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STUDIES ON SHEEP RED CELL MEMBRANE: IDENTIFICATION OF TWO HIGH MOLECULAR WEIGHT INTRINSIC PROTEINS

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Abstract

Sheep red blood cell membrane contained two high molecular weight proteins of 138,000 and 117,000 daltons which were not extractable with 0.1 mM EDTA nor with 0.5 M NaCl but could be solubilized with 0.5% Triton X-100. In the intact cell, these two protein components were susceptible to digestion with papain.

The human red cell membrane has been extensively studied as regards organization and function of its components. Using sodium dodecyl sulfate (SDS) polyacrylamide gel-electrophoresis, some seven major protein bands can be visualized[1]. Band 1,2,2.1,4.1 and 5 (using the nomenclature of Fairbanks *et al.*[1]) are extrinsic proteins and form a cytoskeletal network attached to the cytoplasmic side of the plasma membrane[2]. Band 3, the major intrinsic protein, is believed to be the anion transport protein and also acts as the attachment site for the cytoskeletal network as well as a number of cytoplasmic proteins[2,3]. Band 6 has been identified as glyceraldehyde-3-phosphate dehydrogenase[4]. However, SDS-polyacrylamide gel-electrophoresis also reveals a number of minor bands whose properties and functions have yet to be studied. In sheep red blood cell (SRBC) membrane, there exists two major bands migrating between bands 2 and 3 which are not seen in the human red cell membrane[5]. Since these two protein components, designated in this study S_1 and S_2 (MW of 138,000 and 117,000 respectively, using the major proteins of human red cell membrane as molecular weight markers[2]), constituted up to 10% of the total membrane protein, their properties were investigated and is the subject of this report.

Whole sheep blood were collected in an acid citrate-dextrose solution from animals reared at the Faculty of Science, Mahidol University. SRBC membrane were prepared by hypotonic lysis and extensively washed in 5 mM phosphate buffer at pH 8.0[1]. Membrane preparations were then treated with either 0.1 mM EDTA[1], 0.5 M NaCl[6] or 0.5% Triton X-100[7]. Both treated and untreated membrane preparations

were analyzed by SDS-polyacrylamide gel-electrophoresis[1]. S_1 and S_2 were not extractable with EDTA (which removed band 1,2 and 5) nor with NaCl (which removed band 6) but could be extracted with Triton X-100 which also removed the majority of band 3 (see Fig. 1).

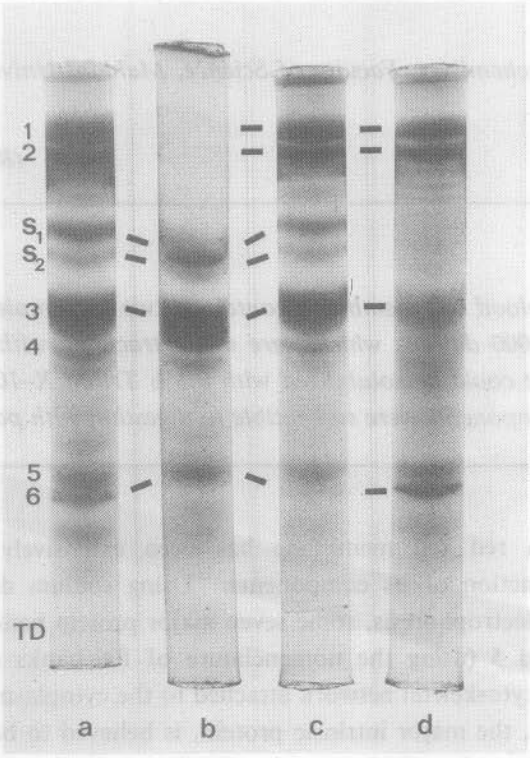

Fig. 1 Protein pattern of SRBC membranes on SDS-polyacrylamide gel-electrophoresis (6.5% gel) stained with Coomassie blue R of a) untreated membrane, b) membrane treated with 0.1 mM EDTA, c) membrane treated with 0.5 M NaCl and d) membrane treated with 0.5% Triton X-100. Each gel was loaded with 50 g protein.

SRBCs have a number of properties distinct from red cells from other mammals. They spontaneously adhere to human T lymphocytes to form E rosettes[9], which may be related to their surface neuraminidase-sensitive sites[10]. SRBC membranes have a high sphingomyelin and a low phosphatidylcholine content[11], and may contain membrane proteins which bind preferentially to sphingomyelin[12]. The relationship of S_1 and S_2 to these characteristics remains to be investigated.

When intact SRBCs were digested with papain (2 mg/ml of cell suspension for 2 h.[8]), conditions which did not produce any cell lysis, there was complete disappearance of S_1 and S_2 bands and a partial decrease in band 3, together with a concomitant increase in peaks of lower molecular weight material (Fig. 2). As expected, band 1 and 2 were not affected by this treatment. Thus S_1 and S_2 behaved as intrinsic proteins which spanned the plasma membrane. S_1 and S_2 have little or no carbohydrates attached to their exposed external portion since no stain could be detected when gels were treated with Periodic acid-Schiff reagent (not shown).

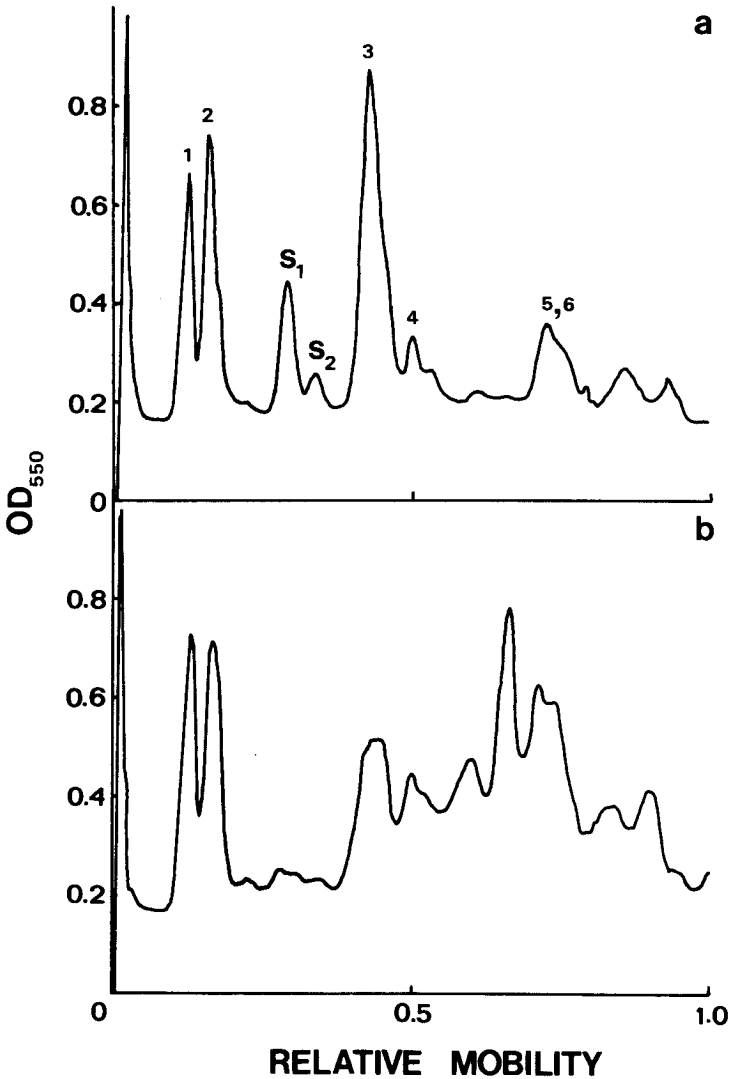

Fig. 2 Coomassie blue R absorption profiles of SDS-polyacrylamide gels of a) membrane of untreated SRBCs and b) membrane of papain digested SRBCs (2 mg enzyme/ml of cell suspension, 2 h.)

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บทคัดย่อ

เยื่อเซลล์เม็ดเลือดแดงของแกะมีโปรตีนเพิ่มขึ้นจากของคน 2 ชนิด น้ำหนักโมเลกุลเป็น 138,000 และ 117,000 daltons ตามลำดับ โปรตีนทั้งสองชนิดฝังตัวแน่นในเยื่อเซลล์เนื่องจากไม่ถูกสกัดด้วย 0.1 mM EDTA และ 0.5 M NaCl แต่ถูกสกัดด้วย 0.5% Triton X-100 โปรตีนทั้งสองมีบางส่วนของโมเลกุลยื่นออกมาภายนอกเซลล์เนื่องจากถูกย่อยด้วย papain เมื่อทำการย่อยเม็ดเลือดแดงกับเอ็นไซม์นี้