

## ISOLATION, PURIFICATION AND MOLECULAR CHARACTERIZATION OF ERYTHROCYTE MEMBRANE SPECTRIN IN BLACK BENGAL GOAT

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### ABSTRACT

Erythrocyte membrane spectrin is a rod shaped tetramer of high molecular weight, composed of two sub units ( $\alpha$  &  $\beta$ ), which intertwine to form  $\alpha\beta$  heterodimer. It plays a crucial role in the maintenance of erythrocyte membrane's deformability and stability. Spectrin, isolated from bovine, ovine, caprine, and camel erythrocyte membranes have showed wide variation in molecular weights. This study was undertaken to isolate, purify and characterize the erythrocyte membrane spectrin from Black Bengal goat (*Capra hircus*) on which no study has been done earlier. Spectrin was extracted from erythrocyte membrane of the goats by treating with 0.1 mM EDTA (pH 7.5) at 37°C for 1 hour, and was purified by gel filtration chromatography on Sephacryl S-200. The molecular weight of purified spectrin was determined by SDS-PAGE using standard molecular weight marker (range, 29 to 205 kDa). The protein concentration of Black Bengal goat erythrocyte membrane, obtained in this study, was 13.5 mg/ml. About 120 mg (24%) of crude spectrin was recovered from 500 ml of whole blood. The crude spectrin primarily consisted of spectrin, but a small amount of actin and ankyrin were also recovered. The proteins of purified erythrocyte membrane spectrin resolved into four peaks, one major (B) and three minor (A, C, D) peaks. Peak A represented the aggregated spectrin and actin, where as peak C and peak D represented ankyrin, actin, and other proteins. Peak B represented the spectrin dimer. SDS-PAGE of erythrocyte membrane protein revealed 11 major bands. The purified spectrin showed  $\alpha\beta$  heterodimer. The molecular weight of purified spectrin obtained in this study was 461 kDa. The molecular weights of  $\alpha$  &  $\beta$  sub-units of the  $\alpha\beta$  heterodimer were 240 kDa and 221 kDa respectively.

### KEY WORDS

Alpha-Beta heterodimer, Black Bengal goat, Erythrocyte membrane, Spectrin

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## INTRODUCTION

Spectrin is a rod shaped tetramer of high molecular weight, composed of two sub units ( $\alpha$  &  $\beta$ ), which intertwine to form  $\alpha\beta$  heterodimer (Figure-1). It plays a crucial role in the maintenance of erythrocyte membrane's deformability and stability (McGough and Joseph, 1990).

Spectrin has been isolated, purified, and characterized in bovines (Ralston, 1975b), ovine (Brenner and Korn, 1979), caprines (Kesh *et al.*, 2009), and camels (Ralston, 1975a). This study was undertaken to isolate, purify, and characterize the erythrocyte membrane spectrin from Black Bengal goat, on which no study has been done earlier.

## MATERIALS AND METHODS

Blood samples collected from healthy Black Bengal goats in acid citrate dextrose (Horne *et al.*, 1989) were used to prepare pure membranes (ghosts) by the method of Dodge *et al.* (1963). The spectrin was extracted by treating pure membrane with 0.1 mM EDTA (pH 7.5) at 37°C for 1 hour followed by centrifugation at 35000 g for 25 minutes at 4°C.

The extracted spectrin ( $\alpha\beta$  heterodimer) was purified by gel filtration chromatography in a column on Sephacryl S-200 (2 cm diameter and 52 cm in length) in a buffer containing 25 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA, 0.03 mM PMSF, and 0.02% sodium azide (pH 8.0) at a flow rate of 18 ml per hour. Fractions were monitored at 280 nm and concentrated by dialysis against sucrose

using dialysis membrane (cut off value 12000). The protein concentration of crude membrane and purified spectrin were estimated by the method of Lowry *et al.* (1951). The banding pattern of crude and purified erythrocyte membrane spectrin was assessed by SDS-PAGE on 7.5% slab gel (Laemmli, 1970). Molecular weight of purified spectrin was determined by SDS-PAGE using standard molecular weight marker (Range, 29 to 205 kDa).

## RESULTS

The protein concentration of Black Bengal goat erythrocyte membrane, obtained in this study, was about 13.5 mg/ml. About 120 mg of crude spectrin was recovered from 500 ml of whole blood. The crude spectrin primarily consisted of spectrin, but a small amount of actin and ankyrin were also recovered.

The proteins of purified erythrocyte membrane spectrin were resolved into four peaks, one major (B) and three minor (A, C, D) peaks (Figure-2). Peak A represented the aggregated spectrin and actin, where as peak C and peak D represented ankyrin, actin, and other proteins. Peak B represented the spectrin dimer.

SDS-PAGE of erythrocyte membrane protein revealed about 11 major bands (Figure-3). The purified spectrin showed  $\alpha\beta$  heterodimer. The molecular weight of purified spectrin ( $\alpha\beta$  heterodimer) was 461 kDa, while the molecular weights of  $\alpha$  &  $\beta$  sub-units were 240 kDa and 221 kDa respectively (Figure-4).

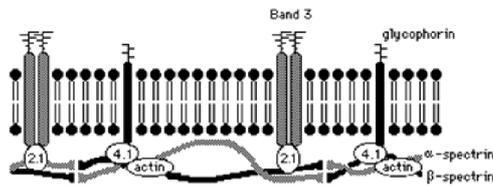


Figure-1. Spectrin complexes of the cell membrane.

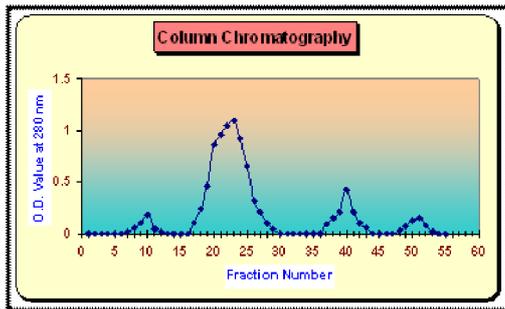


Figure-2. Purification of Black Bengal goat erythrocyte spectrin by gel filtration chromatography on Sephacryl S-200.

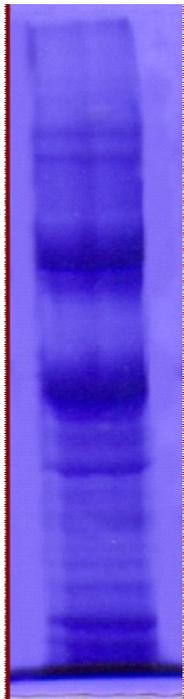


Figure-3. SDS-PAGE (7.5% gel) of normal erythrocyte membrane proteins of Black Bengal goat depicting 11 bands.

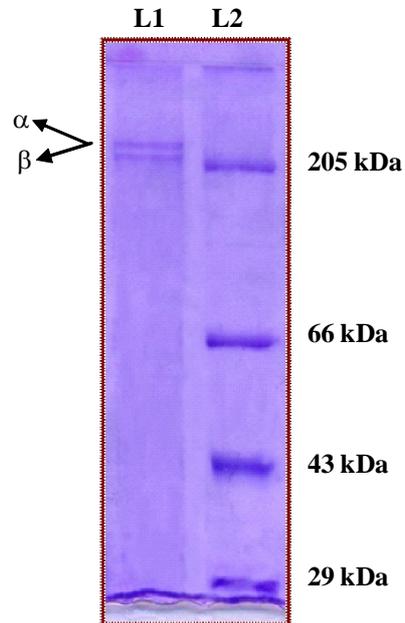


Figure-4. SDS-PAGE (7.5% gel) of purified spectrin ( $\alpha\beta$  heterodimer) of Black Bengal goat erythrocyte membrane. Lane-1: Purified spectrin, Lane-2: Molecular weight marker (Range: 29-205 kDa).

## DISCUSSION

Spectrin can be extracted from the erythrocyte membranes both at 37°C and at 4°C. Spectrin is eluted as dimer at 37°C, and as tetramer at 4°C (Goodman *et al.*, 1988). In the present study spectrin was extracted at 37°C, because prolonged extraction at low ionic strength (4°C) induces the release of only half of the spectrin, as observed in camel ghost, while 2 to 3 extractions are needed for the complete release of spectrin in human and bovine ghosts (Ralston, 1975b). At 37°C, human and bovine ghosts could release all of their spectrin within 1 hour, while camel ghosts released only a fraction of spectrin content, without any apparent change in the shape of the erythrocytes (Ralston, 1975a).

In our study, most of the spectrin from the goat erythrocyte membrane was released within 1 hour like other species such as bovine and human.

The molecular weight of purified spectrin ( $\alpha\beta$  heterodimer) of Black Bengal goat erythrocyte membrane, analyzed by Gel Documentation System using standard protein markers (Range, 29-205 kDa) in 7.5% SDS-PAGE, was found to be of 461 kDa, while it was reported to be 485 kDa in humans (Bennett, 1985), 480 kDa in bovines (Ralston, 1975b), 460 kDa in ovine (Brenner and Korn, 1979), and 460 kDa in chickens (Chan, 1977). Interestingly, it was 435 kDa in Jamunapari goats (Kesh *et al.*, 2009). Breed difference in molecular weight between Jamunapari and Black Bengal goats could be attributed to variation in amino acids of particular proteins.

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