Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis of Purified Casein Fractions Treated with Milk-Clotting Enzymes

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ABSTRACT

Acid casein was fractionated into α_s-, β-, and κ-casein and was treated separately with *Mucor miehei* and *Endothia parasitica* enzymes and calf chymosin. The enzyme treated casein fractions were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the electrophoretic patterns were compared with those of untreated casein fractions. All three enzymes showed proteolytic activity, but *Endothia parasitica* enzyme degraded α_s-casein most.

INTRODUCTION

A recently growing cheese industry and increasing scarcity of calf rennet have stimulated a search for milk clotting enzyme from alternate sources. Microbial clotting enzymes especially are favored because they can be mass produced. Many species of filamentous fungi produce chymosin-like enzymes (8). Microbial enzymes commercially used for cheese making are *Mucor pusillus* enzyme (1), *Mucor miehei* enzyme (9), and *Endothia parasitica* enzyme (6). Sternberg (9) pointed our similarities in the clotting mechanism between *M. miehei* enzyme and calf chymosin. Vanderpoorten and Weckx (10) also reported that microbial rennet from *M. miehei* and *M. pusillus Lindt* produced a pattern of hydrolyzed products similar to calf rennin.

This paper reports the results of a comparison between α_s-, β-, and κ-caseins treated with milk clotting enzymes from *M. miehei, E. parasitica,* and the calf abomasum by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis.

Received February 9, 1981.

MATERIALS AND METHODS

Preparation of Acid Casein

Acid casein was prepared from unpasteurized fresh bovine milk as described by Hipp et al. (4). One liter of milk was centrifuged to remove fatty substance and adjusted to pH 4.5 with 1 N HCl. The precipitated casein was separated by centrifugation and washed three times by suspending it in deionized water. The fresh acid casein was used for further fractionation.

Preparation of α_s- and β-Casein

The α_s- and β-caseins were fractionated in aqueous urea solutions from acid casein as described by Hipp et al. (5).

Preparation of κ-Casein

κ-Casein was prepared from acid casein by the method of Zittle (13) and McKenzie and Wake (7). Alternately, κ-casein also was prepared from acid casein by gel filtration as described by Yaguchi et al. (12).

Milk Clotting Enzymes

Purified *E. parasitica* enzyme was obtained from Pfizer Co., SP. Brazil. *M. miehei* enzyme was prepared by incubating solid wheat bran medium, which was inoculated with spores of *Mucor miehei* NRRL 3420. After incubation at 30°C for 3 days, the aqueous extract was purified as described by Sternberg (9). Chymosin was extracted from calf abomasa and purified as described by Berridge (3).

Preparation of Enzyme-Treated Casein Fractions

Fifty milligrams of α_s-, β-, and κ-casein were dissolved in 4 ml of deionized water with .2 N NaOH added. After dissolution of caseins, solutions were adjusted to pH 6.8 with .2 N HCl and diluted to 5 ml with deionized water. Each solution of α_s-, β-, and κ-casein was mixed with 100 Soxhlet units (one Soxhlet unit is defined as the enzyme activity which clots 1 ml of substrate in 40 min under the conditions described by Sternberg (9) of clotting enzyme from *M. miehei,* or *E. parasitica,* or calf chymosin. The mixtures were incubated at 35°C for 30 min. After incubation, the mixtures immediately were freeze dried.

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was performed as described by Weber and Osborn (11) on enzyme treated casein fractions. One milligram was dissolved in 1 ml of .01 M sodium phosphate buffer at pH 7.0, containing 1% SDS and 1% β-mercaptoethanol and incubated at 37°C overnight. After incubation, .1 ml of sample was mixed with 10 μl of .05% bromphenol blue and .1 ml of 40% sucrose, then 50 μl of mixture were subjected to electrophroesis at a constant current (8 ma/gel tube) for 3 h. After electrophoresis, gels were fixed in a 50% trichloroacetic acid solution overnight and for 2 h in .25% Coomassie blue in 20% trichloroacetic acid solution.

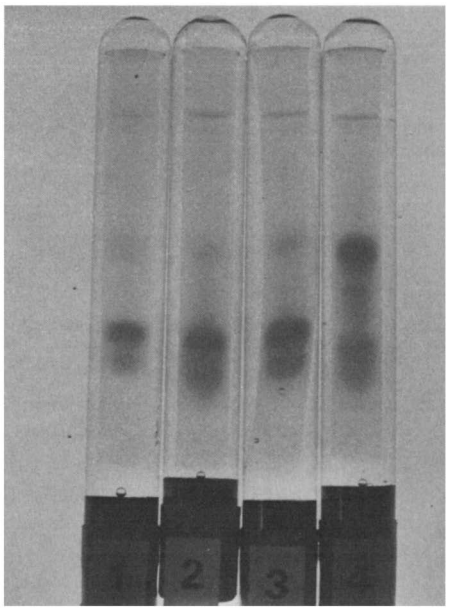

Figure 2. SDS-Electrophoresis of κ-casein treated with various milk-clotting enzymes. 1) Calf rennin; 2) *Mucor miehei* enzyme; 3) *Endothia parasitica* enzyme; 4) untreated κ-casein.

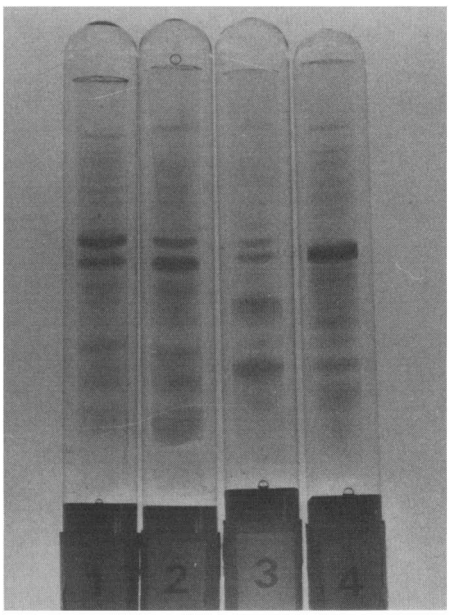

Figure 1. SDS-Electrophoresis of α_s-casein treated with various milk-clotting enzymes. 1) Calf rennin; 2) *Mucor miehei* enzyme; 3) *Endothia parasitica* enzyme; 4) untreated α_s-casein.

RESULTS AND DISCUSSION

α_s-Casein

Effects of milk clotting enzymes on α_s-casein are compared with untreated α_s-casein in Figure 1. All the enzymes catalyzed the degradation of themajor α_s-casein band. When treated with *E. parasitica* protease, the intensity of the major band was decreased markedly.

β-Casein

Differences between electrophoretic patterns of enzyme treated β-casein and untreated controls were not significant.

κ-Casein

Electrophoretic patterns were run on κ-

casein prepared by two procedures. Both κ-casein fractions showed the same electrophoretic patterns when treated with the same enzymes or when used as controls. Figure 2 shows the electrophoretic pattern of κ-casein, prepared by method of Zittle (13). When treated with *M. miehei* or *E. parasitica* enzymes, or chymosin, the major band was decreased substantially. Electrophoretic patterns of κ-casein digested by the three enzymes were similar.

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