Asian-Aust. J. Anim. Sci.
Vol. 24, No. 12 : 1666 - 1673
December 2011

www.ajas.info
http://dx.doi.org/10.5713/ajas.2011.11162

Sialoglycoproteins of Mammalian Erythrocyte Membranes: A Comparative Study

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ABSTRACT : The presence of sialoglycoproteins (SGPs) in the membranes from goat (*Capra aegagrus hircus*), buffalo (*Bubalus bubalis bubalis*) and pig (*Sus scrofa domestica*) erythrocytes was investigated by partial purification with a chloroform-methanol extraction method followed by Sodium dodecyl sulphate - Polyacrylamide gel electrophoresis in comparison to human (*Homo sapiens*) erythrocytes. The results show that mammalian erythrocytes possess clear differences in the SGPs numbers and molecular weights although all animals studied in this experiment are from the same class i.e. mammalia. The SGPs number in human, goat, buffalo and pig are four (PAS-1 to PAS-4), ten (PAS-GI to PAS-GX), seven (PAS-BI to PAS-BVII) and four (PAS-PI to PAS-IV) respectively as indicated by staining the polyacrylamide gel with sialoglycoprotein-specific Periodic acid-Schiff's (PAS) stain. The new SGPs could be observed only after the partial purification of membrane fractions named as PAS-HI with molecular weight (Mr) 190 kDa and PAS-HII 150 kDa in human, PAS-BIA in buffalo and PAS-PIA and PAS-PIVA in pig. The gels were also stained with Coomassie brilliant blue (CBB) and Silver stain to check the contamination of other membrane proteins in the purified fractions. The quantitative distribution of SGPs was also determined by densitometry. Present study indicates that there are some basic differences in mammalian erythrocyte membrane SGPs, especially with respect to their number and molecular weights indicating major structural variations. (**Key Words :** *Bubalus bubalis bubalis*, *Capra aegagrus hircus*, Electrophoresis, Erythrocyte, *Homo sapiens*, Membrane sialoglycoproteins, *Sus scrofa domestica*)

INTRODUCTION

Human erythrocyte membrane is a very well studied biomembrane with respect to structure and organization of proteins. Erythrocyte membrane structure of other mammals is not studied to that extent. The erythrocyte membrane is composed of integral proteins including glycoproteins and a spectrin-based skeletal protein network associated with lipid bilayer (Steck, 1974; Gratzer, 1981; Chasis and Mohandas, 1992). The human erythrocyte membrane contains 10 major polypeptides (Fairbanks et al., 1971) and about 80 minor polypeptides (Kakhniashvili et al., 2004). Similar major polypeptide pattern is also found in other vertebrate erythrocytes (Hamaguchi and Cleve, 1972; Barker, 1991; Matei et al., 2000). A novel glycoprotein gp155 is present in goat erythrocyte membrane and this glycoprotein forms a complex with band 3 and ankyrin (Inaba and Maede, 1988). The sialoglycoproteins (SPGs) which play major role in many physiological processes are the sialic acid rich glycoproteins and get stained only with Periodic acid-Schiff's (PAS) stain. Analysis of human erythrocyte membranes isolated by SDS-PAGE followed by PAS stain, revealed the presence of four SGPs and initially identified as PAS-1, PAS-2 and PAS-3 bands (Fairbanks et al., 1971). Later PAS-4, present between PAS-l and PAS-2, was also identified (Tanner and Boxer, 1972). These SGPs were named as glycophorins (Furthmayr et al., 1975). Glycophorins of human erythrocytes, carry blood group antigenic determinants and serve as receptors for viruses, bacteria and parasites, have been thoroughly studied (Kumar et al., 2006; Spring, 2008). Over 250 blood group determinants are known and most of these are located on integral erythrocyte proteins and glycoproteins. The functions of some of these structures are known (Pasini et al., 2010). The Duffy glycoprotein (GPD) is a chemokine receptor that may act as a scavenger for inflammatory mediators in the peripheral blood, but is also exploited as a receptor by *Plasmodium vivax* and *Plasmodium knowlesi* malarial parasites (Chaudhuri et al., 1989; Daniels, 1999). The human erythrocyte glycophorins known as GPA, GPB,

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Received June 1, 2011; Accepted August 8, 2011

GPC, GPD, and GPE have recently been fully characterized at both the protein and the DNA levels. There are some reports regarding the occurrence of SGPs in horse, ox, sheep, swine, bovine, mouse and rat erythrocyte membranes (Hamaguchi and Cleve, 1972; Murayama et al., 1982; Herraez et al., 1992). The present work aims for a comparative study of partially purified SPGs of goat, buffalo and pig with human erythrocyte membranes by solvent (chloroform-methanol) extraction, separation by SDS-PAGE and followed by staining with PAS, CBB and Silver stains.

MATERIALS AND METHODS

SDS, Phenyl methyl sulfonylflouride (PMSF), N, N'-methylene bisacrylamide, N,N,N,N tetramethyl ethylene diamine (TEMED) and CBB were the products of Sigma chemicals Co., St. Louis, MO, USA. All other chemicals were analytical research grade.

Preparation of erythrocytes

Human blood was obtained from healthy donors (n = 4). The blood of healthy animals (n = 6, each animal) was obtained from local slaughterhouses. All blood samples were collected in Acid/Citrate/Dextrose as anticoagulant. Erythrocytes were obtained by removing plasma and buffy coat from blood by centrifugation at 1,000 xg for 5 min at room temperature (RT) in Remi (Remi R-4C). Erythrocytes pellet was resuspended in 10 vol. of Tris buffered saline (TBS) (10 mM Tris and 150 mM NaCl) and recentrifuged at same xg value. The erythrocytes were thus washed four times with well aspirations and centrifugation, removing a portion of pellet from top each time to ensure maximum removal of leukocytes and pure erythrocytes were obtained.

Membrane preparation

Membranes were prepared from erythrocytes according to Hanahan and Ekholn (1974) with some modifications. Washed erythrocytes were lysed by mixing with 30 vol. of cold 0.01 M Tris-HCl containing 1 mM PMSF, pH-7.4. After 15 min in cold, the suspension was centrifuged at 22,000×g for 15 min in a refrigerated centrifuge (Sorvall RC 5B Plus in an F-28/36 rotor) at 4°C. The resulting deep red supernatant was discarded. The small opaque button seen below the translucent pellet of ghosts was carefully removed. The ghosts were suspended in 20 vol. of cold washing buffer (0.01 M Tris-HCl, pH-7.4) and recentrifuged at same xg value. In this way the membranes were washed four times, when usually a milky white preparation was obtained and stored at 4°C until further use. In fourth washing, 0.05% NaN_3 is added in washing buffer to prevent microbial growth.

Partial purification of erythrocyte membrane sialoglycoproteins

The major SGPs and additional minor SGPs can be recovered in the aqueous phase after extraction of the membranes with a mixture of chloroform-methanol ($CHCl_3$-CH_3OH) at RT. The extracted SGPs remain in the supernatant after centrifugation. For purification of mammalian erythrocyte membrane SGPs (Kornfeld and Kornfeld, 1970), one vol. of membrane (2 mg/ml) was mixed with 9 vol. of $CHCl_3$-CH_3OH mixture (2:1, v/v) and stirred vigorously at RT for 30 min. The mixture was centrifuged at 2,000×g for 10 min in Remi centrifuge at RT. The centrifuged material was separated into two phases; the upper (aqueous) phase was containing most of the soluble SGPs. This phase was collected without disturbing the lipid layer at the interface. The aqueous phase solution was concentrated to $1/10^{th}$ vol. of original membrane vol. used in rotary evaporator at 37°C. The concentrated material was centrifuged at 100,000×g for 1 h in the Sorvall ultracentrifuge (Sorvall WX Ultra 80), at 4°C. The supernatant was collected and stored at -80°C until further use.

Protein estimation using Lowry's method

Protein was estimated according to Lowry et al. (1951). Bovine serum albumin (BSA) standard (0.1 mg/ml to 0.5 mg/ml) was used to plot the standard curve. The protein content of the test samples was determined by extrapolation from the standard curve.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Polyacrylamide gel electrophoresis (one-dimentional) method for discontinuous SDS-PAGE was followed according to Laemmli (1970). Protein samples were solubilized in sample buffer containing 0.031 M Tris, 1% SDS, 0.25% β-mercaptoethanol and 5% glycerol in final volume. Electrophoresis was carried out at constant current of 2 mA/cm using electrode buffer until the bromophenol blue marker head reached the bottom of the gel. The gels were stained with CBB, PAS and Silver stains according to their respective methods of staining.

Staining and destaining of the gels

After electrophoresis, gels were removed from the glass plates and processed further for staining and destaining in a washed plastic container.

Commassie brilliant blue (CBB) staining : Before CBB staining, gel was placed in 200 ml destainer containing 40% methanol-10% acetic acid for 30 min. Gel was stained with 0.1% CBB (R 250) stain for 1-4 h and destaining was done with destainer.

Periodic acid-Schiff's staining : The carbohydrate-

specific staining was performed according to Fairbanks et al. (1971).

Silver staining : The gel was washed with 200 ml distilled water for 30 min on a shaker at RT. The washing was repeated three times. After washing, the gel was incubated with 200 ml solution 1 (0.02% Sodium thiosulphate) for 1 min on the shaker followed by a quick distilled water wash. The gel was then incubated with 200 ml solution 2 (0.2% Silver nitrate containing 0.075% formaldehyde) for 20 min on the shaker followed by a quick distilled water wash. Gel was then placed in 200 ml solution 3 (2% Sodium carbonate, 0.05% formaldehyde, 2% of solution 1) on a shaker until silver stained spots could be seen clearly. The staining was stopped by adding 100 ml stop solution (10% acetic acid). After destaining, the type of gels were scanned on scanner and stored in 10% acetic acid at 4°C until further analysis.

Gel image analysis

The gel images were scanned using Scanner 'HP scanjet 7400c'. Densitometric analysis for quantitation of PAS stained gels was done by using LabWorks^{TM} Image Acquisition and Analysis software, Version 4.0.0.8.

RESULTS

Partial purification of erythrocyte membrane SGPs was done by solvent (chloroform-methanol) extraction method and analyzed by SDS-PAGE. The extraction of mammalian erythrocyte membranes with a mixture of $CHCl_3$-CH_3OH is a relatively simple and efficient procedure for the solubilization of membrane SGPs. The top aqueous phase was containing all of the SGPs and lower precipitated phase containing residual membrane proteins as prepared by centrifugation and separated by SDS-PAGE. For this purpose, three duplicate gels were prepared for staining with three types of stains (PAS, CBB and Silver). All major and minor SGPs were clearly indicated by staining the polyacrylamide gel with PAS stain. The SGPs do not take up intense CBB stain but all proteins and SGPs can be visualized by Silver stain. The SGPs were numbered from PAS-I to PAS-X (according to the number of SGPs) in the order of their decreasing molecular weight. But in case of human erythrocyte membrane, PAS-4 was numbered between the PAS-1 and PAS-2 (as per nomenclature of Fairbanks et al., 1971). Partial purification of membrane fraction showed few new SGPs which could not be observed in unextracted native membranes when stained with PAS stain (Figure 1A to 4A, lane 1).

The electrophoretic patterns of the partially purified SGPs of human (Figure 1A) were showing similar SGPs pattern along with two new SGPs (Figure 1A, lane 2) which were not observed in PAS stained unextracted native membranes (Figure 1A, lane 1). These new bands were named as PAS-HI and PAS-HII with Mr 190 kDa and 150 kDa respectively. They could not be seen in the membranes either because they are less in quantity or may be getting masked with other membrane proteins. This is a surprising observation regarding these two SGPs, as no report is available about the presence of such SGPs in the human erythrocyte membranes. The other four SGPs (PAS-1, PAS-4, PAS-2 and PAS-3) were having same positions as compared to native membranes. There was slight increase in the intensity (amount) of PAS-1 and PAS-4. No changes were observed in PAS-2 and PAS-3. The minor aqueous layer just below the top aqueous layer (containing SGPs) was also loaded (Figure 1A, lane 3) in the gel. This was also showing the presence of PAS-1 and PAS-4 SGPs as present in top layer (Figure 1A, lane 2). The same samples were also loaded on separate gels and stained with CBB and Sliver stain. CBB stain showed all the membrane proteins (Figure 1B, lane 1) which were not stained with PAS stain (Figure 1A, lane 1). The silver stained gel (Figure 1C, lane 2) was confirming the absence of any contamination of membrane proteins in the purified SGPs as similar patterns of SGPs (Figure 1A and C, lane 2) were observed in PAS and silver stained gel. The densitogram (Figure 1D) of PAS stained gel (Figure 1A, lane 1 and 2) clearly indicates the presence of six SGPs in the purified fraction.

The partially purified SGPs of goat erythrocyte membranes (Figure 2A) were showing similar SGPs pattern but having more intensity (amount) of each SGPs (Figure 2A, lane 2). Along with increase in intensity, the changes in Mr of few SGPs were also observed. The major increase in the intensity was observed in the upper six purified SGPs, named PAS-GI to PAS-GVI. The changes in the Mr of PAS-GVII (55 kDa), PAS-GVIII (45 kDa) and PAS-GX (25 kDa) were observed (Figure 2A, lane 2) which were showing Mr 50 kDa, 40 kDa and 23 kDa in the membrane fraction (Figure 2A, lane 1). These upward shifted SGPs PAS-GVII, PAS-GVIII and PAS-GX were not showing any change in the intensity. The densitogram (Figure 2D) of PAS stained gel (Figure 2A, lane 1 and 2) confirms the results given above. Below the top aqueous layer (containing SGPs), the lower precipitated phase containing residual membrane proteins was also collected and analyzed (Figure 2A, lane 3). The same samples (Figure 2A) were also loaded on separate gels and stained with CBB (Figure 2B) and silver stains (Figure 2C). CBB stained gel showed all the membrane proteins (Figure 2B, lane 1) which were not shown with PAS stain (Figure 2A, lane 1) as PAS stain visualize only the membrane SGPs. Similarly, CBB stain was not staining the purified SGPs (Figure 2B, lane 2). The silver stained gel (Figure 2C, lane 2) was visualizing only the purified

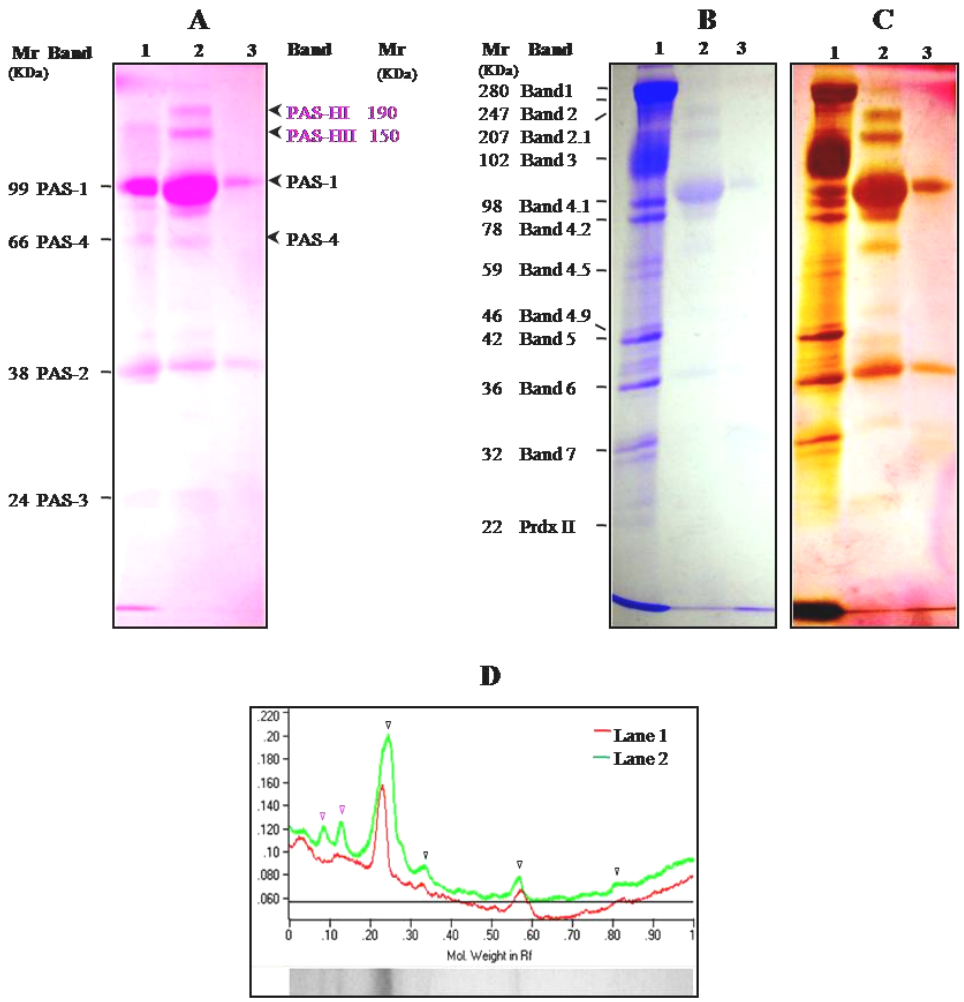

Figure 1. Analysis of partially purified glycoproteins obtained from human erythrocyte (HE) membranes by SDS-PAGE (10% Gel). HE membranes (lane 1), glycoproteins extracted from membranes (lane 2) and minor aqueous layer proteins (lane 3). A: Gel stained with PAS stain (180 µg protein in each lane); B: Gel stained with CBB (60 µg protein in each lane); C: Gel stained with Silver stain (50 µg protein in each lane); D: Densitogram of PAS stained gel. Note: Arrowhead indicates the protein showing major (pink font) or minor (black font) difference/change.

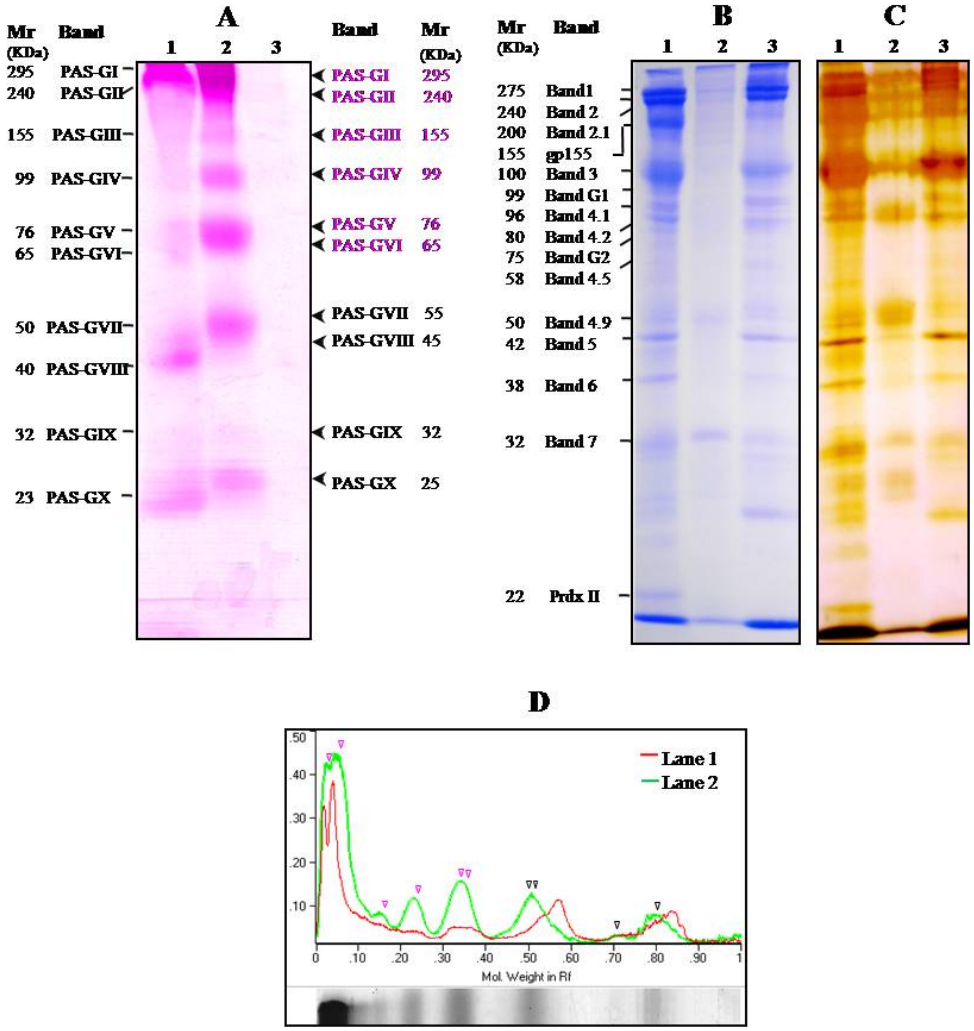

Figure 2. Analysis of partially purified glycoproteins obtained from goat erythrocyte (GE) membranes by SDS-PAGE (10% Gel). GE membranes (lane 1), glycoproteins extracted from membranes (lane 2) and residual proteins (lane 3). A: Gel stained with PAS stain (180 µg protein in each lane); B: Gel stained with CBB (60 µg protein in each lane); C: Gel stained with Silver stain (50 µg protein in each lane); D: Densitogram of PAS stained gel. Note: Arrowhead indicates the protein showing major (pink font) or minor (black font) difference/change.

SGPs, which confirmed the absence of any contamination of membrane proteins in the purified SGPs (Figure 2A and C, lane 2).

The purified SGPs pattern of buffalo erythrocyte membrane as appeared on the gel after staining with PAS stain is illustrated in Figure 3A. The erythrocyte membrane stained with PAS (Figure 3A, lane 1) revealed the presence of seven SGPs but one more SGP, PAS-BIIA (140 kDa) was found to be present after partial purification of SGPs (Figure 3A, lane 2) from erythrocyte membranes. The differences in intensities and Mr of purified SGPs were also observed. PAS-BI (295 kDa) and PAS-BII (175 kDa) were showing decrease in the intensities after purification. PAS-BIII (100 kDa) and PAS-BIV (74 kDa) were appeared with higher intensities but also showing lower shift in their positions as indicated by decrease in Mr. PAS-BV (50 kDa) was showing decreased intensity as well as lower shift in their position. PAS-BVI (30 kDa) and PAS-BVII (20 kDa) also showed slight lower shift in position but no change in the intensities. The densitogram (Figure 3D) of PAS stained gel (Figure 3A, lane 1 and 2) indicated the similar results. The residual membrane proteins in the precipitated phase were also separated by SDS-PAGE (Figure 3A, lane 3). The same samples (Figure 3A) were also loaded on separate gels to stain with CBB (Figure 3B) and silver (Figure 3C) stains. CBB stain gel showed all the membrane proteins (Figure 3B, lane 1) which were not shown with PAS stain (Figure 3A, lane 1). Similarly, CBB stain was not staining the purified glycoproteins (Figure 3B, lane 2), only PAS-BV and PAS-BVII were reflecting their presence. The confirmation of the absence of any contamination of membrane proteins in the purified SGPs (Figure 3A and C,

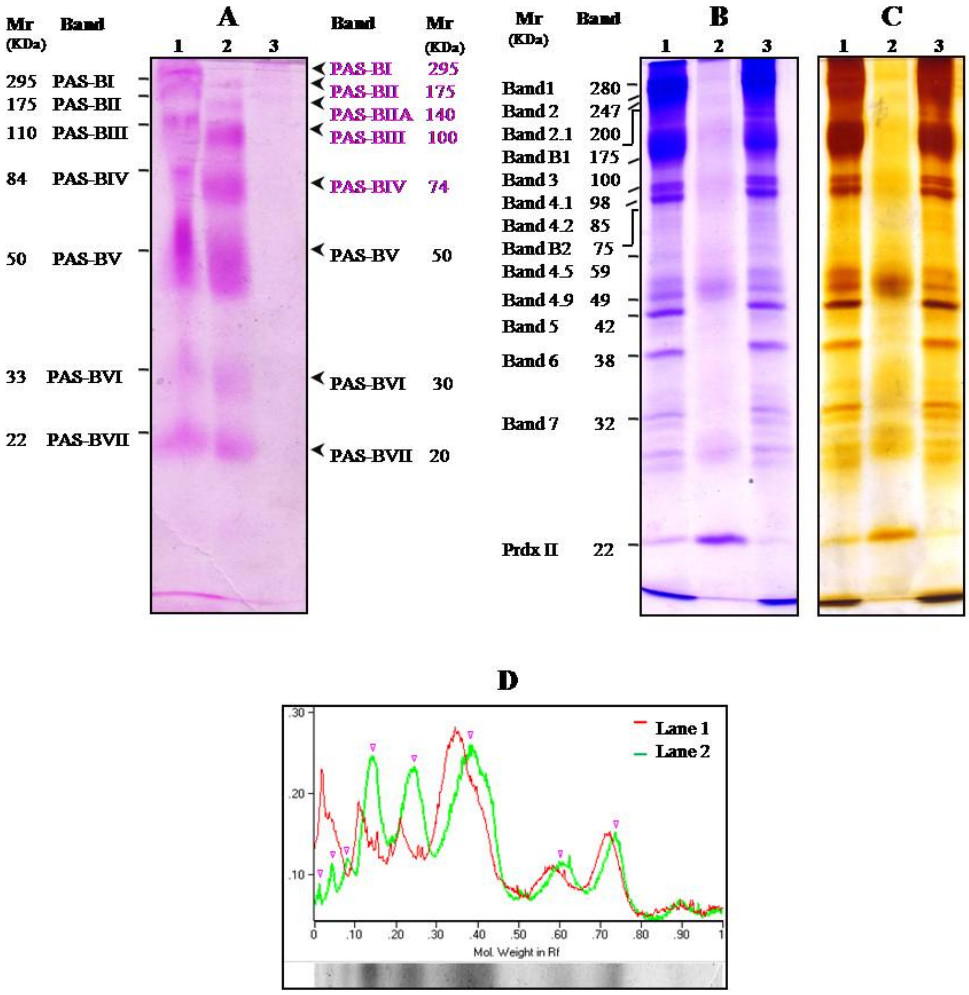

Figure 3. Analysis of partially purified glycoproteins obtained from buffalo erythrocyte (BE) membranes by SDS-PAGE (10% Gel). BE membranes (lane 1), glycoproteins extracted from membranes (lane 2) and residual proteins (lane 3). A: Gel stained with PAS stain (180 µg protein in each lane); B: Gel stained with CBB (60 µg protein in each lane); C: Gel stained with Silver stain (50 µg protein in each lane); D: Densitogram of PAS stained gel. Note: Arrowhead indicates the protein showing major (pink font) or minor (black font) difference/change.

lane 2) was done by silver stain (Figure 3C, lane 2) which is visualizing only the purified SGPs in the gel.

The solubilized SGPs of pig (Figure 4A) were showing changes in SGPs pattern, as two new additional glycoproteins (Figure 4A, lane 2) were observed which were not found to be present in PAS stained membrane fraction (Figure 4A, lane 1). The partially purified SGPs of pig (Figure 4A) erythrocyte membranes were showing two new bands PAS-PIA (160 kDa) on above the other SGPs and PAS-PIVA (25 kDa) at the lower position. PAS-PI (107 kDa) was appeared with low intensity while all other SGPs were with higher intensities. Along with increase in intensity, there was no change in Mr of SGPs. The densitogram (Figure 4D) of PAS stained gel (Figure 4A, lane 1 and 2) clearly indicates the results. The precipitated phase containing residual membrane proteins (Figure 4A, lane 3) revealed the extraction of SGPs from the membranes. The same samples (Figure 4A) were also loaded on separate gels to stain with CBB (Figure 4B) and silver (Figure 4C) stains. CBB stained gel showed all the membrane proteins (Figure 4B, lane 1) which were not shown with PAS stain (Figure 4A, lane 1). Similarly, the purified SGPs were not stained with CBB stain (Figure 4B, lane 2). The silver stained gel (Figure 4C, lane 2) was visualizing only the purified CBB stain, which confirmed the absence of any contamination of membrane proteins in the purified CBB stain (Figure 4A and C, lane 2).

DISCUSSION

Proteomics based analysis by gel electrophoresis helps in understanding the erythrocyte characteristics at molecular level. Comparative studies of erythrocyte SGPs of various mammalian species at molecular levels may provide insight

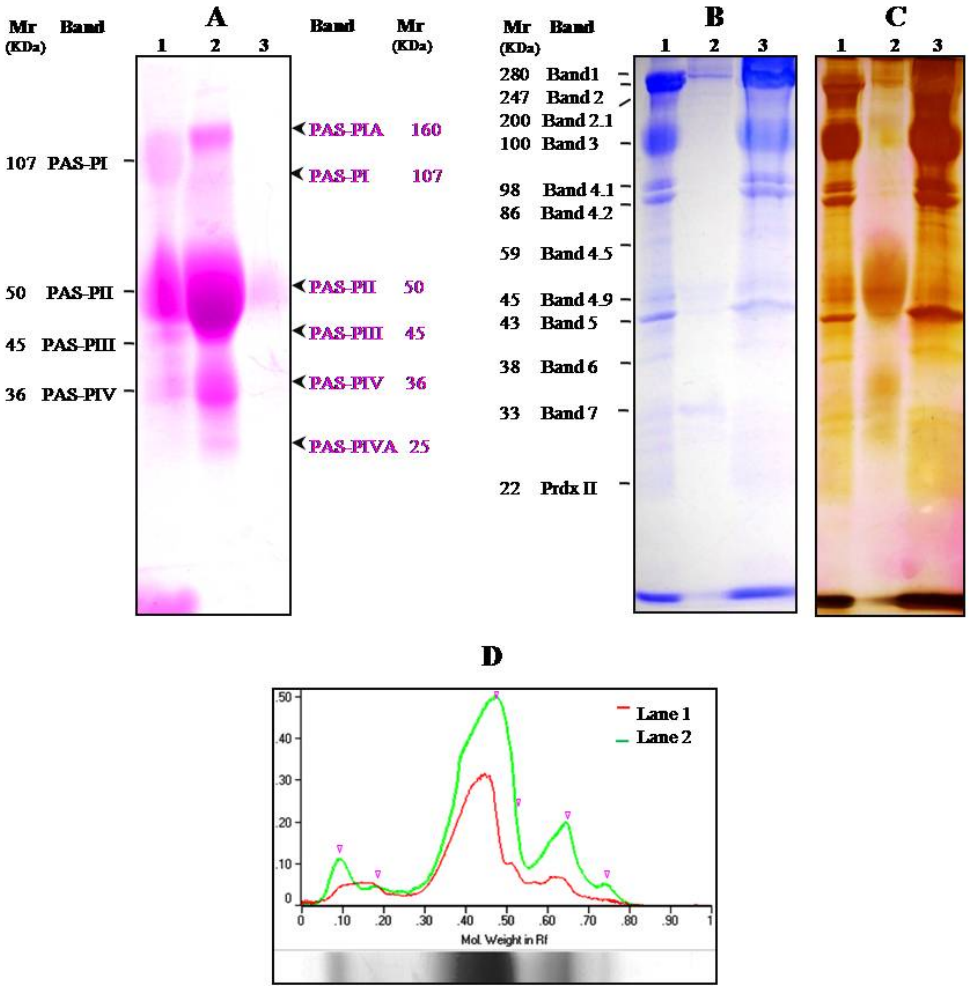

Figure 4. Analysis of partially purified glycoproteins obtained from pig erythrocyte (PE) membranes by SDS-PAGE (10% Gel). PE membranes (lane 1), glycoproteins extracted from membranes (lane 2) and residual proteins (lane 3). A: Gel stained with PAS stain (180 µg protein in each lane); B: Gel stained with CBB (60 µg protein in each lane); C: stained with Silver stain (50 µg protein in each lane); D: Densitogram of PAS stained gel. Note: Arrowhead indicates the protein showing major (pink font) or minor (black font) difference/change.

to know about its architecture among mammals. The species selected in this study are economically important animals, belong to same class and exhibit some phylogenetic relationship. The number of diseases depends on erythrocytes due to alterations in proteins and sialoglycoproteins (Chaudhuri et al., 1989; Daniels, 1999; Kumar et al., 2006). Therefore, from the viewpoint of health of these animals, the study of erythrocyte composition of these organisms has become a matter of concern. So, structural features of erythrocyte proteins of non-human mammals are required to be studied. To best of our knowledge, there is no available data to compare with our results on purified SGPs of erythrocytes for these animals using this electrophoretic technique and buffer system for the separation of SGPs except the isolation of a glycoprotein from goat erythrocyte membrane (Fletcher et al., 1976). The present study was thus designed to investigate the erythrocyte SGPs in three mammals (goat, buffalo and pig) in comparison to human erythrocytes. In this work, attempts are made for understanding the differences in structural features of membrane SGPs of goat, buffalo and pig with human erythrocytes. The membrane SGPs of erythrocytes analyzed by SDS-PAGE showed remarkable differences with PAS staining of gels. The study reveals the presence of some more SGPs in the erythrocyte membranes, which are masked by other membrane proteins as new SGPs appeared in erythrocyte membranes after the purification process. Based on results obtained, it is concluded that the erythrocyte membranes from different species show extensive dissimilarities in SGPs. The differences in SPGs are either due to differences in the sialic acid content or the amino acid content. There is no relationship between the size (surface area) and SPGs number as goat erythrocyte is smallest in size but have more

number of sialoglycoproteins among the species studied. As all the surface SGPs act as blood group antigenic determinants and receptor sites for viruses, bacteria and parasites, the presence of higher number of SGPs within the membrane of goat, buffalo and pig erythrocyte indicates the higher immunological response of the animals as compared to human.

ACKNOWLEDGEMENT

We thank Dr. S. M. Zingde, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Navi Mumbai for her valuable advice and providing the densitometry facilities during the course of this study.

REFERENCES

Barker, R. N. 1991. Electrophoretic analysis of erythrocyte membrane proteins and glycoproteins from different species. Comp. Haematol. Int. 1:155-160.

Chasis, J. A. and N. Mohandas. 1992. Red blood cell glycophorins. Blood 80:1869-1879.

Chaudhuri, A., V. Zbrzezna, C. Johnson, M. Nichols, P. Rubinsteinq, W. L. Marsh and A. O. Pogo. 1989. Purification and characterization of an erythrocyte membrane protein complex carrying Duffy blood group antigenicity. Possible receptor for *Plasmodium vivax* and *Plasmodium knowlesi*. J. Biol. Chem. 264:13770-13774.

Daniels, G. 1999. Functional aspects of red cell antigens. Blood Rev. 13:14-35.

Fairbanks, G., T. L. Steck and D. F. H. Wallach. 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10:2606-2617.

Fletcher, M. A., T. M. Lo and W. R. Graves. 1976. Immunochemical studies of infectious mononucleosis. V. Isolation and characterization of a glycoprotein from goat erythrocyte membranes. J. Immunol. 117:722-729.

Furthmayr, H., M. Tomita and V. T. Marchesi. 1975. Fractionation of the major sialoglycopeptides of the human red blood cell membrane. Biochem. Biophys. Res. Commun. 65:113-121.

Gratzer, W. B. 1981. The red cell membrane and its cytoskeleton. Biochem. J. 198:1-8.

Hamaguchi, H. and H. Cleve. 1972. Solubilization and comparative analysis of mammalian erythrocyte membrane glycoproteins. Biochem. Biophys. Res. Commun. 47:459-464.

Hanahan, D. J. and J. E. Ekholn. 1974. The preparation of red cell ghosts (membranes). Methods Enzymol. 31:168-172.

Herraez, A., J. C. Diez and J. Luque. 1992. Rat erythrocyte glycophorins can be isolated by the lithium diiodosalicylate method used for other glycophorins. Int. J. Biochem. 24:1705-1709.

Inaba, M. and Y. Maede. 1988. A new major transmembrane glycoprotein, gp155, in goat erythrocytes. J. Biol. Chem. 263:17763-17771.

Kakhniashvili, D. G., L. A. Bulla and S. R. Goodman. 2004. The human erythrocyte proteome. Mol. Cell. Proteomics 3:501-509.

Kornfeld, R. and S. Kornfeld. 1970. The structure of a phytohemagglutinin receptor site from human erythrocytes. J. Biol. Chem. 245:2536-2545.

Kumar, K. A., S. Singh and P. P. Babu. 2006. Studies on the glycoprotein modification in erythrocyte membrane during experimental cerebral malaria. Exp. Parasitol. 114:173-179.

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

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

Matei, H., L. Frentescu and Gh. Benga. 2000. Comparative studies of the protein composition of red blood cell membranes from eight mammalian species. J. Cell. Mol. Med. 4:270-276.

Murayama, J., M. Tomita and A. Hamada. 1982. Glycophorins of bovine erythrocyte membranes. Isolation and preliminary characterization of the major component. J. Biol. Chem. 91:1829-1836.

Pasini, E. M., H. U. Lutz, M. Mann and A. W. Thomas. 2010. Red blood cell (RBC) membrane proteomics - Part II: Comparative proteomics and RBC patho-physiology. J. Proteomics 73:421-435.

Steck, T. L. 1974. The organization of proteins in the human red blood cell membrane. J. Cell Biol. 62:1-19.

Spring, F. A. 2008. Characterization of blood-group-active erythrocyte membrane glycoproteins with human antiseras. Transfus. Med. 3:167-178.

Tanner, M. J. A. and D. H. Boxer. 1972. Separation and some properties of the major proteins of the human erythrocyte membrane. Biochem. J. 129:333-347.