SDS-PAGE WITH DISCONTINUOUS BUFFER SYSTEM OF GOAT MILK WHEY

Munazza Shauket, M. Ashfaque, I. Hussain and T. M. Chaudhry
Department of Veterinary Microbiology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

Polyacrylamide gel electrophoresis is a valuable tool for establishing quantitative distribution of milk proteins. The fat free goat milk was obtained by centrifugation. The casein was removed by coagulation with 2 per cent solution of rennin. The whey was purified by filtration and then it was subjected to 12.5 per cent polyacrylamide gel electorphoresis. Six protein markers, bovine serum albumin (dimer), bovine serum albumin (monomer), chicken egg albumin, carbonic anhydrase, haemoglobin and lysozyme were also run in the same way. Two out of five goat milk whey samples, presented five protein bands including α-lactalbumin, ß-lactoglobulin, lactoferrin, serum albumin and unidentified protein with Rf values ranging from 0.34 to 0.72 having molecular weights ranging from 14.3 kDa to 87 kDa. The other three samples had a similar protein pattern except that the protein band with Rf value 0.593 having molecular weight of 30 kDa present in two samples was absent.

INTRODUCTION

Electrophoresis is a widely used chromatographic technique for the separation of mixutres of ionic compounds. Zonal, or gel electrophoresis combines elements of free boundary electrophoresis (separation based on charge) and gel filtration (separation based on size). Gel electrophoresis has been adapted extensively as a tool in preparative and analytical biochemistry. Disk gel electrophoresis containing sodium dodecyl sulphate (SDS) provides improved resolution over a continuous buffer system. Discontinuities in buffer composition, pour size and pH produce an isotachophoretic concentration of the sample in a low percent acrylamide stacking gel, before the size separation in the resolving gel. Because the sample is concentrated into a band of only micron wide before separation, the final degree of diffusion is greatly decreased. Even large amount of sample can be applied without reduction of resolution. As in the continuous buffer system, the density of the gel net work of the resolving gel varies with the molecular weight range of the samples. In addition to this, buffer concentrations and pH values also varies to provide the concentrated effect in the stacking gel and the maximum possible resolution in the resolving gel.

Polyacrylamide gel electrophoresis (PAGE) is a valuable tool for establishing qualitative and quantitative distribution of milk proteins. Five distinct milk proteins by electrophoresis are serum albumin, ß-lactoglobulin, α-lactalbumin, pseudoglobulin and euglobulin (Pearce and Shanley, 1981). The objective of the study was to determine the goat milk whey proteins by a suitable, modern and reliable technique to know whether it could be used as a foetal calf serum substitute for cell culture studies or not. This paper describes only the determination of goat milk whey proteins by SDS-PAGE.

MATERIALS AND METHODS

Purification of Whey

Whey was separated and purified by following the technique described by Akhtar *et al.* (1992). Briefly five fresh goat milk samples were collected and were made fat free by centrifugation at 3,000 rpm for 20-25 minutes. Rennin (2%) at rate of 0.01 ml/ml of milk was added in fat free milk to separate the casein. The sample was incubated at 37°C till curdling. The whey was separated from clotted milk by centrifugation at 2,000 rpm for 20 minutes. The whey thus separated was filtered through Seitz filter assembly and then subsequently through membrane filter of 0.22μm pore size. The purified whey obtained in this way was subjected to 12.5 per cent (SDS-PAGE).

SDS-PAGE

Discontinuous buffer system of SDS-PAGE as described by Laemmli (1970) was used. Separating gel buffer (1.5 M tris HCl, pH 8.8), stacking gel buffer (0.5 tris HCl, pH 6.8), 2X sample buffer (for liquid sample), 1X sample buffer (for solid samples) and runnin buffer (electrode buffer) were prepared following the method described by See and Jackowsky (1989) and stored at 4°C. Vertical gel electrophoresis system (BIORAD-USA) was used for the separation of whey proteins.

The samples were prepared by adding 50μL of 2X samples buffer to 50μL of protein sampel (purified whey) in 1.5 mL eppendorf tubes. A 3μL of 0.2 per cent bromophenol blue was added and kept in water bath at boiling temperature for two minutes and cooled at room temperature. Six protein markers of electrophoresis grade were selected and marked "M" on eppendorf tubes; i.e., M-l: Lysozyme, M-2:Heamoglobin, M-3: Carbonic anhydrase, M-4: Chicken egg albumin, M-5: Boving serum albumin (Monomer) and M-6: Bovine serum albumin (Dimer). Protein marker (2 mg) was mixed with 200μL of 1X sample buffer in eppendorf tube marked "M". A 3μL of 0.2 percent bromophenol blue was added to each tube and was kept in water bath at boiling temperature for two minutes and the cooled at room temperature.

The samples were loaded (20 μL) in to the gel slots. Electrophoresis was carried out at room temperature at 100 V for 6 hours until the bromophenol blue dye was about 1 cm from the bottom of separation gel. After that, the gel was subjected to staining and destaining. (Hames and Rickwood, 1983).

Molecular weights were determined as described by Weber and Osborn (1969) from relative mobility (Rf) by extrapolating from a standard curve of molecular weight markers as shown in Fig 1.

RESULTS

In SDS-PAGE using 12.5 per cent polyacrylamide gel, two out of five goat milk samples, presented five protein bands with Rf values ranging from 0.34 to 0.72 having molecular weights ranging from 14.3 to 87 kDa (Table 1). Protein band with Rf value 0.340 had molecular weight of 87 kDa and that of Rf value 0.373 had molecular weight of 67 kDa. Protein band of Rf value 0.593 had molecular weight of 30 kDa. Protein band of Rf value 0.701 had molecular weight of 19 kDa. Protein band of Rf value 0.72 had molecular weight of 14.3 kDa.

The other three samples had a similar protein pattern except that the protein band with Rf value 0.593 having molecular weight of 30 kDa present in two samples described above was absent. There was a visible difference in concentration of proteins in all five milk whey samples in respect to intensity of staining of individual band (Plate 1).

Plate 1. SDS-PAGE of goat milk whey using 12.5% polyacrylamid gel.

Table 1: Molecular weight of protein bands of goat milk whey with their Rf values

Lane No.	No. of Bands	Rf value	Molecular Weight (kDa)
1	1	0.340	87.0
	2	0.373	67.0
	3	0.593	30.0
	4	0.701	19.0
	5	0.720	14.3
2	1	0.340	87.0
	2	0.373	67.0
	3	0.701	19.0
	4	0.720	14.3
3	1	0.340	87.0
	2	0.373	67.0
	3	0.593	30.0
	4	0.701	19.0
	5	0.720	14.3
4	1	0.340	87.0
	2	0.373	67.0
	3	0.701	19.0
	4	0.720	14.3
5	1	0.340	87.0
	2	0.373	67.0
	3	0.701	19.0
	4	0.720	14.3

DISCUSSION

Out of five milk samples, three had four protein fractions. Almost similar observations have been reported by Stupnitskii and Chenko (1967) that goat milk whey proteins were separated by gel electrophoresis into four fractions corresponding to immunoglobulins (Igs), blood serum albumin (BSA), α-lactalbumin (α-LG). Cossedu and Pisanu (1979), Lim *et al.* (1986) and Mahran *et al.* (1988) also reported the presence of four protein fractions in goat milk whey by using polyacrylamide gel electrophoresis.

The protein fractions with molecular weight of 87 kDa present in all samples of goat milk whey resembled in molecular weight of lactoferrin. Shigeru (1988) also reported the molecular weight of lactoferrin as 87 kDa, while Larson (1985) described the molecular weight of lactoferrin as 90 kDa. Lactoferrin is an iron binding protein present in milk. Transferrin is also an iron binding protein which is common in blood plasma. Lactoferrin is secreted by several other organs besides the mammary glands. Both these differ from each other in composition and electrophoretic mobility and do not

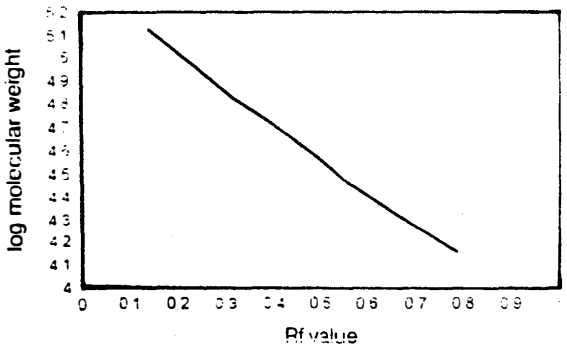

Fig.1: Standard curve of protein markers

cross react with each other immunologically. Transferrin has been isolated from blood of many species but only from the milk of cow and rabbit (Smith, 1959). Lactoferrin has been isolated from milk of humans, mouse, guinea pig, cow and sheep. The protein fractions with molecular weight of 67 kDa present in goat milk whey samples resembled in molecular weight of serum albumin. Larson (1985) described the molecular weight of serum albumin as 66.0 kDa while Shigeru (1988) reported it to be 67 kDa. The protein fraction with molecular weight of 30 kDa present in two samples was an unidentified protein. According to the findings of Larson (1985), when casein is precipitated from milk by acid, the resulting whey contains the soluble proteins including the proteose peptone fragments derived by the cleavage of ß-casein as casein was removed by rennin. The protein bands with molecular weight of 19.0 kDa was present in all five milk whey samples resembled the molecular weight of ß-lactoglobulin (ß-LG). Larson (1985) and Shigeru (1988) observed the molecular weight of ß-lactoglobulin as 18.3 kDa. The protein bands having molecular weight of 14.3 kDa resembled in molecular weight of α-lactalbumin (α-LA). Larson (1985) described the molecular weight of α-lactalbumin as 14.17 kDa, where as Shigeru (1988) observed the molecular weight of α-lactalbumin as 14.2 kDa α -lactalbumin exerts a protective action on the large molecualr aggregations of milk, such as casein and fat globules. Lactalbumin because of its high lysine content also exerts a desirable supplementary effect in improving the nutiritive value of casein. It also plays an essential role in the biosynthesis of lactose. The variation observed in molecular weights of protein fractions exists in literature too.

The intensity of protein bands was different in all goat milk whey samples. Certain physiological and pathological factors influence the concentration of proteins in milk. According to the findings of Birgel *et al.* (1971), the amount of γ-globulin rises in milk with age while albumin and ß-globulin are not influenced by age. Cossedu and Pisanu (1979) observed that immunoglobulin content of goat whey proteins was higher in February and lower in June while ß-lactglobulin and β-lactalbumin contents were lower in February than in other months. Rainard *et al.* (1982) reported that transferrin and bovine serum albumin increased sufficiently in late lactation (270th day of lactation). Lactoferrin concentration increased significantly in quarters infected by major pathogens whereas minor pathogens infection caused no significant increase in lactoferrin concentration (Rainard *et al.*, 1982).

REFERENCES

Akhtar, M., M. Ashfaque, M. Afaque, I. Hussain and A.B. Zahur, 1992. Purification and concentration of colostral immunoglobulins in cross-bred cows. Pakistan Vet. J., 12: 39-41.

Birgel, E.H., S. Ferri, and L.O. Medeiros, 1971. Studies by paper electrophoresis of the proteins of normal serum of Anglo-Nubian female goats. Recl. Med. Vet., 147: 43-48.

Cossedu, A. and S. Pisanu, 1979. Electrophoretic fractionation of goat whey produced in Sardinioa. Archivia Veterinaria Italiana, 30: 79-81.

Hames, B.D. and D. Rickwood, 1983. Gel electrophoresis of proteins. A practical approach. IRL Press Ltd., Oxford pp:230-300.

Laemmli, U.K. 1970. Cleavage of Structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Larson, B.L. 1985. Lactation. 2nd Ed. Lowa state University Press. pp: 139-183.

Lim, J.W., G.W. Park and P.H. Chang, 1986. Fractionation of whey proteins in Korean native goats by polyacrylamide gel electrophoresis and Sephadex gel filtration. Korean J. Dairy Sci., 8: 33-39.

Mahran, G.A., H.A. El-Alamy, M.B. Mahfouz and M.M. El-Loly, 1988. Studies on the chemical composition of Egyptian goat's milk. II. Amino acid composition, protein fraction and non-volatile fatty acids. Egyptian J. Dairy Sci., 36: 110-114.

Pearce, R.J. and R.M. Shanley, 1981. Analytical and preparative separation of whey proteins by chromatofocusing. Australian J. Dairy Tech., 36:110-114.

Rainard, P., B. Poutrel, and J.P. Caffin, 1982. Lactoferrin and transferrin in bovine milk in relation to certain physiological and pathological factors. Annales dc Researches Veterinaires, 13: 321-328.

See, Y.P. and Jackowsky, 1989. Estimating molecular weights of polypeptides by SDS gel electrophoresis. In: Protein structure; A practical approach by T.I. Creighton IRL Press Ltd. Oxford, pp: 1-21.

Shigeru, Y, 1988. Isolation of some minor milk proteins, distributed in acid whey from approximately 100,000 to 250,000 Daltons of particle size. J.Dairy Sci., 71: 109.

Smith, V.R. 1959. Physiology of lactation. 5th Ed. Iowa State University Press. Ames, Iowa, U.S.A., pp: 271-279.

Stupnitskii, R.M. and M.D. Chenko, 1967. Electrophoresis of goat's milk proteins. Fiziol Biokhim Sel'Khozzhivot; Respub. Mezhved Temat. Nauch Sb., 5: 62-65.

Weber, K., and M. Osborn, 1969. The reliability of molecular weight determination by dodecyl sulphate polyacrylamide gel electrophoresis. J. Biol. Chem., 244: 4406.