Note

Analysis of Bovine Whey Protein Components by Two-dimensional Electrophoresis

Kei-ichi SHIMAZAKI, Shunsuke TEZIMA
and Kinjiro SUKEGAWA*Laboratory of Dairy Chemistry,
Department of Animal Science,
Obihiro University of Agriculture
and Veterinary Medicine,
Obihiro 080, Japan*

Received February 14, 1983

Bovine milk contains caseins, β -lactoglobulin, α -lactalbumin, serum albumin (BSA), immunoglobulin (Ig), and other minor proteins and enzymes. Major proteins in whey were separated in good resolution by two-dimensional electrophoresis according to the method of Manabe *et al.*¹⁾ with some modifications. For the first dimensional run, isoelectric focusing (IEF) was employed and the second dimensional run was performed in polyacrylamide gradient gels without any detergents or denaturants.

Acrylamide (AA), N,N' -methylenebisacrylamide (BIS), N,N,N',N' -tetramethylethylenediamine (TEMED) (Wako Pure Chemical Ind., Tokyo), Ampholine (LKB-Produktor AB, Bromma), Coomassie brilliant blue G, BSA, and β -lactoglobulin (A and B) (Sigma Chemical Co., St. Louis, MO) were used. Tris(hydroxymethyl)aminomethane (Tris), ammonium persulfate (AP) and other chemicals were reagent grade. Whey was prepared from fresh bulk milk by an acid precipitation procedure (whey-A) and by centrifugation under $40,000 \times g$ at 20°C (whey-C). BSA from Boehringer Mannheim GmbH. Biochemica (Mannheim) was also used. α -Lactalbumin was kindly supplied from two sources by Dr. R. Niki and Dr. K. Takase and by Dr. K. Nitta.

IEF was carried out with 4% polyacrylamide gel (1.5 ml of AA-BIS (16:0.8% (w/v), 0.75 ml of 0.23% (v/v) TEMED, 0.3 ml of Ampholine, 0.45 ml of water and 3 ml of 0.1% (w/v) AP) in glass tubing (3.3 i.d. $\times$ 100 mm). On the gel surface, 0.05 ml of overlay solution (2% (w/v) Ampholine and 15% (w/v) glycerin) was layered and then pre-focusing was carried out for 30 min with 1 mA/gel. Up to 0.05 ml of sample solution containing 30% (w/v) glycerin and 2% (w/v) Ampholine was added under the surface of the overlay solution. IEF was performed at a constant voltage of 200 V with 0.1 M NaOH solution in the upper buffer chamber and with 0.1 M H_3PO_4 solution in the lower buffer chamber, for 4 hr at 6°C . The positive electrode was in the lower chamber. After IEF was completed, one of the gels was cut into 6 mm sections and each section was

placed in an individual vial containing 0.5 ml of glass-redistilled water. These vials were capped and left to stand overnight, then pH values were measured at $25 \pm 1^\circ\text{C}$.

A couple of slabs (4~30% linear gradient gel) was prepared at a time using a two-chambered gradient mixer where the front chamber contained 4% gel solution (7.5 ml of AA-BIS (16: 0.8% (w/v)), 3.75 ml of 3 M Tris-HCl (pH 8.9)-0.23% (v/v) TEMED, 7.5 ml of 0.2% (w/v) AP, and 11.25 ml of water) and the back chamber contained 30% gel solution (15 ml of AA-BIS (60: 0.4% (w/v)), 3.75 ml of water, and other components the same as in the 4% gel solution). When 8~30% gel was prepared, 8% gel solution (15 ml of AA-BIS (16: 0.8% (w/v)), and other components the same as in the 30% gel solution) was put in the back chamber. The gel size was $90 \times 90 \times 3$ mm. IEF gels were placed on the top of the slabs and then electrophoresis was performed at a constant current of 18 mA/gel at room temperature for 7 hr (4~30% gel) or for 8 hr (8~30% gel) and the positive electrode was in the lower chamber. For gel staining, Coomassie brilliant blue G was used according to Blakesley and Boezi.²⁾

Two-dimensional electrophoretic patterns of whey are shown in Fig. 1-A (4~30% gel, pH 3~8). There are about 12 major protein spots, including BSA, β -lactoglobulin and α -lactalbumin, except Ig area. In Fig. 1-B (8~30% gel, pH 4~6), α -lactalbumin and β -lactoglobulin (A and B) were clearly separated while most of the Ig fractions did not appear. There was a little difference in the appearance of minor protein spots between whey-A and -C. Schematic diagrams of the electrophoretic patterns are in Fig. 2. The various protein spots were identified by running pure preparations and by comparing with the relative mobilities of each protein component and their pI values. Many minor spots were observed and most of them are due to β -lactoglobulin heterogeneities in pI and electrophoretic mobility, because two-dimensional electrophoretic pattern of pure β -lactoglobulin showed a very similar distribution of protein spots. Moreover, the electrophoretic patterns became very simple when whey was developed on a gel containing SDS using the discontinuous buffer system of Laemmli³⁾ after IEF.

From IEF of each whey protein, pI values of β -lactoglobulin B, β -lactoglobulin A, α -lactalbumin, and BSA were estimated to be 5.35 ± 0.03 , 5.23 ± 0.03 , 5.15 ± 0.04 and 4.82 ± 0.04 , respectively. The pI values of β -lactoglobulin and BSA thus obtained are in good agreement with the values reported by other investigators.^{4~7)} The pI value of α -lactalbumin obtained is larger than the value reported by Kronman and Andreotti⁸⁾ but close to the value reported by Klostergaard and Pasternak.⁹⁾

Acknowledgments. We are grateful to Dr. K. Kamashima for useful discussions and to Dr. T. Sato for his technical advice.

FIG. 1. Two-dimensional Electrophoresis of Bovine Whey.

The sample origin is at the upper right.

(A) Linear concentration gradient gel slabs from 4 to 30%, pH 3 to 8. Sample volume applied was 0.05 ml.

(B) Linear concentration gradient gel slabs from 8 to 30%, pH 4 to 6. Sample volume applied was 0.03 ml. A part of gel slab including α -lactalbumin and β -lactoglobulin spots are shown expanded.

FIG. 2. Schemes of the Two-dimensional Electrophoretic Patterns of Bovine Whey. Experimental conditions are the same as in Fig. 1.

REFERENCES

- 1) T. Manabe, K. Tachi, K. Kojima and T. Okuyama, *J. Biochem.*, **85**, 649 (1979).
 - 2) R. W. Blakesley and J. A. Boezi, *Anal. Biochem.*, **82**, 580 (1977).
 - 3) U. K. Laemmli, *Nature*, **227**, 680 (1970).
 - 4) D. Rose, J. R. Brunner, E. B. Kalan, B. L. Larson, P. Melnychyn, H. E. Swaisgood and D. F. Waugh, *J. Dairy Sci.*, **53**, 1 (1970).
 - 5) P. G. Righetti and T. Carabaggio, *J. Chromatogr.*, **127**, 1 (1976).
 - 6) L. J. Kaplan and J. F. Foster, *Biochemistry*, **10**, 630 (1971).
 - 7) M. R. Salaman and A. R. Williamson, *Biochem. J.*, **122**, 93 (1971).
 - 8) M. J. Kronman and R. E. Andreotti, *Biochemistry*, **3**, 1145 (1964).
 - 9) H. Klostergaard and R. A. Pasternak, *J. Am. Chem. Soc.*, **79**, 5674 (1957).
-