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A Comparative Protein Profile of Mammalian Erythrocyte Membranes Identified by Mass Spectrometry

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Abstract A comparative analysis of erythrocyte membrane proteins of economically important animals, goat (*Capra aegagrus hircus*), buffalo (*Bubalus bubalis*), pig (*Sus scrofa*), cow (*Bos tauras*), and human (*Homo sapiens*) was performed. Solubilized erythrocyte membrane proteins were separated by sodium dodecyl sulfate-polyacryamide gel electrophoresis (SDS-PAGE), visualized by staining the gels with Commassie Brilliant Blue (CBB), and identified by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS). Emerging results show that all major erythrocyte membrane proteins present in human are also seen in all the animals except for band 4.5 which could not be identified. Band 3 is seen as more intense and compact, band 4.1 appears as a doublet in all the animal erythrocyte membranes, band 4.2 exhibits a slightly higher molecular weight (Mr) in buffalo, and cow and band 4.9 has a higher Mr in all the animals relative to the human protein. In addition, there are two new bands in the goat membrane, band G1, identified as HSP 90α, and band G2 identified as HSP 70. A new band C2 identified as HSP 70 is also seen in cow membranes. Peroxiredoxin II is of lower intensity and/or higher Mr in the animals. The difference in size of the proteins possibly indicates the variations in the composition of the amino acids. The difference in intensity of the proteins among these mammalians highlights the presence of less or more number of copies of that protein per cell. This data complement the earlier observations of differences in the sialoglycoprotein profile and effect of proteases and neuraminidase on agglutination among the mammalian erythrocytes. This study provides a platform to understand the molecular architecture of the individual erythrocytes, and in turn the dependent disorders, their phylogenetic relationship and also generates a database of erythrocyte membrane proteins of mammals. The animals selected for this study are of economic importance as they provide milk for the dairy industry and raw material for leather industry and are routinely sacrificed to obtain non vegetarian food worldwide.

Keywords Mammalian erythrocytes · Membrane proteins · Electrophoresis · MALDI-TOF/MS

Introduction

Mammalian erythrocytes are heavily studded with the oxygen-binding protein, the hemoglobin, and a set of enzymes, which are involved in the process of transport of respiratory gases. Inspite of common functions, mammalian erythrocytes have variable structure and lifespan. Healthy human erythrocytes have lifespan of 120 days, and that of bovines (buffalo and cow), goat, and pig are 160, 125, and 63 days, respectively (Dukes 1996). Structural studies of erythrocyte proteins are of immense importance for understanding the special features and functions of this cell. Comparative studies of animal erythrocyte proteins

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have been reported earlier (Lenard 1970; Hamaguchi and Cleve 1972; Kobylka et al. 1972; Ralston 1975; Suhail et al. 1988). However, in the early years, characterization of the human erythrocyte proteins was incomplete, and resolution of the different proteins had not reached the stage currently possible. Erythrocyte membrane proteins and sialoglycoproteins from man, rat, mouse, sheep, and dog species have been examined by Barker (1991) using the improved techniques of electrophoresis with more conventional gel stains. A comparative study of human, horse, and rat erythrocytes are also reported (Baskurt et al. 1997). Guerra-Shinohara and Barretto (1999) have reported the absence of band 4.2 (skeletal protein) in several mammalian species. We have earlier reported the difference in profile of sialoglycoproteins (Sharma and Gokhale 2011a) and the effect of proteinases and neuramindase on the agglutination of mammalian erythrocytes (Sharma and Gokhale 2012). A number of diseases depend on erythrocytes due to alterations in their proteins (An and Mohandas 2008; Pasini et al. 2010). Study of erythrocyte membrane provides information for the evaluation of their pathophysiologic significance. Therefore, from the viewpoint of health of these economically important animals, the study of erythrocyte membrane composition has become important. This study was undertaken to profile the major erythrocyte membrane proteins of goat, buffalo, pig, and cow using mass spectrometry so as to obtain their identity.

Materials and Methods

Chemicals

SDS, Phenyl methyl sulfonylflouride (PMSF), acrylamide, *N*,*N*'-methylene bisacrylamide, *N*,*N*,*N*,*N* tetramethyl ethylene diamine (TEMED), and CBB R-250 were purchased from the Sigma Chemicals Co., St. Louis, MO, U.S.A. Trifluoroacetic acid (TFA), α-Cyano-4-hydroxycinnamic acid (CHCA) matrix, and MALDI-TOF/MS calibration standards were obtained from Applied Biosystems. The other chemicals were the products of analytical research grade suitable for gel electrophoresis, staining, and mass spectrometric analysis.

Preparation of Erythrocytes

Human blood was obtained from healthy voluntary donors at Devi Ahilya University, Indore (M.P.). Informed written consent was obtained from voluntary donors. The blood of healthy animals was obtained from local slaughterhouses, Indore. 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×*g* for 5 min at room temperature (RT). Erythrocytes in the pellet were washed with 10 volumes of Tris-buffered saline (TBS; 10 mM Tris–HCl, pH 7.4 with 150 mM NaCl) and recentrifuged at 1,000×*g*. The washing was repeated four times ensuring that the hard pellet of the leukocytes was separated each time to obtain a buffy coat of the erythrocytes.

Preparation of Membranes from Erythrocytes

Membranes were prepared from erythrocytes obtained from six of each of the animals and six voluntary donors according to Hanahan and Ekholm (1974) with some modifications. Washed erythrocytes were lysed by mixing with 30 volume of cold 0.01 M Tris–HCl buffer containing 1 mM PMSF, pH-7.4. After 15 min in cold, the suspension was centrifuged at 30,000×*g* for 15 min in a refrigerated centrifuge 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 volume of cold washing buffer (0.01 M Tris–HCl buffer, pH 7.4) and recentrifuged at the same g value. The membranes were washed four times, when usually a milky white preparation was obtained.

Protein Profiling of Erythrocyte Membranes

Protein was estimated according to Lowry et al. (1951). Polyacrylamide gel electrophoresis in the presence of SDS was performed according to Laemmli (1970)to analyze the membranes. Protein samples were solubilized in sample buffer containing 0.031 M Tris, 1 % SDS, 0.25 % β-mercaptoethanol, and 5 % glycerol. The gels were stained with 0.1 % Coomassie brilliant blue (CBB) staining solution and destained in 40 % methanol and 10 % acetic acid. Erythrocyte membrane proteins from fourteen different animals of each species, and ten voluntary donors were resolved as described; the gels were stained with CBB, and the protein profiles were compared (Supplementary Figures S1 to S9).

Mass Spectrometric (MALDI-TOF/MS) Analysis of Membrane Proteins

Protein bands from a representative CBB-stained gel prepared from each animal (Figs. 1, 2, 3, 4) were cut out and processed for identification by Matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS) according to Shevchenko et al.

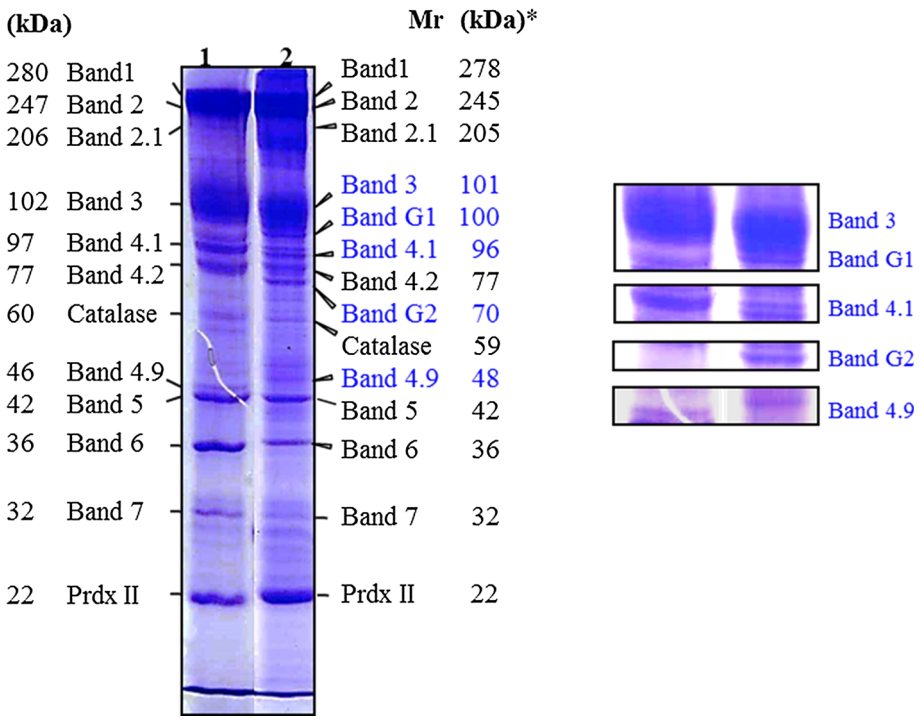

Fig. 1 Analysis of human (HE) and goat (GE) erythrocyte membrane proteins by SDS-PAGE (10 % Gel). HE (*lane 1*) and GE (*lane 2*) membranes stained with CBB (60 µg protein in *each lane*). *Blue font* refers to difference in/new proteins relative to the human erythrocyte membrane protein pattern. *Mr estimated from gel run. The identity of the proteins in *lane 2* is as per mass spectrometry data in Table 1

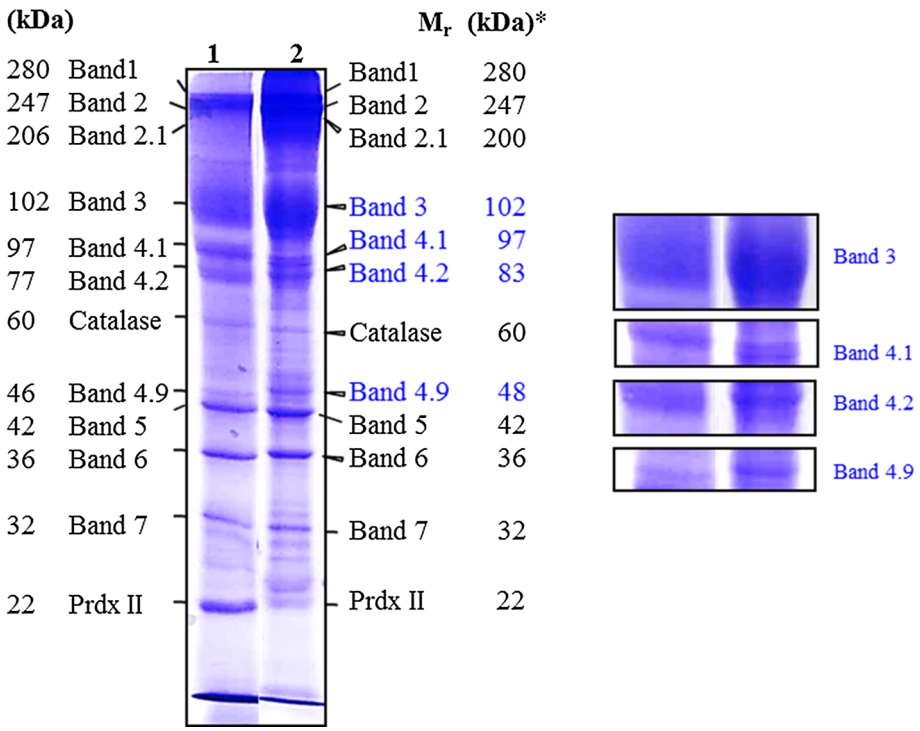

Fig. 2 Analysis of human (HE) and buffalo (BE) erythrocyte membrane proteins by SDS-PAGE (10 % Gel). HE (*lane 1*) and BE (*lane 2*) membranes stained with CBB (60 µg protein in *each lane*). *Blue arrow* head indicates the protein showing difference/new relative to the human erythrocyte membrane protein pattern. *Mr estimated from gel run. The identity of the proteins in *lane 2* is as per mass spectrometry data in Table 2

(1996).The protein digest was premixed with equal volume of CHCA matrix and spotted on MALDI plate (Brucker Daltonik). Peptide mass fingerprint (PMF) data were acquired on MALDI-TOF (Ultraflex II Brucker Daltonik, Germany) in the reflector mode. Mass calibration was carried out using peptide mixture spanning mass range of 800–4,000 m/z, and error was kept to less than 10 ppm. The resulting PMF data were processed and further

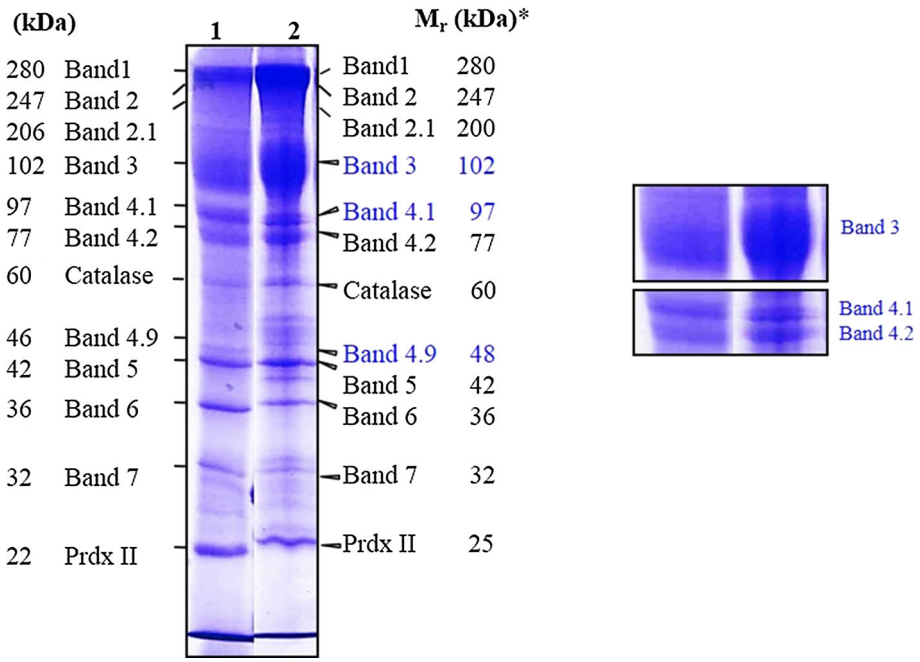

Fig. 3 Analysis of human (HE) and pig (PE) erythrocyte membrane proteins by SDS-PAGE (10 % Gel). HE (*lane 1*) and PE (*lane 2*) membranes stained with CBB (60 µg protein in *each lane*). *Blue font* refers to difference in/new proteins relative to the human erythrocyte membrane protein pattern. *Mr estimated from gel run. The identity of the proteins in *lane 2* is as per mass spectrometry data in Table 3

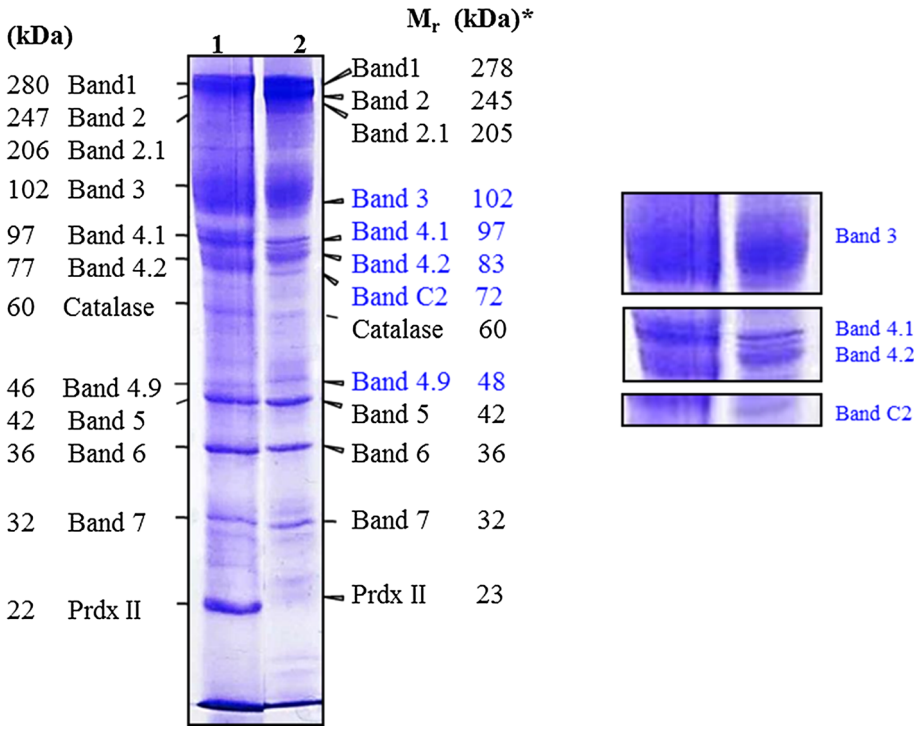

Fig. 4 Analysis of human (HE) and cow (CE) erythrocyte membrane proteins by SDS-PAGE (10 % Gel). HE (*lane 1*) and CE (*lane 2*) membranes stained with CBB (60 µg protein in each lane). *Blue font* refers to difference in/new proteins relative to the human erythrocyte membrane protein pattern. *Mr estimated from gel run. The identity of the proteins in *lane 2* is as per mass spectrometry data in Table 4

analyzed using BioTools Version 3.0 (Brucker Daltonik, Germany) software. The data were searched against Swiss-Prot database (version 57 and 2010_08 as indicated in Tables 1, 2, 3, 4) with taxonomy *Homo sapiens* for human and *Mammalia* for animal species using MASCOT search engine. Only those proteins identified by MASCOT search criteria with the top significant scores were considered as acceptable. The proteins identified were examined for sequence coverage, number of peptides matched, agreement between theoretical and experimental gel molecular weight, and matching of major peaks of PMF with the peptides identified in the protein.

Table 1 Mass spectrometric analysis of goat erythrocyte membrane proteins

Band[a]	Estimated M_r (kDa) on gel	PMF-MASCOT DATA							
		Entry name	Protein name	A.C No.	Matched taxonomy	Mass (kDa)/pI	Score	Sequence coverage (%)	Peptides matched/total peptides
Band 1	278	SPTA1_Human	Spectrin α	P02549	*Homo sapiens*	279.99/4.95	59	14	25/60
Band 2	245	SPTB1_Human	Spectrin β	P11277	*Homo sapiens*	246.46/5.15	132	18	33/58
Band 2.1	205	ANK1_Human	Ankyrin	P16157	*Homo sapiens*	206.26/5.65	87	14	17/66
Band 3	101	B3AT_HUMAN	Anion transporter	P02730	*Homo sapiens*	101.79/5.08	70	24	18/58
Band G1	100	HS90A_HORSE	HSP 90α	Q9GKX7	*Equus caballus*	83.09/5.00	121	26	18/47
Band 4.1	96	41_CANFA	Skeletal protein	Q6Q7P4	*Canis familiaris*	90.68/5.39	96	22	16/50
Band 4.2	77	EPB42_HUMAN	Skeletal protein	P16452	*Homo sapiens*	77.00/8.39	101	22	12/31
Band G2	70	HS71B_BOSMU	HSP 70 1B	Q4U0F3	*Bos mutus grunniens*	70.25/5.54	111	33	17/56
Catalase	59	CATA_BOVIN	Catalase	P00432	*Bos taurus*	59.91/6.79	96	20	7/37
Band 4.9	48	DEMA_HUMAN	Dematin	Q08495	*Homo sapiens*	45.51/8.94	70	24	8/29
Band 5	42	ACTB_CERPY	Actin	P84856	*Cercopithecus pygerythrus*	40.44/5.55	104	32	9/37
Band 6	36	G3P_BOVIN	G3PD	P10096	*Bos taurus*	35.86/8.51	75	31	8/32
Band 7	32	STOM_HUMAN	Stomatin	P27105	*Homo sapiens*	31.73/7.71	63	19	4/14
Peroxiredoxin II	22	PRDX2_BOVIN	Prdx II	Q9BG13	*Bos taurus*	21.94/5.37	38	15	3/30

[a] Refers to bands in lane 2 Fig. 1. The database used was Swiss-Prot version 57.0 except for Band 2.1for which version 2010_08 was used

Table 2 Mass spectrometric analysis of buffalo erythrocyte membrane proteins

Band[a]	Estimated M_r (kDa) on gel	PMF-MASCOT DATA							
		Entry name	Protein name	A.C No.	Matched taxonomy	Mass (kDa)/pI	Score	Sequence coverage (%)	Peptides matched/total peptides
Band 1	280	SPTA1_HUMAN	Spectrin α	P02549	*Homo sapiens*	279.99/4.95	53	7	14/22
Band 2	247	SPTB1_HUMAN	Spectrin β	P11277	*Homo sapiens*	246.46/5.15	56	7	11/26
Band 2.1	200	ANK1_HUMAN	Ankyrin	P16157	*Homo sapiens*	206.26/5.65	65	6	8/15
Band 3	102	B3AT_HUMAN	Anion transporter	P02730	*Homo sapiens*	101.79/5.08	70	24	18/58
Band 4.1	97	41_BOVIN	Skeletal protein	Q9N179	*Bos taurus*	69.25/6.16	80	23	14/37
Band 4.2	83	EPB42_HUMAN	Skeletal protein	P16452	*Homo sapiens*	77.00/8.39	101	22	12/31
Catalase	60	CATA_BOVIN	Catalase	P00432	*Bos taurus*	59.91/6.79	80	20	7/35
Band 4.9	48	DEMA_BOVIN	Dematin	Q08DM1	*Bos taurus*	45.54/8.81	66	18	6/22
Band 5	42	ACTB_BOVIN	Actin	P60712	*Bos taurus*	41.73/5.29	122	41	11/47
Band 6	36	G3P_BOVIN	G3PD	P10096	*Bos taurus*	35.86/8.51	46	31	6/41
Band 7	32	STOM_HUMAN	Stomatin	P27105	*Homo sapiens*	31.73/7.71	37	31	6/37
Peroxiredoxin II	22	PRDX2_BOVIN	Prdx II	Q9BGI3	*Bos taurus*	21.94/5.37	76	29	7/31

[a] Refers to bands in lane 2 Fig. 2. The database used was Swiss-Prot version 57.0 except for Band 2, band 2.1 and band 7 for which version 2010_08 was used

Table 3 Mass spectrometric analysis of pig erythrocyte membrane proteins

Band[a]	Estimated M_r (kDa) on gel	PMF-MASCOT DATA							
		Entry name	Protein name	A.C No.	Matched taxonomy	Mass (kDa)/pI	Score	Sequence coverage (%)	Peptides matched/total peptides
Band 1	280	SPTA1_HUMAN	Spectrin α	P02549	*Homo sapiens*	279.99/4.95	59	14	25/60
Band 2	247	SPTB1_MOUSE	Spectrin β	P15508	*Mus musculus*	246.25/5.19	57	15	26/95
Band 2.1	200	ANK1_HUMAN	Ankyrin	P16157	*Homo sapiens*	206.26/5.65	70	8	11/73
Band 3	102	B3AT_HUMAN	Anion transporter	P02730	*Homo sapiens*	101.79/5.08	70	18	13/35
Band 4.1	97	41_BOVIN	Skeletal protein	Q9N179	*Bos taurus*	69.25/6.16	98	20	14/36
Band 4.2	77	EPB42_HUMAN	Skeletal protein	P16452	*Homo sapiens*	77.00/8.39	64	16	8/58
Catalase	60	CATA_PIG	Catalase	O62839	*Sus scrofa*	59.88/6.32	121	29	13/21
Band 4.9	48	DEMA_BOVIN	Dematin	Q08DM1	*Bos taurus*	45.54/8.81	50	13	4/17
Band 5	42	ACTB_BOVIN	Actin	P60712	*Bos taurus*	41.73/5.29	116	36	11/49
Band 6	36	G3P_HUMAN	G3PD	P04406	*Homo sapiens*	36.05/8.57	45	15	3/14
Band 7	32	STOM_HUMAN	Stomatin	P27105	*Homo sapiens*	31.73/7.71	63	19	4/14
Peroxiredoxin II Fragment	25	PRDX2_PIG	Prdx II	P52552	*Sus scrofa*	14.16/4.70	80	46	5/13

[a] Refers to bands in lane 2 Fig. 3. The database used was Swiss-Prot version 57.0 except for Band 3 and band 4.2 for which version 2010_08 was used

Table 4 Mass spectrometric analysis of cow erythrocyte membrane proteins

Band[a]	Estimated M_r (kDa) on gel	PMF-MASCOT DATA							
		Entry name	Protein name	A.C No.	Matched taxonomy	Mass (kDa)/pI	Score	Sequence coverage (%)	Peptides matched/total peptides
Band 1	278	SPTA1_HUMAN	Spectrin α	P02549	*Homo sapiens*	279.99/4.95	58	9	11/23
Band 2	245	SPTB1_HUMAN	Spectrin β	P11277	*Homo sapiens*	246.46/5.15	44	6	9/65
Band 2.1	205	ANK1_HUMAN	Ankyrin	P16157	*Homo sapiens*	206.26/5.65	67	12	15/71
Band 3	102	B3AT_HUMAN	Anion transporter	P02730	*Homo sapiens*	101.79/5.08	91	24	14/40
Band 4.1	97	41_BOVIN	Skeletal protein	Q9N179	*Bos taurus*	69.25/6.16	88	14	8/28
Band 4.2	83	EPB42_BOVIN	Skeletal protein	O46510	*Bos taurus*	76.61/6.61	70	21	15/44
Band C2	72	HS71B_BOSMU	(HSP 70 1B)	Q4U0F3	*Bos mutus grunniens*	70.25/5.54	89	20	11/24
Catalase	60	CATA_BOVIN	Catalase	P00432	*Bos taurus*	59.91/6.79	80	17	7/37
Band 4.9	48	DEMA_HUMAN	Dematin	Q08495	*Homo sapiens*	45.51/8.94	62	19	5/21
Band 5	42	ACTB_BOVIN	Actin	P60712	*Bos taurus*	41.73/5.29	131	44	10/51
Band 6	36	G3P_HUMAN	G3PD	P04406	*Homo sapiens*	36.05/8.57	50	10	3/3
Band 7	32	STOM_HUMAN	Stomatin	P27105	*Homo sapiens*	31.73/7.71	78	27	6/29
Peroxiredoxin II Fragment	23	PRDX2_BOVIN	Prdx II	Q9BG13	*Bos taurus*	21.94/5.37	78	29	5/10

[a] Refers to bands in lane 2 Fig. 4. The database used was Swiss-Prot version 57.0 except for Band 1, band 3 and band 4.9 for which version 2010_08 was used

Results

Proteins of mammalian erythrocyte membranes were separated by the electrophoresis technique (SDS-PAGE), using discontinuous buffer system. Protein bands were visualized by staining with CBB. All CBB sensitive membrane polypeptides were identified in the human erythrocyte membranes by comparing our own published data (Sharma et al. 2013) and those of others (Fairbanks et al. 1971; Matei et al. 2000; Kakhniashvili et al. 2004). The protein band patterns obtained for each of the fourteen erythrocyte membrane preparations from the animals under study, and ten human volunteers were reproducible as given in supplementary Figs. S1–S9.

Figures 1, 2, 3, and 4 show the representative pattern obtained for each animal and human erythrocyte membrane proteins. The identity of the proteins in the human erythrocyte membranes (lane 1 in Figs. 1, 2, 3, 4) has been marked by comparison to our earlier work (Sharma et al. 2013). The major bands in lane 2, Figs. 1, 2, 3, and 4 have been marked in each of the figures as per the identity obtained by mass spectrometry (details given in Tables 1, 2, 3, 4).

The taxonomy *Mammalia* in MASCOT search engine was used to match PMF data acquired for erythrocyte membrane proteins of these mammalian species due to nonavailability of complete database for erythrocyte proteins of each animal. The CBB stained major membrane protein bands 1 (spectrin α subunit), band 2 (spectrin β subunit), band 2.1 (ankyrin), band 3 (anion exchanger), bands 4.1 and 4.2 (skeletal proteins), band 4.9 (dematin), band 5 (actin), band 6 [Glyceraldehyde-3-phosphate dehydrogenase (G3PD)], and band 7 (stomatin) were found to be present in all mammalian erythrocyte membranes as identified by MALDI-TOF/MS (Tables 1, 2, 3, 4). The cytosolic proteins viz. catalase and peroxiredoxin II (Prdx II) were found to be present in membrane fractions of all mammalian erythrocytes. The band in the position of Band 4.5 could not be identified by MALDI-TOF/MS in all the species examined.

The animal erythrocyte membranes showed differences either in size, shape, or intensity of CBB-stained protein bands as compared to human erythrocyte membranes (Figs. 1, 2, 3, 4). Table 5 summarizes the observations from Figs. 1, 2, 3, and 4. The changes observed are shown in bold. Band 3 is seen as more intense and compact, and band 4.1 appears as a doublet in all the animal erythrocyte membranes. Band 4.2 exhibits a slightly higher Mr in buffalo and cow, and band 4.9 is observed as a broad band of higher Mr in the animals relative to the human proteins. In addition, there are two new bands in the goat membrane, band G1, identified as HSP 90α, and band G2 identified as HSP 70. A new band C2 identified as HSP 70 is also seen in cow membranes. Peroxiredoxin II is of lower intensity and/or higher Mr in the animals.

Table 5 Summary of changes in protein bands as seen from Figs. 1, 2, 3, and 4

Human	Goat (Refer Fig. 1, lane 2 and Table 1)	Buffalo (Refer Fig. 2, lane 2 and Table 2)	Pig (Refer Fig. 3, lane 2 and Table 3)	Cow (Refer Fig. 4, lane 2 and Table 4)
Band 1	NC[a]	NC	NC	NC
Band 2	NC	NC	NC	NC
Band 2.1	NC	NC	NC	NC
Band 3	**Appears compact and intense**	**Appears compact and intense**	**Appears intense and wider**	**Appears compact and intense**
	Band G1 (HSP 90)	–	–	–
Band 4.1	**Doublet**	**Doublet**	**Doublet**	**Doublet**
Band 4.2	NC	**Higher Mr**	NC	**Higher Mr**
	Band G2 (HSP 70)	–	–	**Band C2 (HSP 70)**
Catalase	NC	NC	NC	NC
Band 4.9	**Higher Mr**	**Higher Mr**	**Appears intense/diffused and Higher Mr**	**Appears intense and Higher Mr**
Band 5	NC	NC	NC	NC
Band 6	NC	NC	NC	NC
Band 7	NC	NC	NC	NC
Prdx II	NC	**Same Mr but fainter**	**Higher Mr**	**Higher Mr but fainter**

Bold letters highlight the changes observed

[a] NC no change in band; Identity of the proteins is as given in respective Tables

Discussion

Since the introduction of SDS-PAGE and mass spectrometry, the proteins of human erythrocytes have been extensively studied, and many of their functions are elucidated (Low et al. 2002; Pasini et al. 2006; Mohandas and Gallagher 2008). In contrast, the composition and structure of erythrocytes from other species are less investigated. In the present study, four species (goat, buffalo, pig, and cow) were selected as they are economically important, belong to same class and exhibit some phylogenetic relationship. Our previous studies indicated the differences in the sialoglycoproteins of erythrocyte membranes of goat, buffalo, pig, and cow in comparison to human (Sharma and Gokhale 2011a, b). Our further work, an investigation on differential actions of trypsin, chymotrypsin, and neuraminidase on mammalian erythrocyte surface has also revealed the differences in the surface architecture of erythrocyte membrane proteins and glycoproteins (Sharma and Gokhale 2012). In the present report, an attempt was made for the first time to identify and compare the characteristics of the major membrane proteins of mammalian (goat, buffalo, pig, and cow) erythrocytes with that of human by MALDI-TOF/MS. The data obtained provide a database of erythrocyte membrane proteins of some of the important mammals. Some of the CBB-stained protein bands in the erythrocyte membranes of these animals differ slightly among themselves and with human erythrocyte membranes in sizes (M_r), possibly indicating the differences in the amino acids numbers and types. The intensity difference (decrease or increase) of specific proteins among these mammalian erythrocytes highlights the variations in the number of copies per cell. Besides these differences, the presence of most of the prominent M_r classes of erythrocyte membrane proteins in all the species examined is not unexpected, since most are now known to have important functions in the erythrocyte membrane (Gallagher 2005; Mohandas and Gallagher 2008).

Band 1 and 2, comprising the α and β subunits of spectrin, are the major components of the erythrocyte cytoskeleton, which is essential for the maintenance of the characteristic erythrocyte morphology. Inaba and Maede (1988a) have identified a novel protein, just below spectrin in goat erythrocytes, which they refer to as transmembrane glycoprotein (gp155). This band was seen in the present study but could not be identified by MALDI-MS. The discrete intense appearance of band 3 (anion exchanger) of animal erythrocytes as compared to human, reflects possibly an alteration either in amino acid composition and/or in glycosylation.

Band 4.1 (skeletal protein) appears as two sub-bands in different ratio in all animals. The ratio of splitting of band 4.1 (skeletal protein) into 4.1a and 4.1b is directly correlated with the short life span of pig erythrocytes (Inaba and Maede 1988b). They suggested that the protein 4.1a/b ratio is an internal marker of erythrocyte age. The amount of protein 4.1a initially equals to that of protein 4.1b and amount increases progressively during differentiation and cell aging. Since Band 4.1 (skeletal protein) and band 5 (actin) form part of the cytoskeleton (Bennett 1985), it is possible that the variation in the doublet band 4.1 may have some effect on the fragility of the erythrocyte membrane and hence the cell age.

Band 4.2 (skeletal protein) appears to have lower mobility due to its slightly higher M_r in buffalo and cow as compared to human, goat, and pig. Whether this has any reflection on the longer life span (160 days) of bovine erythrocytes needs attention. The effect of the alterations in bands 4.1 and 4.2 on the skeletal framework in animals would be interesting to pursue to understand their role in erythrocyte membrane architecture and in turn the life span of the erythrocyte.

Band G1 is heat shock protein 90α (HSP 90α) and G2 and C2 are heat shock protein 70 1B (HSP 70 1B). The presence of heat shock proteins in some animal membranes is similar to our earlier observation in human erythrocyte membranes, wherein we have shown the association of cytosolic Hsp 70 and Hsp 90 with the human erythrocyte membrane on exposure to heat stress (Sharma et al. 2013). The presence of heat shock proteins in erythrocytes of animals may be due to the fact these animals are more exposed to heat/environmental stress. Band 4.5 could not be identified by MALDI-TOF/MS in the human and animal erythrocyte membranes. Band 4.9 is observed as broad band with a higher M_r in animal erythrocytes.

In this study, we report for the first time the presence of catalase and Prdx II in the animal erythrocyte membranes. The presence of cytosolic proteins, catalase, and Prdx II in the membrane fraction indicates their distribution in cytosol as well as in membrane of erythrocytes similar to that in human erythrocytes as recently reported from our laboratory (Sharma et al. 2013). Cha et al. (2000) have also reported the association of Prdx II to the human erythrocyte membranes. Prdx II is observed as a single broad band of protein in the animal erythrocyte membrane and is identified as a fragment (14 kDa) by MALDI-TOF/MS in pig erythrocyte membrane.

In summary, the study provides a database of the mammalian erythrocyte membrane proteins which could be useful in understanding any subtle differences in the functions of the erythrocytes from the respective animals.

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