

Preparation of Goat β -Lactoglobulin

Sen and Chaudhuri (11) reported the crystallization of goat β -lactoglobulin from an iso-electric, salt-free solution by a modification of the method of Aschaffenburg and Drewry (1). Phillips and Jenness (8), however, were unsuccessful in their attempts to crystallize goat β -lactoglobulin in a similar fashion. Askonas (2) also failed in an earlier effort. In all cases (2, 8, 11, 12), however, the protein was crystallized from solutions containing ammonium sulfate, as described by Askonas (2).

In the course of an investigation comparing the antigenicity of various β -lactoglobulins, it became necessary to prepare goat β -lactoglobulin from large amounts of pooled herd milk. The method employed is essentially that of Sen and Chaudhuri (11), except for two modifications which enabled us to prepare a large quantity of crystalline material in a relatively short

time. The modifications precede the application of the Sen and Chaudhuri procedure (11). The first modification requires removal of the cream from goat's milk using a DeLaval cream separator;¹ the second requires the acid precipitation of casein at pH 4.2.²

Experimental Procedure

Forty-one liters of pooled herd goat milk were warmed to 40-45 C, and the cream was

¹ It is not implied that the U.S. Department of Agriculture recommends products of companies mentioned, to the possible exclusion of others in the same business.

² It was found that removal of casein by acid precipitation also facilitates the crystallization of cow β -lactoglobulin C prepared according to the method previously described (5).

separated by twice passing the milk through a DeLaval cream separator. The skimmilk, pH 6.6, was then adjusted to pH 4.2 with 1.0 N HCl, to precipitate the casein removed by filtration overnight. The pH of the filtrate (32.3 liters) was readjusted to 6.6 with conc NH_4OH , and the procedure of Sen and Chaudhuri (11) followed. This procedure requires removal of contaminating caseins by addition of 20 g Na_2SO_4 per 100 ml filtrate. The casein-free filtrate is then adjusted to pH 3.0 to remove all whey proteins except β -lactoglobulin. Crude β -lactoglobulin is obtained by raising the pH of the filtrate to 6.6 and adding 20 g $(\text{NH}_4)_2\text{SO}_4$ per 100 ml filtrate. It was found that the filtrate obtained after removal of the β -lactoglobulin was not completely free of protein. Therefore, 15 g $(\text{NH}_4)_2\text{SO}_4$ per 100 ml of filtrate were added, and the precipitated protein removed by filtration. This fraction had a gel-electrophoretic mobility similar to β -lactoglobulin, and no further studies on this fraction have been undertaken. This fraction is discussed further below.

The Sen and Chaudhuri procedure was carried out twice before an effort was made to crystallize the β -lactoglobulin. This ensured removal of contaminating caseins and whey proteins which inhibit crystallization. The final concentrated solution of β -lactoglobulin, pH 5.9, produced a heavy oil after dialysis. The volume of the dialysate was reduced to one-half by pervaporation, and dialysis was then resumed, whereupon crystallization occurred almost immediately. This crystalline material was recrystallized four times from solution in 0.15 N NaCl and subsequent dialysis.

DEAE-cellulose column chromatography was carried out as previously described (3, 6, 9), except that the NaCl gradient extended to only 0.11 M and was contained in six chambers.

Polyacrylamide-gel electrophoresis (10) was performed as described by Peterson (7), using approximately 1% protein solutions. A Tris-EDTA-boric acid buffer (4) was employed with a 7-8% Cyanogum gel.

Results and Discussion

Figure 1 is a gel-electrophoretic pattern of the proteins in the various filtrates obtained during the preparative procedure, except for Figure 1, 4, a preparation of crystalline goat β -lactoglobulin supplied by Miss N. Phillips, and Dr. R. Jenness, Department of Biochemistry, University of Minnesota. Figure 1, 1 is the goat whey after acid precipitation of the caseins at pH 4.2. The whey contains slow-moving substances which tend to streak out from the origin and a substance, probably α -lactalbumin, which migrates faster than β -lactoglobulin. The slower-moving substances are in part removed by precipitation with 20% Na_2SO_4 (Figure 1, 2) and completely removed by adjustment of the Na_2SO_4 filtrate to pH 3.0

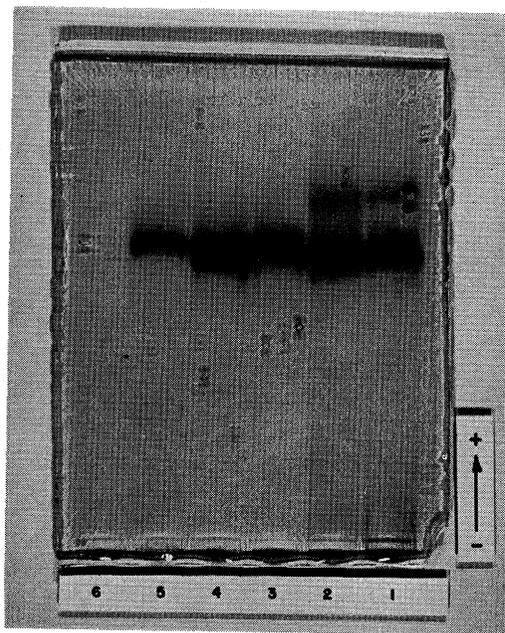


FIG. 1. Polyacrylamide-gel electrophoresis of various filtrates obtained during the preparation of goat β -lactoglobulin. (For explanation, see text.)

(Figure 1, 3). This pH adjustment also removes the fast-moving substances (α -lactalbumin) and leaves a filtrate containing β -lactoglobulin (Figure 1, 3), which is in part removed

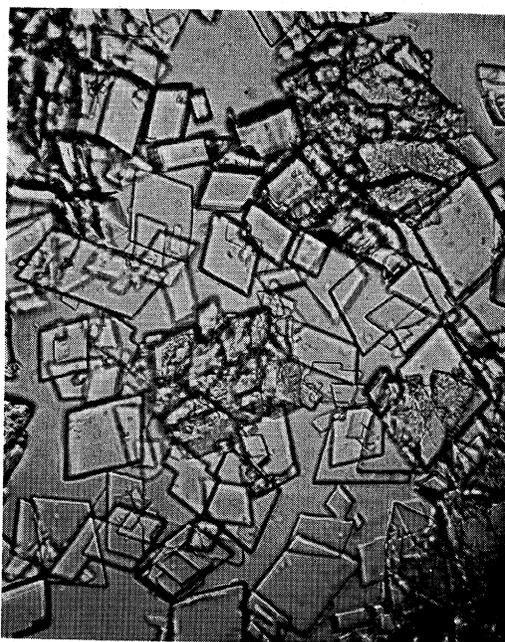


FIG. 2. Four-times-crystallized goat β -lactoglobulin obtained from isoelectric, salt-free solution.

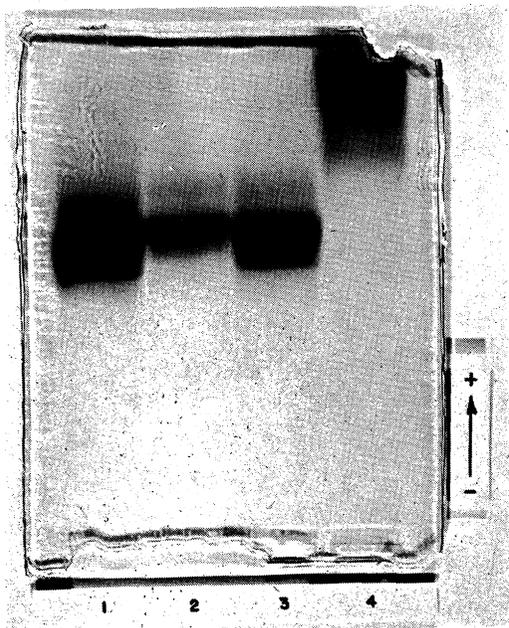


FIG. 3. Polyacrylamide-gel electrophoresis of 1) 35% $(\text{NH}_4)_2\text{SO}_4$ fraction; 2) crystalline goat β -lactoglobulin (Phillips and Jenness); 3) four-times-recrystallized goat β -lactoglobulin (present authors); 4) four-times-recrystallized cow β -lactoglobulin B.

by readjusting the pH 3.0 filtrate to pH 6.6 and adding $(\text{NH}_4)_2\text{SO}_4$ to 20%. The material remaining in the 20% $(\text{NH}_4)_2\text{SO}_4$ filtrate (Figure 1, 5) is completely removed by addition of $(\text{NH}_4)_2\text{SO}_4$ to 35% (Figure 1, 6). The 35% $(\text{NH}_4)_2\text{SO}_4$ filtrate contained a small amount of material after dialysis which did not stain with Amido Black dye. It is obvious from Figure 1 that the procedure gives sharp separations of the major proteins in goat whey, enabling their preparation in good yield. In the present instance, 26 g of four-times-recrystallized β -lactoglobulin (Figure 2) were obtained from 41 liters of pooled milk. In addition, 22.4 g of material, presumably also β -lactoglobulin, was in the mother liquors after crystallization. These yields compare favorably with those obtained in the preparation of cow β -lactoglobulin.

The crystals shown in Figure 2 are similar to those of Sen and Chaudhuri (11), and no effort was made to obtain the hexagonal bipyramids of Askonas (2). Figure 3, 3 shows the four-times-recrystallized goat β -lactoglobulin compared with the preparation of Phillips and Jenness (Figure 3, 2) and four-times-recrystallized cow β -lactoglobulin B (Figure 3, 4) prepared in these laboratories. We have no satisfactory explanation for the fact that the preparation of Phillips and Jenness gives a narrower band after the electrophoresis represented by Figure 3. In agreement with pre-

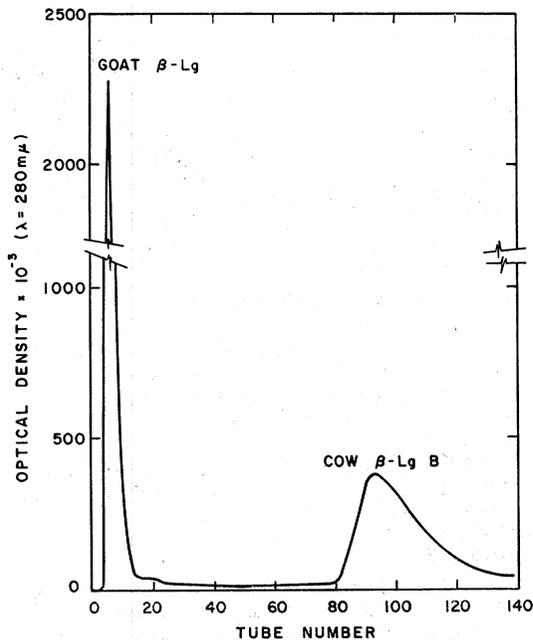


FIG. 4. DEAE-cellulose column chromatography of goat and cow β -lactoglobulins. (For explanation, see text.)

vious findings, goat β -lactoglobulin has a slower mobility than cow β -lactoglobulin B, presumably related to its higher isoelectric point (2, 8, 11, 12); the molecular weight of the two β -lactoglobulins are virtually identical (2, 8, 11, 12). Figure 3, 1 shows that the material precipitating with 35% $(\text{NH}_4)_2\text{SO}_4$ has the same mobility as the crystalline β -lactoglobulin (Figure 3, 3). If these two substances are indeed the same, the goat β -lactoglobulin is much more soluble in $(\text{NH}_4)_2\text{SO}_4$ - Na_2SO_4 solutions at about pH 6.0 than cow β -lactoglobulin. About 7.0 g of this material was obtained in the present instance, whereas a maximum of 3.0 g of material was obtained during preparation of cow β -lactoglobulins.

Figure 4 is the chromatographic separation of cow β -lactoglobulin B and goat β -lactoglobulin. The latter protein is eluted within the holdup volume of the column, probably a verification of its high isoelectric point of 5.9. The buffer employed in the chromatography is pH 5.8, so that the goat protein is probably not sufficiently anionic to exchange with the DEAE-cellulose. Cow β -lactoglobulin B, with a lower isoelectric point, exchanges with the DEAE-cellulose and is eluted in normal fashion (3, 6, 9). The DEAE-cellulose chromatography provides a rapid method for distinguishing and separating cow and goat β -lactoglobulins.

In summary, large quantities of crystalline goat β -lactoglobulin have been prepared in relatively short time, utilizing a modification of the method of Sen and Chaudhuri (11).

Acknowledgments

We thank William C. Wagner of Pure Goat Products, Inc., Boyertown, Pennsylvania, for supplying the goat milk. We also thank Dr. Peter Hoagland for his advice and interest and Miss Ann Neistadt for excellent technical assistance. One of us (E. B. Kalan) wishes to express his gratitude to Dr. Robert Jenness, Department of Biochemistry, University of Minnesota, for a most stimulating experience at the latter's laboratories, where this work was initiated.

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