloma patients were examined by both HRAGE and CE.

3. Results

The quantitative albumin estimations obtained by CE were compared to those obtained from the densitometric tracing of HRAGE. The regression plot of these results is shown in Fig. 1. The coefficient of correlation was 0.93, with a slight positive bias. The regression plot of the

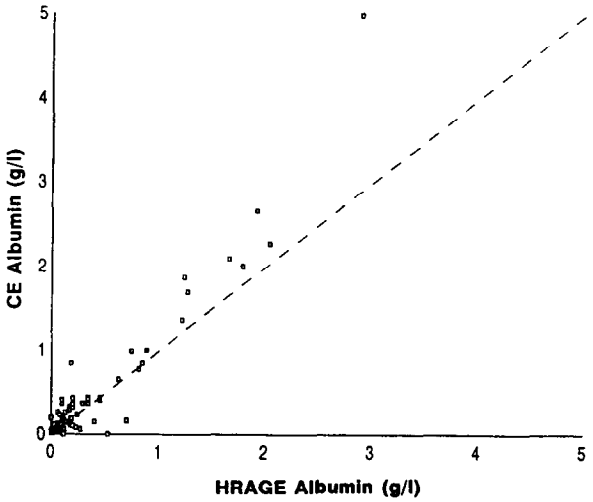

Fig. 1. Quantitative comparison of albumin of 71 urine samples by CE versus densitometric measurement of bands by HRAGE. $y = 1.25x + 0.000$ (as determined by the method of Passing et al. [5]), $r = 0.93$.

quantitation of BJP by CE and HRAGE is shown in Fig. 2. The coefficient of correlation was 0.95.

The location of Bence Jones proteins on HRAGE is known to vary between the alpha 2 region and the slow gamma area. The migration time of these proteins on CE was also found to be variable, ranging between 8.0 and 9.2 min.

Unmodified urine, when treated with strongly basic anion-exchange resin showed a significant reduction in the number of peaks detected. For urine specimens with normal total protein content the number of peaks detected was reduced from 7 to 2 (see Fig. 3).

The migration times of other peaks present in the electropherograms, determined by either standard addition or comparison with known protein containing specimens, are summarised in Table 1.

4. Discussion

Human urine from normal healthy individuals contains small amounts of albumin and other proteins, as well as a large number of small molecular mass molecules. The use of urine concentrators with a M_r cut-off of 15 kDa aided in the identification of protein components by eliminating the majority of smaller species and simplified the identification of the larger molecular mass proteins present in urine.

Previous studies have identified the problem of adsorption of positively charged areas of the proteins to the negatively charged capillary wall when proteins were analysed by CE. This caused reduced resolution and decreased reproducibility. Methods to eliminate this adhesion of proteins to the wall have included the use of buffer at pH extremes, such as pH higher than 9.0 or

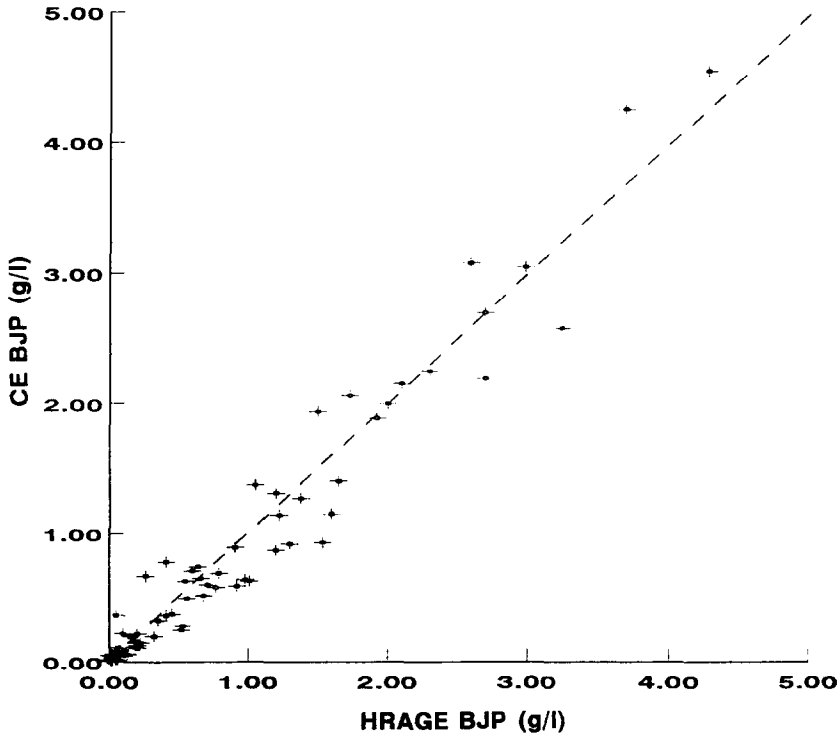

Fig. 2. Quantitative comparison of Bence Jones protein of 71 urine samples by CE versus densitometric measurement of bands by HRAGE. $y = 0.97x + 0.000$ (as determined by the method of Passing et al. [5]), $r = 0.95$.

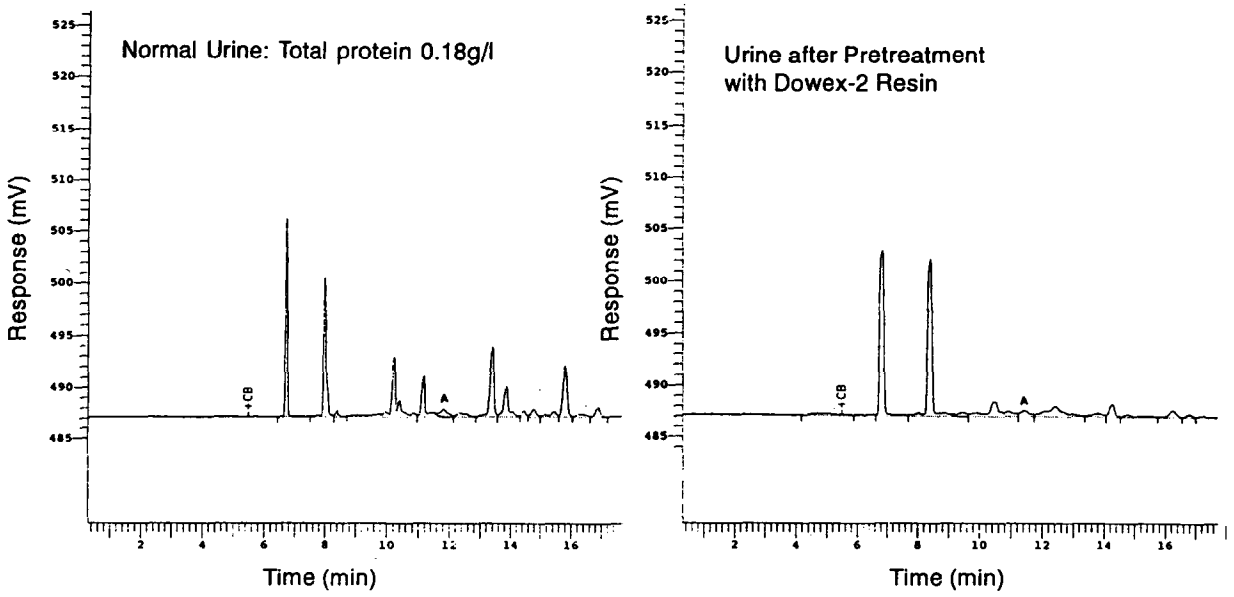

Fig. 3. Capillary electropherogram of normal human urine before and after pre-treatment with Dowex-2 resin. Albumin peak indicated by A at approximately 11.6 min. Bence Jones protein is not found in normal urine specimens. UV detector trace obtained using 18 kV and 54 μA.

lower than 2.5 [6,7]. To facilitate elimination of protein adhesion of the urine to the capillary wall we chose borate buffer at pH 9.7. The use of a buffer with an ionic strength three times higher than that used for serum protein separation [8] aided in the elimination of protein adhesion to the wall of the capillary.

5. Conclusions

CE is an extremely sensitive technique, only nanolitres of sample being required. The high voltages used (>10 kV) produce rapid separations (<20 min) and high separation efficiencies (number of theoretical plates 10^6)

Table 1
Migration times for protein and non-protein components of human urine determined by addition of known substances to boric acid buffer containing 1 m*M* calcium lactate

Compound	Migration time (min)	95% Confidence limits (min)
Pre-albumin	13.8	13.7–14.0
Albumin	11.7	11.5–11.9
Alpha-1-acid glycoprotein	10.5	10.3–10.6
Transferrin	9.0	8.9–9.3
β-Microglobulin	8.3	8.2–8.4
Uromucoid	8.2	8.2–8.4
Immunoglobulin light chains	8.0–9.2	
Urea	6.8	6.7–6.9
Creatinine	6.8	6.7–6.9
Phosphate	14.0	13.9–14.2
Ascorbate	16.0	15.8–16.3
Oxalate	15.0	14.7–15.3

The major advantage of CE over HRAGE for urine analysis relates to the automated nature of CE. The time and skill needed for running, staining, drying, destaining and densitometer scanning of gels is eliminated.

The speed, resolution and automation of CE has allowed it to be introduced as a routine tool in the clinical laboratory. We have been able to further identify peaks in the electrophoretogram produced by capillary electrophoresis of human urine and validate the correlation of urine albumin and light chains between CE and HRAGE.

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