Cellulose Acetate and Polyacrylamide Gel Electrophoresis for Quantification of Milk Protein Fractions

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ABSTRACT

Cellulose acetate electrophoresis and PAGE techniques were compared for fractionation and quantification of milk proteins. Protein bands were stained with Ponceau-S and aniline blue black in the cellulose acetate electrophoresis and PAGE, respectively. αs1-Casein and β-casein absorbed almost equal quantities of Ponceau-S per unit weight, whereas β-casein absorbed more aniline blue black per unit than did αs1-casein. β-Lactoglobulin, α-lactalbumin, and bovine serum albumin absorbed equal amounts of Ponceau-S per unit but differed in absorption of aniline blue black. It was concluded that cellulose acetate electrophoresis was the method of choice for rapid fractionation and quantification of milk proteins.

INTRODUCTION

Several methods have been employed to fractionate milk proteins for identification and quantification (1, 3). The PAGE (4, 6, 7) and starch gel electrophoretic techniques have been used widely but are too time consuming for rapid separation and quantification of large numbers of samples. Electrophoresis of casein (9) and whey protein (1, 5) on cellulose acetate membranes was successful. Electrophoresis of milk proteins on cellulose acetate has eliminated some of the disadvantages of PAGE. This study was conducted to compare cellulose acetate electrophoresis (CAE) with PAGE to fractionate and quantitate milk proteins.

MATERIALS AND METHODS

Preparation of Casein and Whey Proteins

To 10 ml skim milk at 40°C, 1 ml of 1% (vol/vol) acetic acid was added, and after 10 min 1 ml of 1 N sodium acetate was added at the same temperature. The sample was cooled to room temperature and centrifuged at 3800 x g for 30 min. Serum containing whey protein was decanted, measured, and retained for further analysis. The casein precipitate was washed twice with 10 ml distilled water and centrifuged at 3800 x g for 20 min; water was discarded and casein was dissolved in .4 M Tris, 7 M urea buffer, pH 8.2, to a volume of 10 ml and allowed to set at 5°C until it dissolved.

Polyacrylamide Gel Electrophoresis

The equipment used included a vertical gel electrophoresis cell, power supply, and circulating pump, (E-C Apparatus Co., Philadelphia, PA).

Fractionation of Casein. Casein was partitioned by a modified procedure (4). Modifications were 1) .5 ml casein solution was used instead of skim milk; 2) dye used was aniline blue black (ABB); and 3) the gel composition was 12.83 g acrylamide, .64 g bisacrylamide, 31.47 g urea, .3 g ammonium persulfate, dissolved in 130 ml Tris working buffer with six drops of mercaptoethanol added. Polymerization was initiated by adding .2 ml of N',N',N',N'-
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tetramethylethylenediamine to the gel solution. Gel was stained in ABB solution (2 g ABB, 250 ml methanol, and 250 ml distilled water for 15 min; destaining was performed by manual agitation in trays for 1 or 2 d.

Fractionation of Whey Protein. Whey proteins were partitioned by a procedure modified from that at Morr and Manning (6). Gel composition was 14.26 g acrylamide, .74 g bisacrylamide, and .3 g ammonium persulfate dissolved in 150 ml (12 ml stock veronal buffer diluted with distilled water to 150 ml). Polymerization, staining, and destaining were as with casein.

Cellulose Acetate Electrophoresis

Buffers used in fractionation were as described by West and Towers (9), and whey proteins were partitioned by a procedure modified from that of Bell and Stone (1). Casein and whey protein fractionations were conducted in a Beckman (Fullerton, CA) microzone cell using cellulose acetate (CA) as a carrier membrane (13 μ in thickness and pores 2 μ in diameter, obtained from Beckman). A commercial barbital buffer (Beckman, B-2), pH 8.6, was used for whey protein fractionation. A sample applicator (Beckman) was used to apply .25 μl to the CA. For casein and whey protein fractionation, 225 V of constant current was applied from a Beckman power supply for 45 min. The apparatus was kept at 3°C while in use. Ponceau-S (Gelman Scientific Inc., Ann Arbor, MI) was used to stain the membranes. Staining was conducted according to Bell and Stone (1), and the stained membranes were destained in several washings of 5% acetic acid until the background became clear, which took about 5 to 15 min. Then the membranes were air dried and transferred to transparent plastic envelopes.

Scanning

The protein bands on CA (cellulose acetate) and PAG (polyacrylamide gel) were scanned on a Beckman densitometer (Beckman CDC system, model R-112, scanning densitometer permanently mated to a model R-115 computer). Protein bands on CA were scanned at wavelength 520 nm and PAG at 600 nm. The various densitometer peaks for both methods were identified through comparisons with purified αS1- and β-casein (US Biochemicals Corp., Cleveland, OH) and κ-casein (prepared by R. Jenness); β-lactoglobulin (A and B), α-lactalbumin, bovine serum albumin, and immunoglobulins (Sigma Chemical Co., St. Louis, MO). The printout from the densitometer identified the proportional parts of the total area for each fraction in a mixture. In following discussion, values of αS-casein include, αS0-, αS1-, αS2-, and αS3-caseins. After protein bands were isolated by electrophoresis they were stained, and these stained bands were eluted from CA according to Mhatre et al. (5) and from PAG according to El-Shibany and El-Salam (3), and absorbance of the dye was measured on a colorimeter at a wavelengths of 520 and 600 nm for CA and PAG, respectively.

Evaluation of the Cellulose Acetate and Polyacrylamide Gel Electrophoretic Methods

Precision. One hundred twelve milk samples in duplicate were electrophoretically fractionated by both methods. Precision of an analytical value is measured by the deviation of that value from the mean value of replicated observations. Average precision, in this study, was the average deviation of individual results and is expressed as parts per thousand as advocated by Pierce and Haenisch (8). The smaller the value, the higher the precision.

Accuracy. Several known concentrations of αS1- and β-casein; β-lactoglobulin B, α-lactalbumin, and BSA were analyzed by both systems. Five casein samples with known fractional values were made available (D. T. Davies, Hannah Research Institute, UK). They had been prepared and analyzed by ion-exchange and gel chromatography (2). These samples were analyzed by CAE.

RESULTS AND DISCUSSION

Precisions of analyses for αS1-, β-, and κ-casein; β-lactoglobulin, α-lactalbumin, and BSA for CA were 20, 55, 257, 24, 40, and 500 parts per thousand; and for PAG were 10, 28, 113, 24, 40, and 158 parts per thousand, respectively. Neither method had the highest precision for all fractions, but PAG generally was more precise. Smaller fractions of milk proteins resulted in poor precision by both methods.
The results from the two methods differed drastically for both groups of proteins (Table 1). Each value for one method differed from the counterpart value by the other method (P<.05). Linear relationships were observed between concentrations of \( \alpha_{\text{s1}} \) and \( \beta \)-casein and areas under curves when CAE was used. No such relationship was observed with PAGE (Figure 1). When protein fractions were eluted from CA and PAG, there were linear relationships between concentration and absorbance for each protein by both methods (Figure 2). However, the Ponceau-S associated with each unit of \( \alpha_{\text{s1}} \) and \( \beta \)-casein was almost equal, whereas more ABB bound to \( \beta \)-casein than \( \alpha_{\text{s1}} \)-casein per unit. This finding was substantiated when equal quantities of both caseins were mixed and analyzed. The CAE method resulted in 48.5% for \( \alpha_{\text{s1}} \)-casein and 51.5% for \( \beta \)-casein and PAGE resulted 44.5 and 55.5%, respectively. Similar results were observed for whey protein fractions (Figures 3 and 4). When equal quantities of \( \beta \)-lactoglobulin, \( \alpha \)-lactalbumin, and BSA in a mixture were analyzed, their average percentages were 34.61, 31.53, and 33.87, respectively, by CAE method and 31.66, 28.1, and 40.27, respectively, by PAGE method. Protein fractions in casein and whey have different dye binding capacities for anionic dyes, depending upon the dye used (6).

The relationship between casein concentration and absorbance in PAGE is in agreement with El-Shibany and El-Salam (3). The results in CAE method are in agreement with the work of Mhatre et al. (5), who observed that the dye binding capacity of the whey protein components for Ponceau-S was approximately the same and a linear relationship was established between the dye uptake and protein concentration. When dyes were switched in CAE and PAGE methods, background destaining was incomplete in both methods.

The values of the casein fractions of the caseins supplied by Davies and Law (2) are in Table 2 along with the results of CAE. The \( \alpha_{\text{s1}} \) and \( \beta \)-casein values were similar by both methods. The CAE values for \( \alpha_{\text{s2}} \)-casein were higher than reported by Davies and Law (2). This was because \( \gamma \)-casein was included in the \( \alpha_{\text{s2}} \)-casein fraction by CAE analysis but was separate by the chromatographic method of Davies and Law (2). \( \kappa \)-Casein was similar in

| TABLE 1. Means and mean square deviations (MSD) of six milk proteins separated by CAE and PAGE (112 samples from 8 cows). |
|------------------------|------|------|------|------|------|------|
| % of Whey protein      | Bovine serum albumin | Bovine serum albumin | Bovine serum albumin | Bovine serum albumin | Bovine serum albumin | Bovine serum albumin |
| % of casein            | MSD  | \( \alpha \)-Lactalbumin | \( \beta \)-Lactoglobulin | \( \kappa \)-Casein | \( \gamma \)-Casein | \( \alpha_{\text{s2}} \)-Casein |
| CAE                    |      | 4.8a | 2.9b | 8.1b | 2.5  | 2.5  |
| PAGE                   |      | 4.8a | 2.9b | 8.1b | 2.5  | 2.5  |

\( \alpha_{\text{s2}} \)=Values within columns not sharing common superscript differ significantly (P<.05).
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Figure 1. Relationship of $\alpha_{SI}$- and $\beta$-casein concentration to the area under curve. CA = Cellulose acetate.

Figure 2. Relationship of $\alpha_{SI}$- and $\beta$-casein concentration to the absorbance of their eluted samples. CA = Cellulose acetate, CAE = cellulose acetate electrophoresis.
Figure 3. Relationship of whey proteins to the area under curve. CA = Cellulose acetate.

Figure 4. Relationship of whey protein fractions to the absorbance of their eluted samples. CA = Cellulose acetate, CAE = cellulose acetate electrophoresis.
four samples but differed considerably in sample 4. Sample 4 was unique in that the CAE method did not detect α_s2-casein but recorded high values for κ-casein relative to what Davies and Law (2) reported.

**CONCLUSIONS**

The precision of CAE analyses of milk proteins was satisfactory but was less precise for casein fractions and one of the whey protein fractions than by PAGE. Smaller fractions were not precisely determined by either method. Values from PAGE and CAE differ for each casein and whey protein fractions. The CAE results were considerably more accurate than those from PAGE. The CAE technique was simple, more rapid, and less expensive than the PAGE method. The method of choice to fractionate and quantitatively analyze milk proteins was CAE.

**REFERENCES**


